Natronococcus amylolyticus sp. nov., a Haloalkaliphilic Archaeon

HARUHIKO KANAI,¹ TETSUO KOBAYASHI,^{2*} RIKIZO AONO,¹ AND TOSHIKI KUDO²

Department of Bioengineering, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku,
Yokohama, Kanagawa 227,¹ and Laboratory of Microbiology, Institute of Physical
and Chemical Research (RIKEN), 2-1 Hirosawa, Wako,
Saitama 351-01,² Japan

The α -amylase-producing haloalkaliphilic archaeon *Natronococcus* sp. strain Ah-36^T (T = type strain) was isolated previously from a Kenyan soda lake, Lake Magadi. Most cells of strain Ah-36^T occurred in irregular clusters, and the colonies were orange-red. The polar lipids of this organism were composed of C₂₀, C₂₀ and C₂₀, C₂₅ derivatives of phosphatidylglycerol and phosphatidylglycerophosphate. Phosphatidylglycero-(cyclo-)phosphate, which is characteristic of *Natronococcus occultus*, was not detected. The complete nucleotide sequence of the 16S rRNA gene revealed that the closest relative of strain Ah-36^T is *N. occultus* ATCC43101^T (level of similarity, 96.4%), an extremely halophilic archaeon. However, strain Ah-36^T did not exhibit a significant level of DNA homology to *N. occultus* ATCC43101^T, which represents the only previously described species in the genus *Natronococcus*. We describe a new species for strain Ah-36^T, for which we propose the name *Natronococcus amylolyticus*.

The extremely halophilic archaea are currently placed in six validly described genera, including two genera of haloalkaliphiles, which are characterized by their alkaliphily and very low Mg²⁺ requirements (3). The haloalkaliphile genera form a distinct group within the halophilic archaea, as determined by a 16S rRNA phylogenetic analysis (9). The two haloalkaliphilic genera are the genera *Natronobacterium* and *Natronococcus*, whose cells are rod shaped and cocci, respectively. The genus *Natronobacterium* comprises four species, *Natronobacterium magadii*, *Natronobacterium pharaonis*, *Natronobacterium gregoryi* (15), and *Natronobacterium vacuolatum* (11), and the genus *Natronococcus* includes only one previously described species, *Natronococcus occultus*. The haloalkaliphilic archaea exhibit characteristically simple total-lipid patterns, in which the major polar lipids are C₂₀, C₂₀ and C₂₀, C₂₅ derivatives of phosphatidylglycerol and phosphatidylglycerophosphate (15). These organisms also contain minor phospholipids which can be easily detected by two-dimensional thin-layer chromatography (10, 11); *Natronobacterium magadii* contains PL3, *Natronobacterium pharaonis* contains PL1, *Natronobacterium gregoryi* contains PL1 and PL3, *Natronobacterium vacuolatum* contains PL3 and PL4, and *Natronococcus occultus* contains PL2. PL2 has been identified as C₂₀, C₂₀ and C₂₀, C₂₅ derivatives of phosphatidylglycero-(cyclo-)phosphate (8), but the other minor phospholipids have not been identified yet. So far, phospholipid PL2 has been found only in *Natronococcus occultus* among the halophilic archaea.

In the course of screening halophiles, we isolated strain Ah-36^T (T = type strain), an amylase-producing haloalkaliphilic archaeon, and classified this organism in the genus *Natronococcus* on the basis of some morphological and physiological properties. We purified and characterized the amylase produced by strain Ah-36^T (6) and determined its primary structure (5). In this paper we describe additional morphological, physiological, and genetic properties of strain Ah-36^T and de-

scribe a new species for this organism, for which we propose the name *Natronococcus amylolyticus*.

MATERIALS AND METHODS

Organisms and growth media. Isolation and preliminary characterization of *Natronococcus* sp. strain Ah-36^T (= JCM9655^T [Japan Collection of Microorganisms, The Institute of Physical and Chemical Research]) have been described previously (6). *Natronococcus occultus* ATCC 43101^T, *Natronobacterium magadii* ATCC 43099^T, *Natronobacterium gregoryi* ATCC 43098^T, and *Natronobacterium pharaonis* ATCC 35678^T were obtained from the American Type Culture Collection.

The organisms were grown in natronobacteria medium (1).

Microscopy. Cell size and shape were determined by phase-contrast microscopy.

Lipid analyses. Polar lipids were extracted and were analyzed by thin-layer chromatography by using the method of Collins et al. (2).

Molecular cloning of the 16S rRNA gene. The 16S rRNA gene was amplified by PCR by using total DNA from strain Ah-36^T as the template. The following PCR primers were used: 5'-C(C/T)G(G/T)TTGATCC(C/T)G(G/C)C(A/G)GAGG-3' (corresponding to the 5' end of the 16S rRNA) and 5'-CCATTGTAGC CCGCGT-3' (complementary to the 16S rRNA at around position 1200). The amplified DNA fragment (length, approximately 1.2 kb) was labeled with digoxigenin dUTP by using a DNA labeling kit supplied by Boehringer Mannheim Biochemicals. The labeled DNA was used as the probe to detect the 16S rRNA gene in the cloning procedure.

Total DNA which was isolated from strain Ah-36^T by a previously described method (5) was digested with *Eco*RI and analyzed by Southern hybridization to determine the size of the DNA fragment carrying the 16S rRNA gene. A DNA fragment which was approximately 6.5 kb long and hybridized to the probe was cloned in *Escherichia coli* MV1184 [*ara* Δ (*lac*-*proAB*) Δ (*srl*-*recA*)306::Tn10 ϕ 80*lacZ* Δ M15 *rpsL* *thi* [F' *lac*⁺ *lacZ* Δ M15 *proAB* *trd*36]] with pUC119 as the vector by using a previously described procedure (7).

DNA sequencing. The DNA sequence was determined with an Applied Biosystems model 373A DNA sequencer.

Phylogenetic analysis. A phylogenetic tree based on levels of divergence was constructed by the neighbor-joining method (13) by using the Clustal V package (4). All sites with gaps and all sites where nucleotides were not determined were omitted from the comparison.

The nucleotide sequences of the 16S rRNA genes of the following organisms were obtained from the EMBL database (Release 37) and were used for comparison: *Natronococcus occultus*, *Natronobacterium magadii*, *Haloferax volcanii*, *Halobacterium halobium*, *Halococcus morrhuae*, *Haloarcula marismortui*, *Methanococcus vannielii*, and *Methanobacterium formicium*.

DNA-DNA homology. Total DNAs from the haloalkaliphilic archaea were isolated by a previously described procedure (5, 15). DNA-DNA hybridization experiments were performed by the dot blot technique (7). Hybridization was carried out in a solution containing 50% formamide and 5% blocking reagent (Boehringer Mannheim) in 2 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 56°C (stringent conditions; denaturation temperature – 19°C) by using digoxigenin-labeled total DNAs as the probes. The blots were analyzed with a DIG DNA detection kit supplied by Boehringer Mannheim Biochemicals.

* Corresponding author. Present address: Department of Applied Biological Sciences, School of Agricultural Sciences, Nagoya University, Chikusa, Nagoya, Aichi 464-01 Japan. Phone: 81-52-789-4086. Fax: 81-52-789-4087. Electronic mail address: i45557a@nucc.cc.nagoya-u.ac.jp.

FIG. 1. Phase-contrast micrographs of *Natronococcus occultus* (A) and *Natronococcus* sp. strain Ah-36^T (B) in the logarithmic growth phase. Bars = 10 μ m.

Nucleotide sequence accession numbers. The nucleotide sequence of the 16S rRNA gene of strain Ah-36^T has been deposited in the DDBJ database under accession number D43628. The EMBL accession numbers for the sequences used for comparison are as follows: *Natronococcus occultus*, Z28378; *Natronobacterium magadii*, X72495; *Haloferax volcanii*, K00421; *Halobacterium halobium*, M11583; *Halococcus morrhuae*, X00662; *Haloarcula marismortui*, X61688; *Methanococcus vannielii*, M36507; and *Methanobacterium formicium*, M36508.

RESULTS

Morphology. As reported previously (6), strain Ah-36^T cells are nonmotile cocci that are 1 to 2 μ m in diameter and occur in irregular clusters, in pairs, and as single cells. Additional morphological studies of Ah-36^T revealed that strain Ah-36^T cells occurred mostly in irregular refractile clusters throughout each culture, while the cells of *Natronococcus occultus*, the type and only previously described species of the genus *Natronococcus*, occurred in pairs and as single cells, as shown in Fig. 1. The Ah-36^T colonies were circular, entire, and orange-red and varied in diameter from 1 to 5 mm, which may have

reflected differences in cell numbers in the cell clusters. On the other hand, *Natronococcus occultus* produced pale brown colonies that were uniform in size (diameter, about 1 mm). The orange-red color of Ah-36^T colonies suggests that bacterioruberin or another orange-red pigment is present in this organism.

Physiological and biochemical features. Some physiological and biochemical features of strain Ah-36^T have been described previously (6). In addition, Ah-36^T did not require a high magnesium ion concentration (>5 mM). Cell growth was observed in the presence of MgCl₂ concentrations of less than 1 mM. This organism was partially susceptible to tetracycline (MIC, 25 μ g/ml) while for *Natronococcus occultus* the MIC was 100 μ g/ml; slight growth of strain Ah-36^T and *Natronococcus occultus* was observed in the presence of 12.5 and 50 μ g of tetracycline per ml, respectively.

Lipids. The polar lipid pattern of Ah-36^T was the simplest pattern found in the halophilic archaea (Fig. 2). This organism contained C₂₀, C₂₀ and C₂₀, C₂₅ derivatives of phosphatidylglycerophosphate and phosphatidylglycerol. Phospholipid PL2, which is characteristic of *Natronococcus occultus* (8), was not present in this organism.

16S rRNA gene sequence. The nucleotide sequence of the 16S rRNA gene and its flanking regions is shown in Fig. 3. The 16S rRNA gene was estimated to be 1,474 nucleotides long by comparing it with 16S rRNA genes of other halobacteria and was followed by a putative alanine tRNA gene after a 133-nucleotide space. The sequence GTTAAG, which resembles the boxA sequence found in members of the family *Halobacteriaceae* ([T/C]TTAAG) (1a), was observed 214 bp upstream of the putative 5' end of the 16S rRNA. In addition, the sequence TTCGAnnnnnTTAA (n denotes any nucleotide) at positions 268 to 281 was identical to the promoter consensus sequence of ribosomal protein genes in *Halobacterium cutirubrum* (14). The flanking regions of the 16S rRNA gene could form an extensive double-helix structure which could act as a substrate for an RNase III-like enzyme, as demonstrated in *E. coli* (16).

Comparisons with 16S rRNA sequences from other haloalkaliphilic archaea revealed that *Natronococcus occultus* is the closest relative of strain Ah-36^T (Fig. 4), indicating that this strain belongs to the genus *Natronococcus*. However, the low level of sequence similarity (96.4%) may indicate that strain Ah-36^T is a representative of a new species. Strain Ah-36^T exhibited levels of sequence similarity

FIG. 2. Thin-layer chromatogram of polar lipids extracted from *Natronococcus occultus* (lane 1) and *Natronococcus* sp. strain Ah-36^T (lane 2). PG, phosphatidylglycerol; PGP, phosphatidylglycerophosphate; PL2, phosphatidylglycero-(cyclo)-phosphate.

Promoter Region	
<u>TTCGA</u> aggggTTAAgtactcctcgaggcttatgaatacgtccgaaggaaatgaggatcct	60
accctcgcggtccgcccgtacagatgggatctgatgtgagccttggtagttcggtgacgcc	120
cgatcggtccgcgattcggcaccaccgaactggaccatatacagatagacgattcaagtg	180
16S rRNA	
agtgtaaccgatcatactccgccaacctcccccttttgggggaagagcACTCCGGTTGA	240
TCCTGCCGGAGGCCATTGCTATTGGAGTCCGATTTAGCCATGCTAGTTGTACGAGTTTAG	300
ACTCGTAGCAGATAGCTCAGTAACACGTGGCCAACTACCCTCTGGAGCGCGACAACCTC	360
GGGAACTGAGGCTAATCGTGCATACCGCTCTCATGCTGGAAGTTGCAGAGAGCTCGAAA	420
TGCCCCGGCGCCAGAGGATGTGGCTGCGGCCGATTAGGTAGACGGTGGGGTAACGGCCCA	480
CCGTGCCAGTAATCGGTACGGGTTGTGAGAGCAAGAGCCCGAGACGGTATCTGAGACAA	540
GATACCGGGCCCTACGGGGCGCAGCAGGCGGAAACCTTTACACTGCACGCCAGTGCGAT	600
AAGGGGACTCCAAGTGCAGGGGCATAGAGCCCTCGCTTTTCACGACCGTAAGGAGGTCGT	660
AGAATAAGTGCTGGGCAAGACCGGTGCCAGCCGCGGGTAATACCGGCAGCACGAGTGA	720
TGGCCGCTCTTATTGGGCCTAAAGCGTCCGTAGCTGGCCGCGCAAGTCCATCGGGAAATC	780
CGCACGCTTAACGTGCGGGCGTCCGGTGGAAACTGCGTGGCTTGGGACCGGAAGATCCAG	840
AGGGTACGTCCGGGGTAGGAGTGAAATCCTGTAATCCTGGACGGACCACCGGTGGCGAAA	900
GCGCTCTGGAAAGACGGATCCGACGGTGAGGGACGAAAGCTCGGGTCACGAACCGGATTA	960
GATACCCGGGTAGTCCGAGCTGTAAACGATGTCTGCTAGGTGTGCCACAGGCTACGAGCC	1020
TGTGGTGTGCCGTAGGGAAGCCGTGAAGCAGACCGCCTGGGAAGTACGTCCGCAAGGATG	1080
AAACTTAAAGGAATTGGCGGGGAGCACTACAACCGGAGGAGCCTGCGGTTTAATTGGAC	1140
TCAACGCCGGACATCTCACCAGCATCGACAGTAGCAATGAAAATCAGTGTGATGAGCTTA	1200
TTGGAGCTACTGAGAGGAGGTGCATGGCCGCCGTGAGCTCGTACCGTGAGGCGTCTGTT	1260
AAGTCAGGCAACGAGCGAGACCCGCACTCCTAATTGCCAGCAACACCCTTGTGGTGGTTG	1320
GGTACATTAGGAGGACTGCCAGTGCCAAACTGGAGGAAGGAACGGGCAACGGTAGGTACAG	1380
TATGCCCCGAATGTGCTGGGCAACACGCGGGCTACAATGGTTCGAGACAGTGGGACGCAAC	1440
CCCAGAGGGGGCGCTAATCTCCGAAACTCGATCGTAGTTCCGGATTGCGGACTGAAACTC	1500
GTCCGCATGAAGCTGGATTCCGTAGTAATCGCCGTTTCAAGACGGCGGTGAATACGTCC	1560
CTGCTCCTTGACACACCGCCCGTCAAAGCACCCGAGTGAGGTCCGGATGAGGCCCTGT	1620
ACGGGGGTTCGAATCTGGGCTTCGCAAGGGGGCTTAAGTCGTAACAAGGTAGCCGTAGGGG	1680
AATCTGCGGCTGGATCACCTCCAcagaccgagaccaccccgacggggtggctcaccacat	1740
gcgggtcccgttcgggttagcgcccatcatgggcccgggacacgttgaactaccgaggc	1800
Alanine tRNA	
tcacaccatattacgctctccacctcgcggtggagAGTGGGCCCATAGCTCAGTGGGAGA	1860
GTGCTCCTTTGCAAGGAGGATGCCAGGGTTTGAATCCCTGTGGGTCCATactcgggtt	1920
ggatcgaatc	1930

FIG. 3. Nucleotide sequence of the 16S rRNA gene from *Natronococcus* sp. strain Ah-36^T. The 16S rRNA sequence and the alanine tRNA sequence are shown in capital letters. Nucleotide substitutions in *Natronococcus occultus* are shown above the sequence. The parts of the nucleotide sequence that are similar to the promoter consensus sequence of the extremely halophilic archaea are underlined.

of 92.9, 90.0, 89.6, 89.4, and 88.6% with *Natronobacterium magadii*, *Haloferax volcanii*, *Halobacterium halobium*, *Halococcus morrhuae*, and *Haloarcula marismortui*, respectively (Table 1).

DNA-DNA homology. Ah-36^T did not exhibit significant levels of DNA-DNA homology with *Natronococcus occultus*, *Natronobacterium magadii*, *Natronobacterium gregoryi*, and *Natronobacterium pharaonis* as determined by dot blot hybridization experiments (Fig. 5). Under the same experimental

conditions, *Halobacterium halobium* CCM2090 gave strong hybridization signals with *Halobacterium salinarum* CCM2148 and NCMB764 (levels of DNA-DNA homology, 60 and 77%, respectively) (data not shown) (12).

DISCUSSION

On the basis of morphological, physiological, and genetic properties, Ah-36^T is a member of the genus *Natronococcus*,

FIG. 4. Phylogenetic location of *Natronococcus amylolyticus* on a neighbor-joining tree based on 16S rRNA gene sequences. The 16S rRNA gene sequences from *Methanococcus vannielii* and *Methanobacterium formicium* were used to root the tree. The numbers at the nodes are bootstrap values for the nodes (based on 1,000 bootstrap resamplings). Bar = 0.01 nucleotide substitution per site. Abbreviations: Hc., *Halococcus*; Ha., *Haloarcula*; Hb., *Halobacterium*; Hf., *Haloferax*; Nb., *Natronobacterium*; Nc., *Natronococcus*.

but this strain differs from the only previously described species, *Natronococcus occultus* as follows: (i) the colonies of Ah-36^T are orange-red, while *Natronococcus occultus* produces pale brown colonies; (ii) Ah-36^T cells occur mostly in irregular clusters (Fig. 1); (iii) starch is hydrolyzed by Ah-36^T and gelatin is not liquefied (6), while *Natronococcus occultus* liquefies gelatin and does not hydrolyze starch; (iv) the polar lipids of Ah-36^T consist of only C₂₀, C₂₀ and C₂₀, C₂₅ derivatives of phosphatidylglycerol and phosphatidylglycerophosphate, and the minor polar lipid phosphatidylglycero-(cyclo-)phosphate is not detected; (v) the 16S rRNA gene sequences of Ah-36^T and *Natronococcus occultus* exhibit a level of similarity of only 96.4%; and (vi) no DNA-DNA homology is observed between Ah-36^T and *Natronococcus occultus*. Thus, we propose that Ah-36 should be designated the type strain of a new species, *Natronococcus amylolyticus*.

Description of *Natronococcus amylolyticus* Kanai, Kobayashi, Aono, and Kudo sp. nov. *Natronococcus amylolyticus* (am.y.lo.ly'ti.cus. Gr. n. *amylum*, starch; Gr. adj. *lyticus*, dissolving; M.L. adj. *amylolyticus*, starch dissolving). Cells are nonmotile cocci that are 1 to 2 µm in diameter and occur mostly in irregular clusters. Colonies are circular and orange-red. Cell lysis does

FIG. 5. Dot blot analysis to determine DNA-DNA homology. Abbreviations: Nb., *Natronobacterium*; Nc., *Natronococcus*; E., *Escherichia*.

not occur in distilled water. Extremely halophilic; growth occurs in the presence of NaCl concentrations between 8 and 30% and optimum growth occurs in the presence of 15 to 20% NaCl. The temperature range for growth is 22 to 50°C, and the optimum temperature is 40 to 45°C. The pH range for growth is 8.0 to 10.0, and the optimum pH is around 9.0. The polar lipids consist of C₂₀, C₂₀ and C₂₀, C₂₅ derivatives of phosphatidylglycerol and phosphatidylglycerophosphate. Does not contain phosphatidylglycero-(cyclo-)phosphate. Chemoorganotrophic. Obligatory aerobic. Nitrate and nitrite reduction positive. Starch is hydrolyzed. Gelatin is not liquefied. Susceptible to anisomycin, bacitracin, erythromycin, novobiocin, and tetracycline. Resistant to ampicillin, chloramphenicol, polymyxin B, and streptomycin. The G+C content of the DNA is 63.5 mol%. Inhabits a Kenyan soda lake, Lake Magadi.

The type strain is *Natronococcus amylolyticus* Ah-36 (= JCM 9655).

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Biodesign Program to T. Kudo.

TABLE 1. Matrix of levels of relatedness between species of archaea based on levels of similarity of 16S ribosomal DNA sequences^a

Species	% Sequence similarity								
	<i>Natronococcus amylolyticus</i>	<i>Natronococcus occultus</i>	<i>Natronobacterium magadii</i>	<i>Halobacterium halobium</i>	<i>Haloferax volcanii</i>	<i>Halococcus morrhuae</i>	<i>Haloarcula marismortui</i>	<i>Methanobacterium formicium</i>	<i>Methanococcus vannielii</i>
<i>Natronococcus amylolyticus</i>	100	96.4	92.9	89.6	90.0	89.4	88.6	76.8	75.6
<i>Natronococcus occultus</i> ^b		100	92.6	89.8	89.0	88.2	87.8	77.6	76.2
<i>Natronobacterium magadii</i> ^b			100	90.1	90.0	88.9	89.5	77.6	75.4
<i>Halobacterium halobium</i>				100	88.3	88.9	90.6	78.4	75.1
<i>Haloferax volcanii</i>					100	88.4	88.2	77.0	75.9
<i>Halococcus morrhuae</i> ^b						100	89.6	76.8	75.1
<i>Haloarcula marismortui</i> ^c							100	77.8	75.1
<i>Methanobacterium formicium</i>								100	81.0
<i>Methanococcus vannielii</i> ^b									100

^a Sites with gaps and sites where nucleotides were not determined were not included in the comparison.

^b The 16S ribosomal DNA sequence of the type strain was used.

^c The *mna* gene sequence was used.

We thank Michael Travisano for carefully reading the manuscript.

REFERENCES

1. American Type Culture Collection. 1989. Catalogue of bacteria and phages, 17th ed. American Type Culture Collection, Rockville, Md.
- 1a. Arndt, E., W. Krömer, and T. Hatakeyama. 1990. Organization and nucleotide sequence of a gene cluster coding for eight ribosomal proteins in the archaeobacterium *Halobacterium marismortui*. J. Biol. Chem. **265**:3034–3039.
2. Collins, M. D., M. Goodfellow, and D. E. Minnikin. 1980. Fatty acid isoprenoid quinone and polar lipid composition in the classification of *Curtobacterium* and related taxa. J. Gen. Microbiol. **118**:29–37.
3. Grant, W. D., and H. Larsen. 1989. Order *Halobacteriales* ord. nov., p. 2216–2233. In J. T. Staley, M. P. Bryant, N. Pfennig, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 3. The Williams and Wilkins Co., Baltimore.
4. Higgins, D. G., A. J. Bleasby, and R. Fuchs. 1992. CLUSTAL V: improved software for multiple sequence alignment. CABIOS **8**:189–191.
5. Kobayashi, T., H. Kanai, R. Aono, K. Horikoshi, and T. Kudo. 1994. Cloning, expression, and nucleotide sequence of the α -amylase gene from the haloalkaliphilic archaeon *Natronococcus* sp. strain Ah-36. J. Bacteriol. **176**:5131–5134.
6. Kobayashi, T., H. Kanai, T. Hayashi, T. Akiba, R. Akaboshi, and K. Horikoshi. 1992. Haloalkaliphilic maltotriose-forming α -amylase from the archaeobacterium *Natronococcus* sp. strain Ah-36. J. Bacteriol. **174**:3439–3444.
7. Kobayashi, T., Y. S. Kwak, T. Akiba, T. Kudo, and K. Horikoshi. 1994. *Thermococcus profundus* sp. nov., a new hyperthermophilic archaeum isolated from a deep-sea hydrothermal vent. Syst. Appl. Microbiol. **17**:232–236.
8. Lanzotti, V., B. Nicolaus, A. Trincone, M. De Rosa, W. D. Grant, and A. Gambacorta. 1989. A complex lipid with a cyclic phosphate from the archaeobacterium *Natronococcus occultus*. Biochim. Biophys. Acta **1001**:31–34.
9. McGenity, T. J., and W. Grant. 1993. The haloalkaliphilic archaeon (archaeobacterium) *Natronococcus occultus* represents a distinct lineage within the *Halobacteriales*, most closely related to the other haloalkaliphilic lineage (*Natronobacterium*). Syst. Appl. Microbiol. **16**:239–243.
10. Morth, S., and B. J. Tindall. 1985. Variation of polar lipid composition within the haloalkaliphilic archaeobacteria. Syst. Appl. Microbiol. **6**:247–250.
11. Mwatha, W. E., and W. D. Grant. 1993. *Natronobacterium vacuolata* sp. nov., a haloalkaliphilic archaeon isolated from Lake Magadi, Kenya. Int. J. Syst. Bacteriol. **43**:401–404.
12. Ross, H. N. M., and W. D. Grant. 1985. Nucleic acid studies on halophilic archaeobacteria. J. Gen. Microbiol. **131**:165–173.
13. Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**:406–425.
14. Shimmin, L. C., and P. P. Dennis. 1989. Characterization of the L11, L1, L10 and L12 equivalent ribosomal protein cluster of the halophilic archaeobacterium *Halobacterium cutirubrum*. EMBO J. **8**:1225–1235.
15. Tindall, B. J., H. N. M. Ross, and W. D. Grant. 1984. *Natronobacterium* gen. nov. and *Natronococcus* gen. nov., two new genera of haloalkaliphilic archaeobacteria. Syst. Appl. Microbiol. **5**:41–57.
16. Young, R. A., and A. Steitz. 1978. Complementary sequences 1700 nucleotides apart form a ribonuclease III cleavage site in *Escherichia coli* ribosomal precursor RNA. Proc. Natl. Acad. Sci. USA **75**:3593–3597.