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ORIGINAL PAPER

Biodegradation of crude oil and pure hydrocarbons by extreme halophilic archaea from hypersaline coasts of the Arabian Gulf

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Abstract Two extreme halophilic *Haloferax* strains and one strain each of *Halobacterium* and *Halococcus* were isolated from a hypersaline coastal area of the Arabian Gulf on a mineral salt medium with crude oil vapor as a sole source of carbon and energy. These archaea needed at least 1 M NaCl for growth in culture, and grew best in the presence of 4 M NaCl or more. Optimum growth temperatures lied between 40 and 45°C. The four archaea were resistant to the antibiotics chloramphenicol, cycloheximide, nalidixic acid, penicillin, streptomycin and tetracycline. The strains could grow on a wide scope of aliphatic and aromatic (both mono-and polynuclear) hydrocarbons, as sole sources of carbon and energy. Quantitative measurements revealed that these extreme halophilic prokaryotes could biodegrade crude oil (13–47%, depending on the strain and medium salinity), *n*-octadecane (28–67%) and phenanthrene (13–30%) in culture after 3 weeks of incubation. The rates of biodegradation by all strains were enhanced with increasing NaCl concentration in the medium. Optimal concentration was 3 M NaCl, but even with 4 M NaCl the hydrocarbon-biodegradation rates were higher than with 1 and 2 M NaCl. It was concluded that these archaea could contribute to self-cleaning and bioremediation of oil-polluted hypersaline environments.

Keywords Archaea · Bioremediation · Crude oil · Extreme halophiles · Hydrocarbon biodegradation

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Introduction

The biodegradation and bioremediation of hydrocarbons by non-extremophilic microorganisms have already been the subject of extensive research (for reviews see Rehm and Reiff 1981; Van Hamme et al. 2003; Rosenberg 2006; Radwan 2009). On the other hand, only little information is available on hydrocarbon biodegradation and bioremediation by extremophilic microorganisms especially archaea (for review see Margesin and Schinner 2001; Le Borgne et al. 2008). Hypersaline areas, although of wide global distribution and of being exposed to pollution, including hydrocarbon-pollution (Lefebvre and Moletta 2006), are typical examples of rarely studied extreme environments. Some earlier reports assume that bacteria with their greater metabolic diversity should be more promising degraders than archaea (Oren et al. 1992). Furthermore, since there is an inverse relationship between salinity and hydrocarbon solubility (Whitehouse 1984), the substrate availability to microorganisms should be lower in saline than nonsaline environments. This is probably in part why earlier workers assumed an inverse relationship between hydrocarbon biodegradation and salinity (Ward and Brock 1978). However, some other earlier reports indicated that even in the presence of high NaCl concentrations, actinomycetes (Kunznetsov et al. 1992; Al-Mueini et al. 2007) and archaea (Bertrand et al. 1990; Kulichevskaya et al. 1992; Tapilatu et al. 2010) could oxidize petroleum hydrocarbons.

The self-cleaning and bioremediation of polluted hypersaline environments does not seem to be possible with "conventional" microorganisms (Pieper and Reineke 2000; Oren 2002). The potential alternative microorganisms in these applications are probably the extreme halophilic archaea. Since the major objective of this paper was to study the hydrocarbon-attenuation potential of four

archaeal isolates from a hypersaline coastal area of the Arabian Gulf, the following is a brief review of relevant studies on this group of procaryotes.

As already mentioned, information on hydrocarbon biodegradation in hypersaline environments is limited and in part contradictory. Literature reports are available on the adaptation of hydrocarbon-utilizing microbial consortia to high salinity (Riis et al. 2003; Kleinsteuber et al. 2006). Other reports indicate that salinity did not affect microbial hydrocarbon biodegradation (Kerr and Capone 1988). Still other reports show that hydrocarbon biodegradation was enhanced by salinity increases (Diaz et al. 2000; Yang et al. 2000). This diversity reflects the complexity of the microbial consortia involved. Extreme halophilic archaea have been reported by only a few investigators to degrade both aliphatic hydrocarbons and aromatic compounds. For instance, several species belonging to *Halobacterium* were reported to degrade a wide range of *n*-alkanes and polynuclear aromatic hydrocarbons at about 30% NaCl concentration (Kulichevskaya et al. 1992). Also the haloarchaea *Haloferax* (Emerson et al. 1994) and *Haloarcula* (Oren et al. 1992; Fairley et al. 2002) could degrade aromatic compounds. Cuadros-Orellana et al. (2006) suggested that the ability to degrade *p*-hydroxybenzoic acid is a widespread feature among Halobacteriaceae.

In this paper we describe the isolation of four extreme halophilic archaeal strains from a hypersaline coastal area of the Arabian Gulf on a saline mineral medium with oil vapor as sole source of carbon and energy. We also studied the effect of increasing medium salinity on the hydrocarbon-biodegradation potential of the archaea.

Materials and methods

Sampling

The Arabian Gulf is a saline basin in which evaporation exceeds fresh water influx (Al-Zamel 1983). Hypersaline coastal areas are the supertidal "sabkha" in the north (Kuwait) and south (Abu Dhabi, Qatar) of the Gulf.

Five soil and five pond-water samples were collected at random from the sabkha of the southern (Khiran) coast of Kuwait in sterile plastic bags and vials, kept in an ice-box (8°C), transported to the laboratory and processed the same day. Relevant environmental parameters viz pH value and salinity, dissolved oxygen, total organic carbon, nitrate, ammonium and phosphate contents were recorded for the sampling sites using a water quality checker (WQC-24, Japan). Total hydrocarbons in the samples were extracted with three successive aliquots of pentane and determined quantitatively by gas–liquid chromatography (GLC) (see later) using *n*-hexadecane solution (30 μg ml^{-1} diethyl-ether) as an external standard.

Archaeal numbers and identities

Cultivable extreme halophilic oil-utilizing microorganisms in the soil and water samples were counted by the dilution plating method using, as a medium, the constituent mineral compounds only of the medium described by Mevarech and Werczberger (1985) and crude oil (light Kuwait oil) vapor as a sole source of carbon and energy. The complete medium had the following composition (g l^{-1}): 240 NaCl; 30 $MgCl_2 \cdot 6H_2O$; 35 $MgSO_4 \cdot 7H_2O$; 7 KCl; 8.5 yeast extract; 1.7 peptone; 1.7 casamino acids; 20 agar; pH 7.5. To prepare the oil medium, yeast extract, peptone and casamino acids were deleted and instead 2 g l^{-1} NH_4NO_3 was added and oil vapor was made available (see below). One gram of soil or water was suspended in 99 ml sterile pond-water giving the stock (10^{-2}) from which down series of dilutions (till 10^{-4}) were prepared. Aliquots, 0.25 ml of each dilution was spread on the solid mineral medium in Petri-dishes and crude oil vapor was made available as a sole carbon and energy source from 3 ml oil-impregnated filter papers fixed in the dish lids. Dishes were sealed with cello tape and incubated at 37°C for 3 weeks. Five parallel plates were prepared for every dilution. The colony forming units (CFU) were counted. Strains in the pooled replicate plates were categorized according to their colony and cell morphologies, counted and three representative colonies were isolated, purified and maintained on the above complete (with yeast extract, peptone and casamino acids) medium. The isolates were subcultured every another week, and identified by sequencing their 16S rRNA coding genes.

The GeneElute Bacterial Genomic DNA Miniprep Kit (Sigma–Aldrich, St. Louis, MO, USA) was used to extract total genomic DNA and the 16S rRNA genes were amplified by polymerase chain reaction (PCR) using the primers 0018F (5′-ATTCCGGTTGAGCC TGCC) and 1518R (5′-AGGAGGTGAGCCAGCCGC). The PCR products were purified using the QIA quick PCR purification kit (Qiagen, Valencia, CA, USA) in order to remove the Taq polymerase, primers, and dNTPs that might interrupt the sequencing procedure. Partial sequences of the 16S rRNA encoding genes were obtained by applying the BigDye version 3.1 Terminator Kit (Applied Biosystems, Warrington, UK); 20 ng of the DNA template was added to 8 μL of BigDye version 3.1 terminator; 1 μL of the primers was added to the mixture and the final volume was brought up to 20 μL with sterile molecular water. The vials were incubated in a GeneAmp PCR system 9700 thermocycler (Applied Biosystems, Foster City, CA, USA). The standard PCR program was conducted; it consisted of 1 cycle of

denaturation for 5 min at 94°C, annealing at 65°C for 1 min, followed by 25 cycles with denaturation temperature at 55°C for 30 s and extension step at 72°C for 30 s followed by 7 min at 72°C. Purification was done by adding 2 µl of 3 M sodium acetate pH 5.2 and 50 µl of 100% ethanol to each sample followed by incubation for 20 min at room temperature before centrifugation for 20 min at 13,000 relative centrifugal force (RCF). The pellet was washed with 50 µl of 70% ethanol and recentrifuged at room temperature for 5 min at 13,000 RCF. The pellet was left to dry in the hood for 10 min in the dark and 20 µl Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) was added to each sample prior to the denaturing step, where samples were incubated in a thermocycler for 2 min at 94–95°C before being loaded in the 3130*xl* genetic analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing analysis version 5.2 software (Applied Biosystems, Foster City, CA, USA) was used to analyze the results. Sequences were subjected to basic local alignment search tool analysis with the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) GenBank database (Altschul et al. 1997). The sequences were deposited in the GenBank database under the accession numbers cited in Table 1. For further confirmation of the strain identities, the sequences were compared with actual type strain sequences accessible through the "Ribosomal Database Project".

Effect of sodium chloride concentration on growth

Growth of archaeal isolates in the complete medium and the mineral medium with oil vapor as sole source of carbon and energy in the presence of 1 through 4 M NaCl was measured. Aliquots, 10 ml of the media were dispensed in test tubes, sterilized and inoculated each with 0.1 ml of a common inoculum prepared by suspending a loopful of the biomass in 5 ml sterile pond-water. To exclude the possibility that traces of organic matter in the pond-water might have had served as alternative carbon sources, it was made sure in a parallel experiment that no archaeal growth occurred when oil vapor was not provided to the culture. Tubes containing the mineral medium were provided with crude oil vapors created from 3 ml crude oil-impregnated cotton plugs and tightly coating the plugs with multiple layers of cello tape to prevent volatilization in the open atmosphere. The tightness of these stoppers was checked by the lack of the typical oil vapor smell at the tube mouths after cello tape coating. In this experiment cells made use of the amount of oxygen that was available in the sealed tubes. Plugs of tubes containing the complete medium were coated similarly, but without oil impregnation. Incubation was done at 37°C (which although not optimal, yet supported satisfactory growth) for 3 weeks and growth was measured in terms of optical density at 600 nm. Optical density measurements were also used to examine growth at different temperatures and in media containing benzene, toluene or *p*-hydroxybenzoic acid as sole sources of carbon and energy.

Table 1 Environmental parameters of the sampling sites

Parameters	Water samples	Soil samples
pH value	7.2–7.3	7.2–7.4
Salinity (M)	1.5–2.0	1.5–4.5
Dissolved oxygen (mg l^{-1})	2.3–5.1	2.1–5.2
Total organic carbon (%)	2.4–2.5	2.4–2.9
Total hydrocarbons (µg g^{-1})	22.1–35.3	28.9–59.1
Nitrate (mg kg^{-1})	1.4–2.2	1.6–2.0
Ammonium (mg kg^{-1})	2.1–2.2	2.1–2.4
Phosphate (mg kg^{-1})	1.5–1.7	1.5–1.9

Hydrocarbon-utilization and biodegradation

To study the potential of individual archaea for the utilization, as sole carbon and energy sources, of the *n*-alkanes with chain lengths from C_8 through C_{40} and of the aromatics benzene, toluene, phenanthrene, biphenyl, naphthalene and *p*-hydroxybenzoic acid, a common cell suspension was prepared for every strain by suspending a biomass-loopful from a 3-day culture in 5 ml sterile pond-water. A loopful of the suspension was streaked onto the above mineral salt medium containing 0.5% w/v of the individual hydrocarbons. After incubation for 3 weeks at 37°C, cultures were examined for growth.

The effect of salinity on the hydrocarbon-biodegradation potential of the pure archaeal cultures was determined quantitatively. Aliquots, 200 ml of the mineral medium containing 1, 2, 3 and 4 M NaCl were dispensed in 500 ml conical flasks, and provided with, 0.2% w/v aliquots of crude oil, *n*-octadecane (an alkane) or phenanthrene (an aromatic hydrocarbon) separately. For each organism a common inoculum was prepared by suspending 1 loopful of cells in 5 ml pond-water, and each flask was inoculated with 1 ml of this suspension. Three replicates were used throughout, and the flasks were incubated on an electric shaker, 180 rpm, at 37°C for 3 weeks. The control samples were prepared by following the same procedure, but using autoclaved instead of fresh biomass. At the end of the incubation period, residual hydrocarbons were recovered from each medium aliquot by three 30-ml aliquots of diethyl ether. The combined extract was completed to 90 ml and 1 µl was analyzed by gas–liquid chromatography (GLC), using a Chrompack (Chrompack, Middelburg, the Netherlands) CP-9000 instrument equipped with a FID, a WCOT-fused silica CP-SIL-5CB capillary column, 15 m × 0.25 mm, and a temperature program which raised

Table 2 Numbers, identities and growth requirements of extreme halophilic oil-utilizing archaeal isolates from the hypersaline coast of the Arabian Gulf

Description	Isolate identities			
	HA-1[a]	HA-2[b]	HA-3[c]	HA-4[c]
Isolated from	Water	Water	Soil	Soil
CFU g^{-1}	1500	1200	710	240
16S rRNA-gene sequencing				
Total bases compared	405	512	356	367
Nearest GenBank match	*Haloferax* sp.	*Haloferax* sp.	*Halobacterium* sp.	*Halococcus* sp.
% of similarities	99	99	100	99
Subdivision	Halobacteria	Halobacteria	Halobacteria	Halobacteria
GenBank accession numbers	GU550441	GU573917	GU550442	GU550443
Salinity and temperature requirements				
Minimum NaCl (M)	1	1	1	1
Optimum NaCl (M)	>4	>4	4	4
Growth temperature range (°C)	35–50	30–45	30–50	30–50
Optimum growth temperature (°C)	40	40	45	45
Antibiotic resistance				
Chloramphenicol (50 mg l^{-1})	+	+	+	+
Cycloheximide (60 mg l^{-1})	+	+	+	+
Nalidixic acid (30 mg l^{-1})	+	+	+	+
Penicillin (50 mg l^{-1})	+	+	+	+
Streptomycin (30 mg l^{-1})	+	+	+	+
Tetracycline (50 mg l^{-1})	+	+	+	+
Utilization of hydrocarbons as sole carbon and energy sources				
Crude oil volatile portion	+	+	+	+
The *n*-alkanes				
C_8 to C_{18}	+	+	+	+
C_{19} to C_{21}	+	+	+	−
C_{22} to C_{34}	+	+	−	−
C_{35} to C_{40}	−	−	−	−
The aromatics				
Benzene	+	−	+	+
Toluene	+	+	+	+
Phenanthrene	+	+	−	−
Biphenyl	+	+	+	−
Naphthalene	+	+	−	+
p-Hydroxybenzoic acid	−	−	+	+

[a] Deep pink colonies
[b] Pale pink colonies
[c] Pale red

the temperature from 45 to 310°C at 10°C min^{-1}. The peak areas of residual hydrocarbons were compared to the areas of the control peaks enabling the calculation of decrease percentages. The values obtained were taken as quantitative measure of the hydrocarbon biodegradation.

Results and discussion

The environmental parameters that were measured in the sampling sites are recorded in Table 1. These data imply that oil bioremediation in situ via extreme halophilic archaea can potentially occur under suitable environmental conditions as far as the pH value, salinity aeration and nitrogen and phosphate contents are concerned. The site was not pristine, although the total hydrocarbon contents were low. Probably these pollutants had their origin in the permanently polluted water body of the Gulf, although some may be of biogenic origin. This low hydrocarbon content was reflected in rather low numbers of hydrocarbon-utilizing archaea, which expectedly may be enriched after any potential oil spill in this site.

The results in Table 2 show that both the soil and pondwater samples from the hypersaline sabkha area contained several to many hundreds of CFU g^{-1} of archaea capable of growth on oil vapor as a sole source of carbon and

energy. These numbers, although low are considerable given that workers on archaea with "conventional" requirements routinely have to enrich them in culture before isolation (Goh et al. 2006; Usami et al. 2005; Vetriani et al. 1999). As mentioned above, the low numbers may probably be due to the low hydrocarbon content of the site. Presumably the numbers would be enhanced in situ following potential oil spill there. The plates contained of course in addition to the archaea colonies of extreme halophilic bacteria, in the magnitude of 10^4 CFU g^{-1}, but the numbers given in Table 2 were these of archaea affiliated to *Haloferax* (2 strains), *Halobacterium* and *Halococcus* only. This table contains also data related to the 16S rRNA gene partial sequencing of the four strains. The comparison of the sequences of the four archaeal strains with those of strains in the "Ribosomal Database Project" confirmed their identities. The PCR fragments compared for *Halobacterium* sp. (356 bp) and *Halococcus* sp. (367 bp) with the GenBank references were not particularly long. However, corresponding fragments for *Halobacterium* sp. SP3 (2) (Akolkar et al. 2008) and *Halococcus* sp. JCM 8979 (Brito-Echeverria et al. 2009) with 359 and 257 bp, respectively, were as short or even considerably shorter. It is to be noted that these fragments were long enough to affiliate the isolates on the genus level, but would probably be too short to affiliate them on the species level.

The four hydrocarbon-utilizing archaea grew best in the presence of 4 M NaCl or more, and failed to grow at concentrations less than 1 M NaCl, i.e., their salt requirements were similar to those recorded for the same genera growing on "conventional" carbon and energy sources (e.g., Bertrand et al. 1990; Aono et al. 1991; Magot et al. 2000; Ramos et al. 2002). In addition to their extreme halophilic nature, these oil-utilizing archaea, with 40 and 45°C, had rather elevated optimum temperature requirements. Their temperature range was 30–50°C. Thus, they are also similar in their temperature requirements to their counterparts with conventional nutritional requirements (e.g., Falb et al. 2008).

Similar to other archaea (Denner et al. 1994; Grant et al. 2001; Stan-Lotter et al. 2002), the four hydrocarbon-utilizing archaeal strains were resistant to the antibiotics chloramphenicol, cycloheximide, nalidixic acid, penicillin, streptomycin and tetracycline.

Table 2 shows further that the four archaeal strains had the potential for utilization of a wide scope of aliphatics and aromatics as sole sources of carbon and energy. Short and medium-chain *n*-alkanes with chain lengths up to C_{18} supported the growth of the four isolates, longer *n*-alkanes supported the growth of *Haloferax* only, but not of *Halobacterium* and *Halococcus.* The mononuclear aromatics benzene, toluene and *p*-hydroxybenzoic acid supported the growth of *Halobacterium* and *Haloccoccus.* The growth of

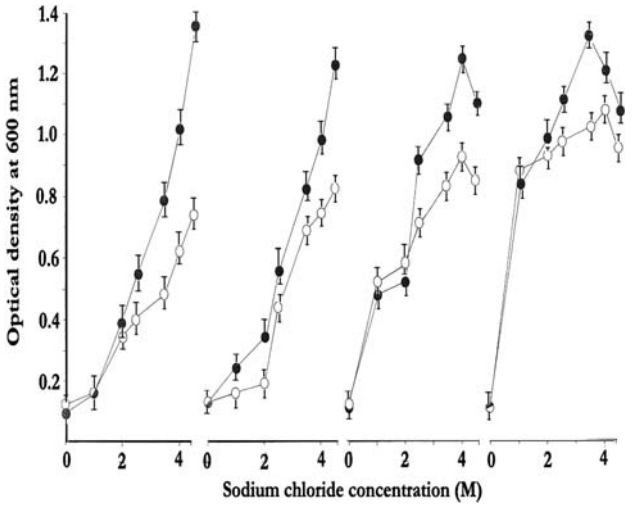

Fig. 1 Effect of medium salinity on growth of archaeal isolates for 3 weeks at 37°C in media containing either peptone (+ yeast extract) or crude oil vapor as sole sources of carbon and energy. *Closed circles* conventional medium, *open circles* oil medium. From *left to right* *Haloferax* sp. HA-1, *Haloferax* sp. HA-2, *Halobacterium* sp. HA-3, *Halococcus* sp. HA-4, respectively. Each reading was the mean of three replicates, the standard deviation values were <5% of the mean values

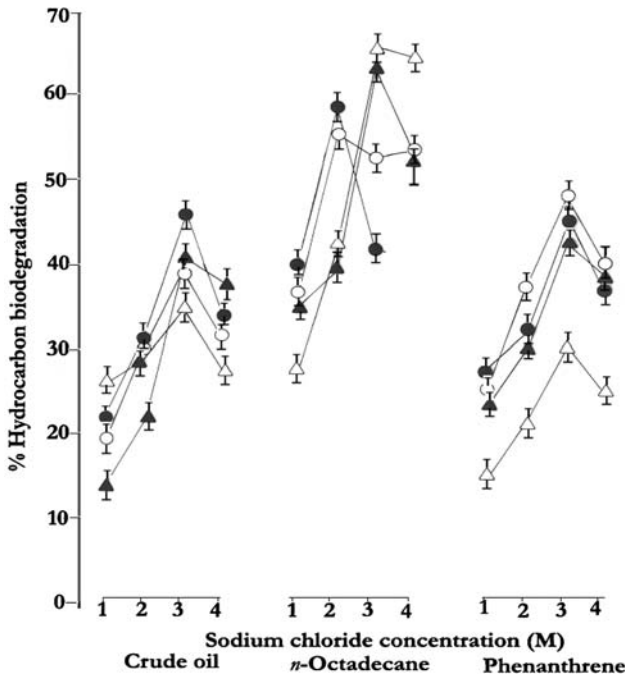

Fig. 2 Effect of medium salinity on crude oil and hydrocarbon biodegradation by archaea. *Open circles Haloferax* sp. HA-1, *closed circles Haloferax* sp. HA-2, *open triangles Halobacterium* sp. HA-3, *closed triangles Halococcus* sp. HA-4. Each reading was the mean of three replicates, the standard deviation values were <5% of the mean values. Incubation period, 3 weeks at 37°C

the two *Haloferax* strains was supported by toluene and one was supported by benzene, but both failed to grow on *p*-hydroxybenzoic acid. Differences between different strains of the same taxon are quite common among prokaryotes. In this context, Cuadros-Orellana et al. (2006) suggested that the ability to degrade *p*-hydroxybenzoic acid is a widespread feature among Halobacteriaceae.

Figure 1 confirms that the growth of the four studied archaea in media containing either peptone and amino acids or crude oil as sole sources of carbon and energy was enhanced by increasing the NaCl concentration in the medium. Both strains of *Haloferax* grew best at NaCl concentration of 4.5 M, *Halobacterium* at 4 M and *Halococcus* at 3.5–4 M. These optima were similar irrespective of whether the carbon source was organic nitrogenous compounds or oil vapor. It is however obvious in Fig. 1 that the growth of the four archaea was better with nitrogenous compounds than with hydrocarbons as substrates, at NaCl concentrations >2 M. The weaker growth on hydrocarbons might have been due to that the isolates required specific growth factors (e.g., amino acids and vitamins) which we avoided to add, or else they might have interfered with the hydrocarbons as sole carbon and energy sources.

The results in Fig. 2 indicate that the tested archaea biodegraded crude oil, pure *n*-octadecane and pure phenanthrene in culture. The biodegradation rates increased with increasing NaCl concentration in the medium. However, the optimum NaCl concentration of 3 M for hydrocarbon biodegradation, with the only exceptions of 2 M for the two *Haloferax* strains on *n*-octadecane as substrate, was considerably lower than the optimum NaCl concentration for growth, 3.5–4.5 M. A probable explanation for the better hydrocarbon biodegradation at lower salinity may be related to the oxygen availability. It is known that oxygen

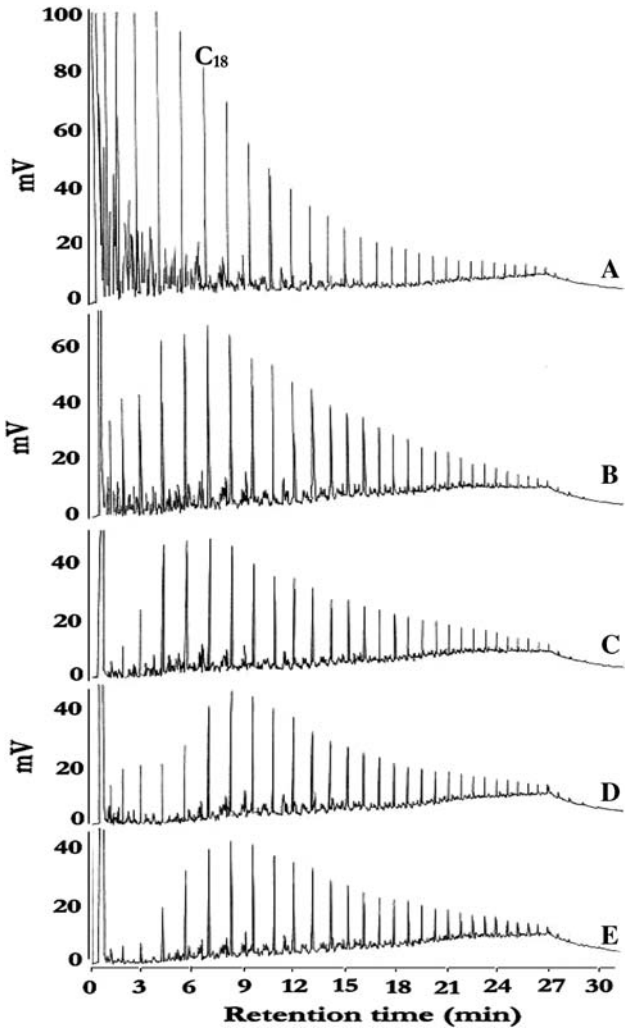

Fig. 3 Typical GLC profiles of residual crude oil hydrocarbons in media that had supported archaeal isolates for 3 weeks at 37°C. Profiles from the *top downward*: *A* control (autoclaved culture) nonbiodegraded hydrocarbons, medium; *B* medium with 1 M NaCl that supported *Haloferax* sp. HA-1; *C* medium with 3 M NaCl that supported *Haloferax* sp. HA-2; *D* medium with 3 M NaCl that supported *Halobacterium* sp. HA-3; *E* medium with 3 M NaCl that supported *Halococcus* sp. HA-4

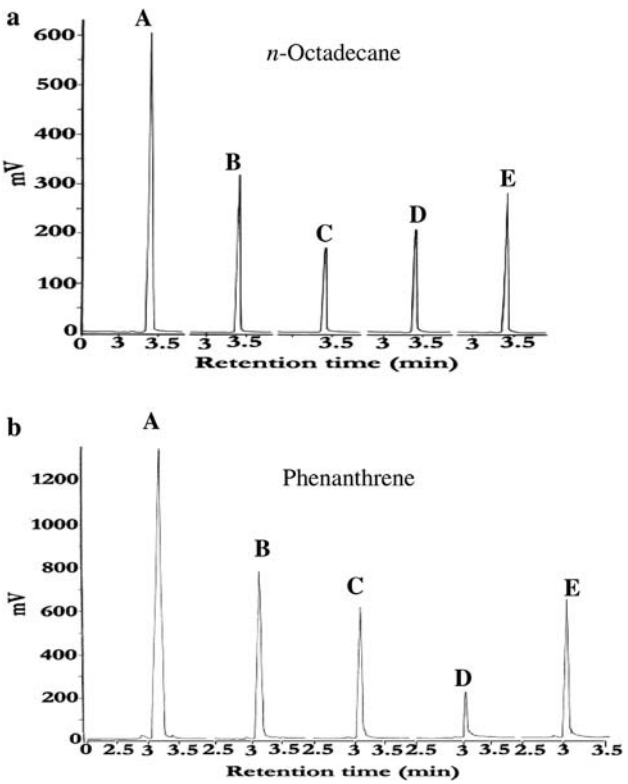

Fig. 4 Typical GLC profiles of residual pure hydrocarbons in saline media that had supported archaeal isolates for 3 weeks at 37°C. **a** *n*-octadecane, **b** phenanthrene. Profiles from *left to right*: *A* control (autoclaved cultures) nondegraded hydrocarbons medium, *B* medium with 1 M NaCl that supported *Haloferax* sp. HA-1, *C* medium with 3 M NaCl that supported *Haloferax* sp. HA-2, *D* medium with 3 M NaCl that supported *Halobacterium* sp. HA-3, *E* medium with 3 M NaCl that supported *Halococcus* sp. HA-4

solubility in water decreases with increasing salinity (Whitehouse 1984). Our measurements showed that raising the medium salinity from 1 to 4 M NaCl was associated with a decrease of the dissolved oxygen content from 5.3 to 2.2 mg l^{-1} medium. Molecular oxygen is involved in the initial step of attack by the microorganisms on hydrocarbons using the hydroxylase (oxygenase) systems (Cerniglia 1984; Von Wedal et al. 1988). With all the tested strains on all the tested hydrocarbons the biodegradation rates in the presence of 4 M NaCl were obviously higher than in the presence of 1 and even 2 M NaCl. This result confirms and consolidates the halophilic nature of these archaea, not only as far as growth rates are concerned, but also regarding the hydrocarbon biodegradation rates.

The typical GLC profiles of oil fractions (Fig. 3) and pure hydrocarbons (Fig. 4) recovered from control (autoclaved) and experimental cultures reveal that the four archaeal strains biodegraded the lower molecular weight hydrocarbons (with peaks closer to the gas peak) more efficiently than the higher molecular weight hydrocarbons as well as the pure aliphatic and aromatic hydrocarbons. The hydrocarbon decrease could have not been by volatilization, because the culture tubes were perfectly sealed, as obvious from the comparatively larger profiles of the autoclaved controls. This result coordinates with that in Table 1 indicating that the four archaea could all grow on lower molecular weight alkanes (C_8–C_{18}), but varied in their potential for utilizing high molecular weight compounds as sole sources of carbon and energy.

In conclusion, the extreme halophilic archaea *Haloferax*, *Halobacterium* and *Halococcus* from a hypersaline coastal area of the Arabian Gulf could utilize (as sole carbon and energy sources) and biodegraded (as indicated by the quantitative GLC-analysis) both aliphatic and aromatic hydrocarbons in the presence of up to 4.5 M NaCl or higher. The individual strains could utilize a wide range of individual *n*-alkanes with different chain lengths and mono- and polynuclear hydrocarbons as sole sources of carbon and energy. These strains grew optimally at rather elevated temperatures, 40–45°C. Such properties make these extreme halophilic procaryotes suitable biological materials for self-cleaning and bioremediation of oil-polluted hypersaline environments, especially in hot regions.

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References

Akolkar AV, Deshpande GM, Ravel KN, Durai D, Nerurkar AS, Desai AJ (2008) Organic solvent tolerance of *Halobacterium* sp. SP1(1) and its extracellular protease. J Basic Microbiol 48:421–425

Al-Mueini R, Al-Dalali M, Al-Amri IS, Patzelt H (2007) Hydrocarbon degradation at high salinity by a novel extremely halophilic actinomycete. Environ Chem 4:5–7

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Al-Zamel AZ (1983) Geology and oceanography of recent sediments of Jazirat Bubiyan and Ras As-Sabiyah, Kuwait, Arabian Gulf. Unpublished PhD thesis, University of Sheffield, Sheffield, pp 273–274

Aono R, Aibe K, Inoue A, Horikoshi K (1991) Preparation of organic solvent-tolerant mutants from *Escherichia coli* K 12. Agric Biol Chem 55:1935–1938

Bertrand JC, Almallah M, Acquaviva M, Mille G (1990) Biodegradation of hydrocarbons by an extremely halophilic archaebacterium. Lett Appl Microbiol 11:260–263

Brito-Echeverria J, Lopez-Lopez A, Yarza P, Anton J, Rosello-Mora R (2009) Occurrence of *Halococcus* spp in the nostrils salt glands of the seabird *Calonectris diomedea*. Extremophiles 13: 557–565

Cerniglia CE (1984) Microbial transformation of aromatic hydrocarbons. In: Atlas RM (ed) Petroleum microbiology. Macmillan, New York

Cuadros-Orellana S, Pohlschro M, Durrant LR (2006) Isolation and characterization of halophilic archaea able to grow in aromatic compounds. Int Biodeterior Biodegrad 57:151–154

Denner EBM, McGenity TJ, Busse HJ, Grant WD, Wanner G, Stan-Lotter H (1994) *Halococcus salifodinae* sp nov., an archaeal isolate from an Austrian salt mine. Int J Syst Bacteriol 44:774–780

Diaz MP, Grigson SJW, Peppiatt C, Burgess JG (2000) Isolation and characterization of novel hydrocarbon degrading euryhaline consortia from crude oil and mangrove sediments. Mar Biotechnol 2:522–532

Emerson D, Chauhan S, Oriel P, Breznak JA (1994) *Haloferax* sp. D1227, a halophilic Archaeon capable of growth on aromatic compounds. Arch Microbiol 161:445–452

Fairley DJ, Boyd DR, Sharma ND, Allen CCR, Morgan P, Larkin MJ (2002) Aerobic metabolism of 4-hydroxybenzoic acid in Archaea via an unusual pathway involving an intramolecular migration shift (NIH shift). Appl Environ Microbiol 68:6246–6255

Falb M, Muller K, Königsmaier L, Oberwinkler T, Horn P, Gronau SV, Gonzalez O, Pfeiffer F, Bauer EB, Oesterhelt D (2008) Metabolism of halophilic archaea. Extremophiles 12:177–196

Goh F, Leuko S, Allen MA, Bowman JB, Kamekura M, Neilan BA, Burns PB (2006) *Halococcus hamelinensis* sp nov., a novel halophilic archaeon isolated from stromatolites in Shark Bay. Australia Int J Syst Evol Microbiol 56:1323–1329

Grant WD, Kamekura M, McGenity TJ, Ventosa A (2001) Class III. Halobacteria class. nov. In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology, 2nd edn. Springer, New York, 1:294–334

Kerr RP, Capone DG (1988) The effect of salinity on the microbial mineralization of two polycyclic aromatic hydrocarbons in estuarine sediments. Mar Environ Res 26:181–198

Kleinsteuber S, Riis V, Fetzer I, Harms H, Müller S (2006) Population dynamics within a microbial consortium during growth on diesel fuel in saline environments. Appl Environ Microbiol 72:3531–3542

Kulichevskaya IS, Milekhina EI, Borzenkov IA, Zvyagintseva IS, Belyaev SS (1992) Oxidation of petroleum hydrocarbons by extremely halophilic archaeobacteria. Microbiology 60:596–601

Kunznetsov VD, Zaitesve TA, Vakulenko LV, Filippova SN (1992) *Strepromyces albiazalis* sp nov. L a new petroleum hydrocarbon degrading species of thermo-and halotolerant *Streptomyces*. Microbiology 61:62–67

Le Borgne S, Paniagua D, Vazquez-Duhalt R (2008) Biodegradation of organic pollutants by halophilic bacteria and archaea. J Mol Microbiol Biotechnol 15:74–92

Lefebvre O, Moletta R (2006) Treatment of organic pollution in industrial saline wastewater: a literature review. Water Res 40:3671–3682

Magot M, Ollivier B, Patel BKC (2000) Microbiology of petroleum reservoirs. Antonie van Leeuwenhoek 77:103–116

Margesin R, Schinner F (2001) Biodegradation and bioremediation of hydrocarbons in extreme environments. Appl Microbiol Biotechnol 56:650–663

Mevarech M, Werczberger R (1985) Genetic transfer in *Halobacterium volcanii*. J Bacteriol 162:461–462

Oren A (2002) Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. J Ind Microbiol Biotechnol 28:56–63

Oren A, Gurevich P, Azachi M, Hents Y (1992) Microbial degradation of pollutants at high salt concentrations. Biodegradation 3:387–398

Pieper D, Reineke W (2000) Engineering bacteria for bioremediation. Curr Opin Biotechnol 11:262–270

Radwan SS (2009) Phytoremediation for oily desert soil. In: Singh A, Kuhad RC, Ward OP (eds) Advances in applied bioremediation. Springer, Berlin

Ramos JL, Duque E, Gallegos MT, Godoy P, Ramos-Gonzalez MI, Rojas A, Teran W, Segura A (2002) Mechanisms of solvent tolerance in gram-negative bacteria. Annu Rev Microbiol 56: 743–768

Rehm HJ, Reiff I (1981) Mechanisms and occurrence of microbial oxidation of long-chain alkanes. Adv Biochem Eng 19:175–216

Riis V, Kleinsteuber S, Babel W (2003) Influence of high salinities on the degradation of diesel fuel by bacteria consortia. Can J Microbiol 49:713–721

Rosenberg E (2006) Hydrocarbon-oxidizing bacteria. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes, a handbook on the biology of bacteria, vol Vol. 2, 3rd edn edn. Springer, Berlin, pp 564–577

Stan-Lotter H, Pfaffenhuemer M, Legat A, Busse HJ, Radax C, Gruber C (2002) *Halococcus dombrowskii* sp nov., an archaeal isolate from a Permian alpine salt deposit. Int J Syst Evol Microbiol 52:1807–1814

Tapilatu YH, Grossi V, Acquaviva M, Militon C, Bertrand J-C, Cuny P (2010) Isolation of hydrocarbon-degrading extremely halophilic archaea from an uncontaminated hypersaline pond (Camarque, France). Extremophiles 14:225–231

Usami R, Fukushima T, Mizuki T, Yoshida Y, Inoue A, Horikoshi K (2005) Organic solvent tolerance of halophilic archaea, *Haloarcula* strains: effects of NaCl concentration on the tolerance and polar lipid composition. J Biosci Bioeng 99:169–174

Van Hamme JD, Singh A, Ward O (2003) Recent advances in petroleum microbiology. Microbiol Mol Biol Rev 67:503–549

Vetriani C, Jannasch HW, MacGregor BJ, Stahl DA, Reysenbach AL (1999) Population structure and phylogenetic characterization of marine benthic archaea in deep-sea sediments. Appl Environ Microbiol 65:4375–4384

Von Wedal RJ, Mosquera JF, Goldsmith CD, Hater GR, Wong A, Fox TA, Hunt WT, Paulies MS, Quiros JM, Wiegand JW (1988) Bacterial biodegradation of petroleum hydrocarbons in ground water: in situ augmented bioreclamation with enrichments isolates in California. Water Sci Technol 20:501–503

Ward DM, Brock TD (1978) Hydrocarbon biodegradation in hypersaline environments. Appl Environ Microbiol 35:85–90

Whitehouse BG (1984) The effects of temperature and salinity on the aqueous solubility of polynuclear aromatic hydrocarbons. Mar Chem 14:319–332

Yang L, Lai CT, Shieh WK (2000) Biodegradation of dispersed diesel fuel under high saline conditions. Water Res 34:3303–3314