ISSN 0026-2617, Microbiology, 2006, Vol. 75, No. 6, pp. 739–746. © Pleiades Publishing, Inc., 2006.
Published in Russian in Mikrobiologiya, 2006, Vol. 75, No. 6, pp. 849–856.

EXPERIMENTAL ARTICLES

Characterization of Extremely Halophilic *Archaea* Isolated from Saline Environment in Different Parts of Turkey[1]

B. Ozcan[a, 2], C. Cokmus[b], A. Coleri[b], and M. Caliskan[a]

[a] *Department of Biology, Faculty of Sciences and Letters, Mustafa Kemal University, 31040, Hatay, Turkey*
[b] *Department of Biology, Faculty of Science, Ankara University, 06100, Tandogan, Ankara, Turkey*

Received November 15, 2005

Abstract—Ninety-five extremely halophilic strains were isolated from six distinct saline regions of Turkey by using complex medium containing 25% NaCl. The selected regions are Tuz Golu (salt lake), Ankara; Aci Lake, Denizli; Salda Lake, Denizli; Seyfe Lake, Kyrsherhir; Tuzla Lake, Kayseri; and Bolluk Lake, Konya. The isolated strains were tested for motility, gram reaction, cell and colony morphologies, pigmentation, biochemical characteristics, and antibiotic sensitivities. According to membrane glycerol diether moieties and antibiotic susceptibilities, all isolated strains were found to belong to the domain *Archaea*. All isolates were examined for the presence of plasmids by agarose gel electrophoresis and it was established that most isolates contained plasmids that varied in number and whose molecular sizes ranged from 1 to 36.9 kbp. Whole-cell protein profiles from isolates were analyzed by SDS-PAGE and a similarity dendogram was constructed using the UPGMA method. Significant similarities and differences were observed among the isolates. The strains were clustered in eight groups and ten of our isolates were placed in the same group with the standard strains. The current study represents the first isolation and characterization of such a large collection of archeal strains from Turkey.

DOI: 10.1134/S002626170606018X

Key words: characterization, halophilic archaea, plasmids, SDS-Page (protein profile), Turkey.

INTRODUCTION

The increasing interest, in recent years, in microorganisms from hypersaline environments has resulted in the discovery of several new species and genera belonging to *Bacteria* and *Archaea*. Halobacteria are a group of microorganisms forming a part of the domain *Archaea* that require high salt concentration for growth [1]. Members of aerobic chemoorganotrophic halophilic archaea that require at least 12% (2M) NaCl for growth and that contain diether-linked lipids are classified in the family *Halobacteriaceae* [2]. *Halobacteriaceae* presently contains 18 genera and 56 species have been described, including some exclusively alkaliphilic species [2–7]. Genera containing mostly neutrophilic species include *Halobacterium, Haloferax, Haloarcula*, and *Natrialba* [8]. Strains of mostly red–orange pigmented extreme halophiles among the *Archaea* have been isolated from various hypersaline environments such as hypersaline lakes [9, 10], soda lakes [11, 12], solar salterns [13], and saline soils [14, 15].

Halophilic archaea have a number of useful applications and potential new applications in biotechnological process are being investigated. They produce products such as bacteriorhodopsin for information processing and ATP generation; novel extracellular polysaccharides; exoenzymes such as amylase, cellulase, xylanase, lipase, and proteases; poly-β-hydroxyalkanoate for biodegredable plastic production; and a protein from *Halobacterium salinarum* with significance for cancer research [8, 16–18]. Extremely halophilic archaea with economic importance continue to be isolated from different saline habitats in the world and new taxa in the *Halobacteriales* are still being described.

Hypersaline environments are commonly present in Turkey. Until now, only a few reports have existed on the characterization of halophilic Turkish archaeal communities [19, 20]. The main purpose of the current study is to isolate and characterize archeal strains from samples of brine and saline soil collected from six distinct saline regions of Turkey. The halophilic archaeal strains isolated were characterized for their morphological and biochemical properties, plasmid contents, and protein profiles.

MATERIALS AND METHODS

Collection of samples and isolation of halophilic Archaea. Brine, salt and saline soil samples were collected in sterile plastic bags and bottles from Tuz Golu (Salt lake), Ankara; Aci Lake, Denizli; Salda Lake, Denizli; Seyfe Lake, Kyrsherhir; Tuzla Lake, Kayseri;

[1] The text was submitted by the authors in English.
[2] Corresponding author; e-mail: birgulozcan@gmail.com.

and Bolluk Lake, Konya, in September 2000 and 2001. Each sample was either diluted in sterile Sehgal–Gibbons medium without organic material [21] or each sample was inoculated for enrichment and was streaked on SG agar medium. This medium contained (g/l^{-1}): NaCl, 250; $MgSO_4 \cdot 7H_2O$, 20; KCl, 2; Sodium citrate (trisodium salt), 3; Casamino acids, 7.5; Yeast extract, 1; and $FeSO_4 \cdot 7H_2O$, 0.0023. The pH was adjusted to 7.3 with 1M KOH. For solid medium, 20 g agar 1^{-1} was added. Plates were incubated at 37°C. After two weeks incubation, representative colonies were transferred to fresh SG medium and isolated in pure culture.

The strains of *Halobacterium salinarum*, *Haloferax mediterranei*, *Haloferax volcanii*, *Haloarcula marismortui*, and *Natrialba asiatica* were obtained courtesy of Prof. Dr. Aharon Oren (Jerusalem, Israel) and strains of *Haloarcula vallismortis* DSM 3756, *Halococcus morrhuae* CCM 537 were used as reference strains.

Morphological, cultural and biochemical tests. All tests for determining the phenotypic properties of isolates were carried out as specified in the proposed minimal standards for the description of new taxa in the order *Halobacteriales* [22].

Isolates were examined for motility and morphological features in wet mounts. Gram staining was carried out by using acetic acid fixed samples, as described by Dussault [23]. Colony appearance and pigmentation were determined on SG medium agar after growth for seven days.

Nitrate reduction was tested in liquid SG medium supplemented with 0.5% $NaNO_3$. Formation of gaseous products from nitrate was detected by the presence of gas bubbles of Durham tubes [24]. Anaerobic growth in the presence of L-arginine was tested according to Oren and Litchfield [25]. Production of acids from sugars was examined on SG medium supplemented with 0.5% (w/v) of sugars tested [26]. Indole production was determined by adding the Kovac's reagent to the SG broth supplemented with 1% (w/v) tryptone. To determine starch hydrolysis, the strains were streaked onto SG medium with 1% (w/v) soluble starch and plates were flooded with iodine solution after growth was obtained. Catalase was detected by adding a 1% (v/v) H_2O_2 solution to colonies on SG agar medium. The presence of oxidase was determined with tetramethyl-*p*-phenylendiamine-HCl [27]. Tween 80 and gelatine hydrolysis were tested as recommended by Gutierrez and Gonzales [28]. SG agar with 0.5% sodium thiosulfate was used to test production of H_2S. Casein hydrolysis was determined by observing the formation of clear zones around colonies on agar medium with 0.15% (w/v) skimmed milk powder [27]. Appropriate positive and negative controls were run for all of the above tests and they were carried out at least three times.

Lipid chromatography. Total lipid analysis of isolates was carried out as described by Ross et al. [29]. Extracted lipid fractionates from dry cells were applied to silica gel plates and then plates were developed in petroleum ether/diethyl ether (85 : 15, v/v). Archaeal lipids were revealed by spraying with dodecamolybdophosphoric acid and heating.

Plasmid isolation. Plasmid DNA was isolated by using a modified method of Anderson and McKay [30]. Electrophoresis was performed in 0.7% (w/v) agarose gels in TAE buffer (pH 8) at 120 V for 3.5 h. Plasmid sizes were determined by using a commercial plasmid size marker (Sigma D5292).

Whole cell protein SDS gel electrophoresis. SDS-PAGE of whole-cell proteins was performed as described by Laemmli [31] and Hesselberg and Vreeland [32]. The haloarchaeal cells were collected from synchronized SG broth cultures and the total cell proteins were extracted with SDS-sample buffer. Equal quantities of protein were applied to wells and electrophoresis was carried out at 30 mA for 3.5 h. Gels were stained with Coomassie Brillant Blue. Molecular weights were calculated for each protein band by comparison with SDS-Molecular weight markers (Sigma SDS–200) that were included with each gel run.

The protein profiles were scored manually as present (1) or absent (0) across all the archaeal samples, and the values were used to compile a binary data matrix. The MVSP software package version 3.1 [33] was used to calculate Nei and Li [34] similarity coefficients. A dendogram was constructed using the Unweighted Pair-Group Method of the Arithmetic Average (UPGMA) [35].

Antibiotic susceptibility. Antibiotic susceptibility was tested by spreading cell suspensions on plates of SG medium and applying antibiotic discs (ampicillin, 10 μg; norfloxacin, 10 μg; tetracycline, 30 μg; bacitracin, 10 IU; rifampicin, 5 μg; azithromycine, 15 μg; neomycin, 30 μg; chloramphenicol, 30 *v*; penicillin G, 10 IU; vancomycin, 30 μg; novobiocin, 30 μg) [36, 21]. The results were recorded as sensitive or resistant after 14 days of incubation at 37°C.

RESULTS

Cell and colony morphology. In this study, a total of 980 isolates were obtained from the regions described in Materials and Methods. From these, 95 archaeal colonies were selected on the basis of their different colony and cell morphologies. Seventy-two archaeal strains were obtained from saline soil samples, twenty-one from brine, and two from salt samples.

All archaeal colonies that developed on the plates were circular with mostly entire edges, convex, non-mucoid, opaque, and of different shades of red. However, high-convex, raised, transparent, translucent, and colorless colonies were also observed. Cells of isolates were found by phase-contrast microscopy to vary from rods, cocci, or pleomorphic rods, to flat extremely pleomorphic shapes when growing on SG broth (Fig. 1). Five isolates had rod-cocci cell morphologies during

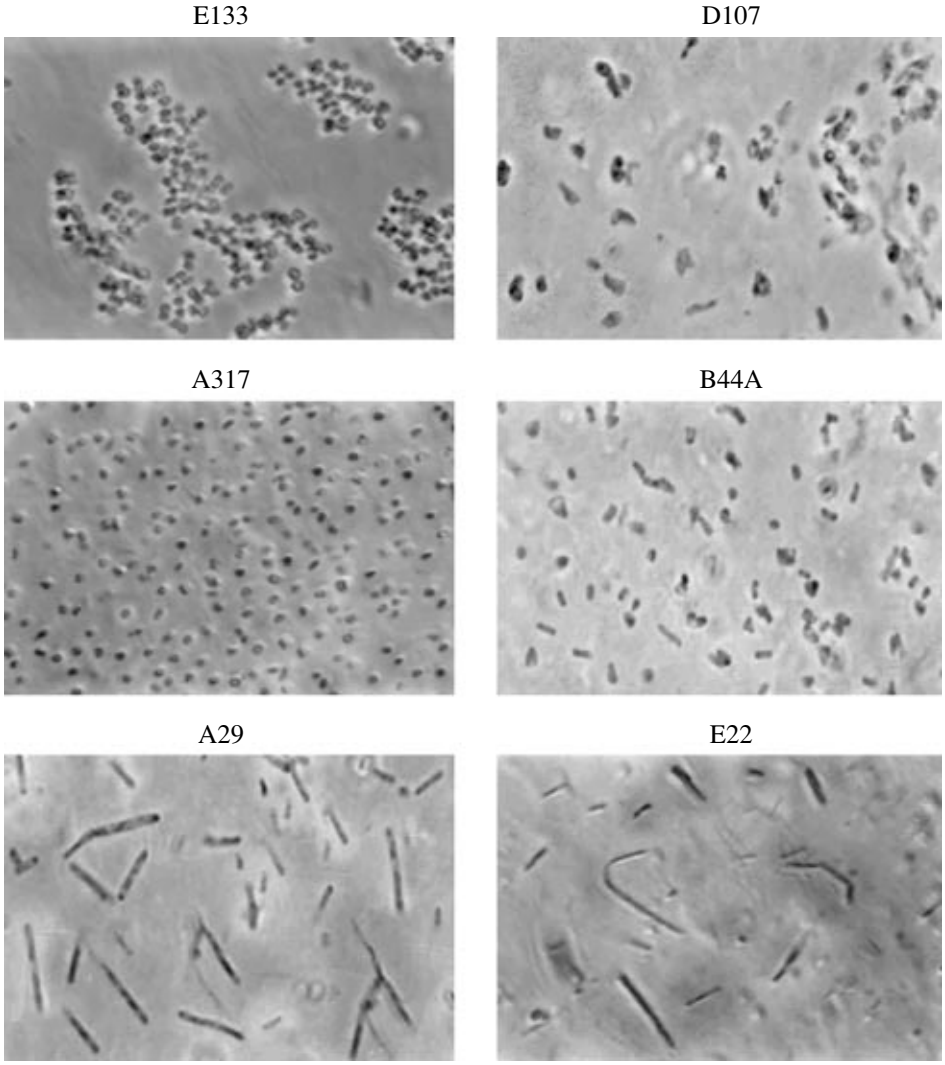

Fig. 1. Phase-contrast micrograph of isolated archaeal strains showing different cell morphologies (X2680).

their growth period, being rod-shaped and derived from colorless colonies at the early stage of colony growth, but becoming cocci-shaped and turning red at a later period of colony growth. Most of the strains stained gram-negative, a few were gram-variable. Further information on the cell and colony morphology of the isolates is given in Table 1.

Biochemical and antibiotic sensitivity characteristics. All isolated strains were catalase- and oxidase-positive. Most isolates produced hydrogen sulfide from sodium thiosulphate and reduced nitrate to nitrite, while a few produced gas from nitrate. Only two isolates grew anaerobically on arginine. Different reactions were noticed for starch, gelatin, Tween 80, casein hydrolysis, indole production and acid formation from several sugars. A summary of the biochemical properties of the strains is presented in Table 2. All strains isolated were found to be resistant to ampicillin, norfloxacin, tetracycline, azithromycine, neomycin, chloramphenicol, penicillin G, and vancomycin. All were sensitive to novobiocin, bacitracin, and rifampicin.

Ether lipid analysis and plasmid contents. All isolated strains were found to have phytanyl diether moieties according to the results of thin layer chromatography. Sixty of the ninety-five isolates were found to contain plasmids that varied in both number and size. Twenty-three isolates harbor a single plasmid, twenty isolates contained two, five isolates contained three, seven isolates contained four, and one isolate harbored as many as six plasmids. Plasmids sizes varied between 1 and 36.9 kbp.

Protein profiles. The electrophoretic conditions used in this work allowed resolution of up to 51 protein bands among the isolated strains. An example of a typical electrophoretic gel is presented in Fig. 2. The strains used in this study produced between 19 and 36 major protein bands that were used in computer comparisons.

Table 1. Morphological characteristics of the isolated halophilic archaeal strains

Characteristic	Isolate number
Gram (–)	92
Gram (+/–)	3
Colonial properties	
Circular	95
Mucoid colony	17
Highly convex	7
Convex	80
Raised	8
Entire edge	90
Irregular edge	5
Opaque	59
Transparent	4
Translucent	32
Motility	79
Cell morphology	
Rod	59
Pleomorphic	18
Cocci	5
Cocci-rod	5
Pleomorphic rod	8

Table 2. Biochemical properties of the isolated halophilic archaeal strains

Tests (positive result)	Isolate number
Catalase activity	95
Oxidase ativity	95
H_2S production	81
Nitrate reduction	78
Tween 80 hydrolysis	51
Indole production	44
Gelatin hydrolysis	44
Casein hydrolysis	30
Starch hydrolysis	29
Anaerobic growth on L-arginine	2
Gas from nitrate	2
Fermentation of sugars	
Glucose	53
D(+)-xylose	45
L(+)-arabinose	34
Sucrose	32
Maltose	28
D(–)-fructose	20
D(+)-galactose	15

The computer analysis of the protein profiles clustered these isolates into eight groups. The computer analysis was carried out at several clustering levels before selection of the eight clusters presented in Fig. 3. This cluster level was selected since it represented the most stable grouping of the strains.

The similarity dendogram identified two major clusters. The first one comprised groups 7 and 8 and the second comprised groups 1 to 6. Only ten isolates were clustered with known haloarchaeal strains, which are clustered in group 1. Two strains (A263A and A263B) have 100% similarity in group 1, showed 80% similarity to *Halobacterium salinarum*, and also share the same colony and cell morphology and biochemical features, and have one common plasmid. Furthermore, only these two isolates and *Halobacterium salinarum* could grow anaerobically in the presence of L-arginine [25]. Another high similarity level (81%) was found between *Haloferax mediterranei* and strain A317. They are similar in terms of colony and cell morphologies, pigmentation, biochemical properties, and having no plasmids. However, isolate A317 produced H_2S, but *Haloferax mediterranei* does not [26]. Additionally, all of the cocci cell-shaped isolates showing different colony morphology, pigmentation, and biochemical characteristics were clustered with *Halococcus morrhuae* CCM 537.

Group 2 included 16 isolates. They were rod-shaped and showed similar colony morphologies and red pigmentation except for one isolate which appeared pink. Most of the isolates did not hydrolyze gelatin, Tween 80, starch, or casein; did not produce indole; and did not reduce nitrate. Twenty-two isolates clustered in group 3 but exhibited some differences in morphological and biochemical features. Cells of isolates were rod-shaped, pleomorphic rod, or pleomorphic showing different shapes. Group 4 included 16 isolates of which 13 were pleomorphic cell-shaped, while three had rod-shaped cells. The isolates in this group showed different biochemical characteristics. Four isolates with rod-shaped cells clustered in group 5. The colonies of three isolates were mucoid and two of the isolates were colorless. Indole formation, starch hydrolysis, and acid production from sugars were not usually observed among the strains in this group. Each isolate contained plasmids in varying numbers and of different sizes.

Group 6 contained 15 strains that differed in biochemical properties and plasmid content, and that had colony and cell morphologies that were rod-shaped or pleomorphic. Ten isolates clustered in group 7. While nine isolates had a similar colony morphology (mucoid, highly convex, opaque, and pink–red-colored), the remaining isolate was red, transparent, and nonmucoid. Five isolates had rod-shaped cells and colorless colonies at the beginning of their growth periods and then

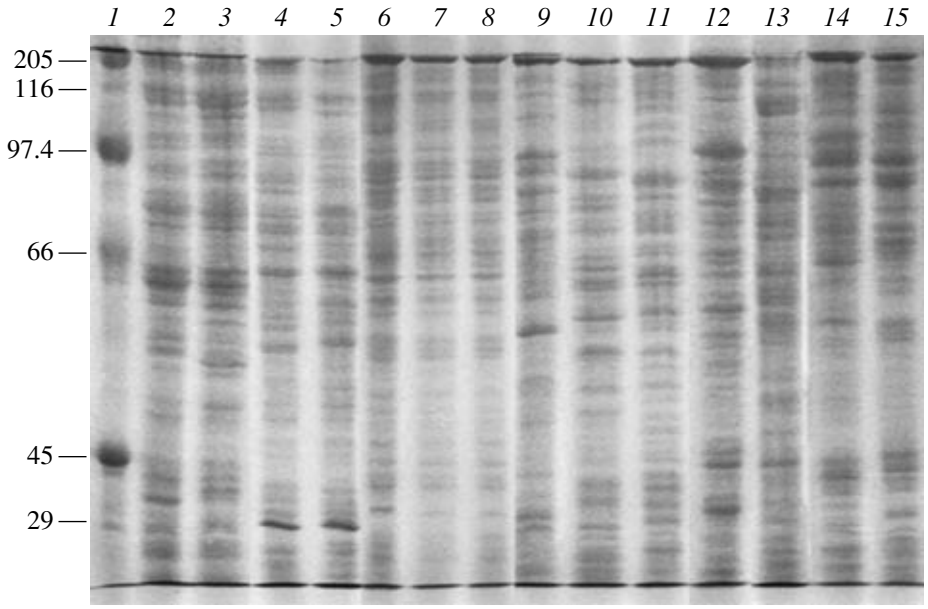

Fig. 2. Protein profiles of the archaeal strains isolated from distinct saline environments. Lane *1*, molecular weight standard mixture (Sigma Chem. Co., MW-SDS–6H), (in kilodalton, kDa); Lane *2*, *Halobacterium salinarum*; Lane *3*, A263A; Lane *4*, *Haloferax mediterranei*; Lane *5*, A317; Lane *6*, D83A; Lane *7*, D113; Lane *8*, F98; Lane *9*, D31; Lane *10*, E55; Lane *11*, A43; Lane *12*, C13; Lane *13*, F4A; Lane *14*, B54A; Lane *15*, E57.

changed into red colonies with cocci-shaped cells during later growth. Most of the isolates in this group reduced nitrate and did not produce H_2S. Group 8 included only two strains that showed different colony and cell morphologies. One was orange–red in color and had a pleomorphic cell shape, the other produced pink colonies with rod-shaped cells. Both strains exhibited further differences in their biochemical characteristics and plasmid content.

DISCUSSION

In the current study, 95 archaeal strains were isolated from several distinct parts of Turkey. All 95 isolates examined belong to the archaeal domain, family *Halobacteriaceae*. Evidence supporting this was obtained from the extremely halophilic nature of the cultures, the red color formation [8], ether-lipid contents [32], and resistance to antibiotics that target the bacterial peptidoglycan [36, 37]. Besides their ether-lipid content, antibiotic sensitivity data is one of the most important criteria among the proposed minimal standards for the description of new isolates in the order *Halobacteriales* [22]. The information obtained for the antibiotic sensitivity of these strains may serve as a guideline for the enrichment and isolation of archaeabacteria from natural sources. It was previously reported that the *Halobacteriaceae* are generally resistant to ampicillin, cycloserine, chloramphenicol, kanamycin, polymixin, erythromycin, neomycin, penicillin G, tetracycline, and streptomycin, and are sensitive to anisomycin, bacitracin, rifampicin, and novobiocin [36, 6]. In the current study, our isolates were also resistant to ampicillin, norfloxacin, tetracycline, azithromycine, neomycin, chloramphenicol, penicillin G, and vancomycin, and sensitive to novobiocin, rifampicin, and bacitracin.

Phenotypic characterization, as well as protein elctrophoresis profiles and plasmid characterization, are useful tools for isolation and preliminary clustering of unknown halophilic archaeal strains [13, 32, 38]. Although protein profiles of new isolates have seldom been included in species description [39], they are helpful for preliminary grouping of a large number of unknown strains [35]. In the case of our 95 isolates, the data obtained by protein profiling enabled us to construct a similarity dendogram that clustered our isolates into eight groups and that associated ten halophilic archaeal isolates with known halophilic archaeal strains.

The occurrence of plasmids and megaplasmids in halophilic archaea has been reported previously [40, 41]. In the description of halobacterial strains, the determination of the plasmid content has been recommended [22]. However, their presence cannot be considered as a definite characteristic for the classification of halophilic archaea. The plasmid numbers and sizes determined in this study showed differences between the isolated strains. No megaplasmids were detected and the plasmid size was between 1 and 36.9 kbp.

In the current study, we have described a large collection of halophilic archaeal strains collected from various saline parts of Turkey. Now, we plan to further characterize the isolates by determining properties such

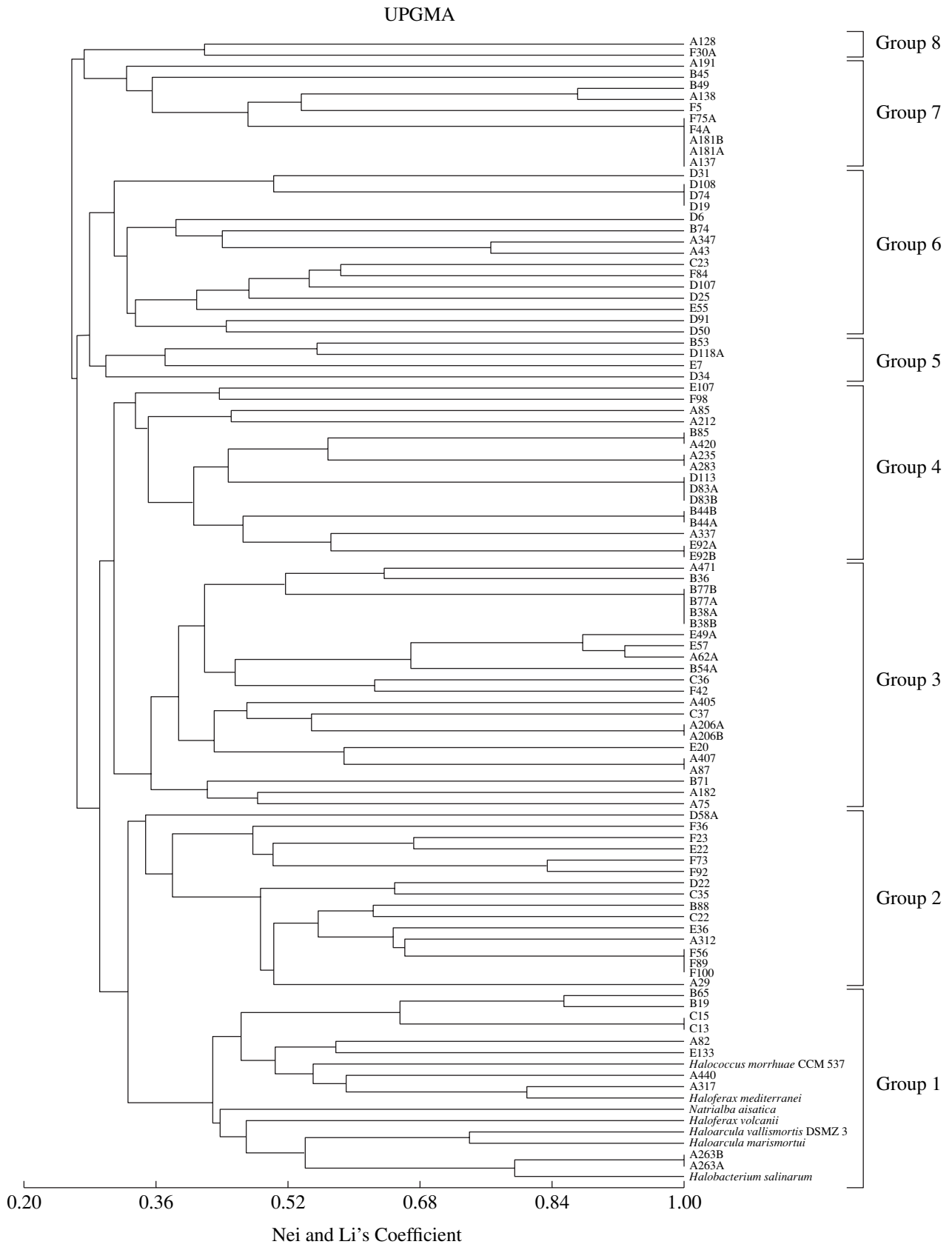

Fig. 3. Similarity dendogram between isolated halophilic archaeal strains based upon protein profiles.

as G+C content of the DNA, sequence of 16 S rRNA, and polar-lipid characterization in order to make a more precise classification of our isolates.

ACKNOWLEDGMENTS

We would like to thank prof. Dr. Aharon Oren for the standard archaeal strains and helpful discussions.

REFERENCES

1. Kamekura, M., Diversty of Extremely Halophilic Bacteria, *Extremophiles*, 1998, vol. 2, pp. 289–295.
2. Grant, W.D., Kamekura, M., McGenity, T.J., Ventosa, A., and Order, I., Halobacteriales, *Bergey'S Manual of Systematic Bacteriology V.I. The Archaea and Deeply Branching and Phototrophic Bacteria. 2nd ed.*, Garrity G.M., Ed., New York: Springer, 2001.
3. Elshahed, M.S., Savage, K.N., Oren, A., Gutierrez, M.C., Ventosa, A., and Krumholz, L.R., *Haloferax sulfurifontis* sp. nov., a Halophilic Archaeon Isolated from a Sulfide- and Sulfur-Rich Spring, *Int. J. Syst. Evol. Microbiol.*, 2004, vol. 54, pp. 2275–2279.
4. Fan, H., Xue, Y., Ma, Y., Ventosa, A., and Grant, W.D., *Halorubrum tibetense* sp. nov., a Novel Haloalkaliphilic Archaeon from Lake Zabuye in Tibet, China, *Int. J. Syst. Evol. Microbiol.*, 2004, vol. 54, pp. 1213–1216.
5. Feng, J., Zhou, P., and Liu, S., *Halorubrum xinjiangense* sp. nov., a Novel Halophile Isolated from Saline Lakes in China, *Int. J. Syst. Evol. Microbiol.*, 2004, vol. 54, pp. 1789–1791.
6. Feng, J., Zhou, P., Zhou, LiuY.S., and Warren-Rhodes, K., *Halorubrum alkaliphilum* sp. nov., a Novel Haloalkaliphile Isolated from a Soda Lake in Xinjiang, China, *Int. J. Syst. Evol. Microbiol.*, 2005, vol. 55, pp. 149–152.
7. Kamekura, M, Alkaliphilic Microorganisms, *Enigmatic Microorganisms and life in extreme environments*, Seckbah, J., Ed., Kluwer Acad. Publishers, Netherland, 1999.
8. Oren, A, The Order *Halobacteriales, The Prokaryotes. A handbook on the Biology of Bacteria: Ecophsiology, Isolation, Identifications, Applications,* Dwarkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., and Stackebrandt, E., Eds., 3rd ed., Springer: New York, 2001 (electronic publication).
9. Franzmann, P.D., Stackebrandt, E., Sanderson, K., Volkman, J.K., Cameron, D.E., Stevenson, P.L., McMeekin, T.A., and Burton, H.R., *Halobacterium lacusprofundi* sp. nov., Halophilic Bacterium Isolated from Deep Lake, Antarctica, *Syst. Appl. Microbiol.*, 1988, vol. 11, pp. 20–27.
10. Oren, A., Gurevich, P.R., Gemmel, T., and Teske, A., *Halobaculum gomorrense* gen. nov., sp. nov., a Novel Extremely Halophilic Archaeon from Dead Sea, *Int. J. Syst. Bact.*, 1995, vol. 45, pp. 747–754.
11. Soliman, G.S.H. and Truper, H.G., *Halobacterium pharaonis* sp. nov., a New Extremely Haloalkaliphilic Archaebacterium with Low Magnesium Requirement, *Zbl. Bakteriol. I. Abt. Orig.*, 1982, vol. C, pp. 318–329.
12. Tindall, B.J., Ross, H.N.M., and Grant, W.D., *Natronobacterium* gen. nov. and *Natronococcus* gen. nov., Two New Genera of Haloalkaliphilic Archaebacteria, *Syst. Appl. Microbiol.*, 1984, vol. 5, pp. 41–57.
13. Asker, D. and Ohta, Y., *Haloferax alexandrinus* sp. nov., an Exteremely Halophilic Cathaxanthin-Producing Archaeon from a Solar Saltern in Alexadria (Egypt), *Int. J. Syst. Evolut. Microbiol.*, 2002, vol. 52, pp. 729–738.
14. Ihara, K., Watanabe, S., and Tamura, T., *Haloarcula argentinensis* sp. nov. and *Haloarcula mukohatei* sp. nov., Two Extremely Halophilic Archaea Collected in Argentina, *Int. J. Syst. Bacteriol.*, 1997, vol. 47, pp. 73–77.
15. Zvyagintseva, I.S. and Tarasov, A.L., Extreme Halophilic Bacteria from Saline Soils, *Microbiology*, 1988, vol. 56, pp. 664–668.
16. Chaga, G., Porath, J., and Illeni, T., Isolation and Purification of Amyloglucosidase from *Halobacterium Sodomonse, Biomed. Chromatogr.*, 1993, vol. 7, pp. 256–261.
17. Rodriguez-Valera, F, Biotechnological Potential of Halobacteria, *The Archaebacteria: Biochemistry and Biotechnology*, Danson, M.J., Hough, D.W., and Lunt, G.G., Eds., The Biochemical Society, Portland Press: L., 1992, ISBN:1-85578-010-0.
18. Ventosa, A. and Nieto, J.J., Biotechnological Applications and Potentialities of Halophilic Microorganisms, *J. Microbiol. Biotechnol.*, 1995, vol. 11, pp. 85–94.
19. Birbir, M. and Sesal, C., Extremely Halophilic Bacterial Communities in Sereflikohisar Salt Lake in Turkey, *Turk. J. Biol*, 2003, vol. 27, pp. 7–22.
20. Elevi, R., Assa, P., Birbir, M., Ogan, A., and Oren, A., Characterization of Extremely Halphilic Archaea Isolated from the Ayvalik Saltern, Turkey, *World J. Microbiol*, 2004, vol. 20, pp. 719–725.
21. Montalvo-Rodriguez, R., Lopez-Garriga, J., Vreeland, H., Oren, A., Ventosa, A., and Kamekura, M., *Haloterrigena thermotolerans* sp. nov., a Halophilic Archaeon from Puerto Rico, *Int. J. Syst. Evolut. Microbiol.*, 2000.
22. Oren, A., Ventosa, A., and Grant, W.D., Proposed Minimal Standarts for Description of New Taxa in the Order *Halobacteriales, Int. J. Syst. Bacteriol.*, 1997, vol. 47, pp. 233–238.
23. Dussault, H.P., An Improved Technique for Staining Red Halophilic Bacteria, *J. Bacteriol.*, 1955, vol. 70, pp. 484–485.
24. Mancinelli, R.L. and Hochstein, L.I., The Occurrence of Denitrification in Extremely Halophilic Bacteria, *FEMS Microbiol. Letts.*, 1986, vol. 35, pp. 55–58.
25. Oren, A. and Litchfield, D.C., A Procedure for the Enrichment and Isolation of *Halobacterium, FEMS Microbiol. Letts.*, 1999, vol. 173, pp. 353–358.
26. Rodriguez-Valera, F., Juez, G., and Kushner, D.J., *Halobacterium mediterranei* sp. nov., a new Carbohydrate-Utilizing Extreme Halophile, *Syst. Appl. Microbiol.*, 1983, vol. 4, pp. 369–381.
27. Holding, A.J. and Collee, J.G., Routine Biochemical Tests, *Meth. Microbiol.*, 1971, vol. 6A, pp. 11–20.
28. Gutierrez, C. and Gonzalez, C., Method for Simultaneous Detection of Proteinase and Esterase Activities in Extremely Halophilic Bacteria, *Appl. Microbiol.*, 1972, vol. 24, pp. 516–517.
29. Ross, H.N.M., Collins, M.D., Tindall, B.J., and Grant, W.D., A Rapid Procedure for the Detection of Archaebacterial Lipids in Halophilic Bacteria, *J. Gen. Microbiol.*, 1981, vol. 123, pp. 75–80.

30. Anderson, D.G. and MacKay, L.L, Simple and Rapid Method for Isolating Large Plasmid DNA from *Lactic Streptococci*, *Appl. Environ. Microbiol.*, 1983, vol. 46, pp. 549–552.

31. Laemmli, U.K., Clevage of Structural Proteins During the Assembly of the Head of Bacteriophage T4, *Nature*, 1970, vol. 227, pp. 680–685.

32. Hesselberg, M. and Vreeland, R.H., Utilization of Protein Profiles for the Characterization of Halophilic Bacteria, *Curr. Microbiol.*, 1995, vol. 31, pp. 158–162.

33. Kovach, W.L., MVSP–A Multivariate Statistical Package for Windows, ver.3.1, Kovach Computing Services, Pentraeth, Wales, UK, 1999, p. 133.

34. Nei, M. and Li, W., Mathematical Model for Studying Genetic Variation in Terms of Restriction Endonucleases, *Proc. Natl. Acad. Sci. U.S.A.,* 1979, vol. 76, pp. 5269–5273.

35. Sneath, P.H.A. and Sokal, R.R., *Principles of Numerical Taxonomy*, Freeman W.H., Ed., San Francisco, 1973.

36. Bonelo, G., Ventosa, A., Megias, M., and Ruiz-Berraquero, F., The Sensitivity of Halobacteria To Antibiotics, *FEMS Microbiol. Letts.*, 1984, vol. 21, pp. 341–345.

37. Böck, A. and Kandler, O., Antibiotic Sensitivity of Archaebacteria, *The Bacteria - a Treatise on Structure and Function, Archaebacteria*, Woese, C.R. and Wolfe, R.S., Eds., Academic Press Orlando, FLVol.III, 1985, pp. 525–541.

38. Stan-Lotter, H., Pfaffenhuemer, M., Legat, A., Busse, H.J., Radax, C., and Gruber, C., *Halococcus dombrowskii* sp. nov., an Archaeal Isolate from a Permian Alpine Salt Deposit, *Int. J. Syst. Evol. Microbiol.,* 2002, vol. 52, pp. 1807–1814.

39. Kamekura, M. and Dyall-Smith, M.L., Taxonomy of the Family *Halobacteriaceae* and Description of Two New Genera *Halorubrobacterium* and *Natrialba, J. Gen. Appl. Microbiol.,* 1995, vol. 41, pp. 333–350.

40. Gutierrez, M.C., Garcia, M.T., Ventosa, A., Nieto, J.J., and Ruiz-Berraquero, F., Occurence of Megaplasmids in Halobacteria, *J. Appl. Bacteriol.*, 1986, vol. 61, pp. 67–71.

41. Ng, W.V., Ciufo, S.A., Smith, T.M., Rumgarner, R.E., Baskin, D., Faust, J., Hall, B., Loretz, C., Seto, J., Slagel, J., Hood, I., and DasSarma, S., Snapshot of a Large Dynamic Replicon in a Halophilic Archaeon: Megaplasmid Or Minichromosome?, *Genome Res.*, 1998, vol. 8, pp. 1131–1141.