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Response of the Archaeal Community to Simulated Petroleum Hydrocarbon Contamination in Marine and Hypersaline Ecosystems

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Abstract Petroleum hydrocarbons are among the most important contaminants in aquatic ecosystems, but the effects of different petroleum components on the archaeal communities in these environments are still poorly investigated. Therefore, the effects of representative alkanes, polycyclic aromatic hydrocarbons and crude oil on archaeal communities from marine (Massambaba Beach) and hypersaline waters (Vermelha Lagoon) from the Massambaba Environmental Protection Area, Rio de Janeiro, Brazil, were examined in this study. Hydrocarbon contamination was simulated in vitro, and the resulting microcosms were temporally analyzed (4, 12 and 32 days after contamination) using molecular methods. DNA and RNA extractions were followed by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analyses and by the further molecular identification of selected DGGE bands. Archaeal communities could not be detected in the marine microcosms after contamination with the different hydrocarbons. In contrast, they were detected by DNA- and RNA-based methods in hypersaline water. Dendrogram analyses of PCR-DGGE showed that the archaeal communities in the hypersaline water-derived microcosms selected for by the addition of heptadecane, naphthalene or crude oil differed from the natural ones observed before the hydrocarbon contaminations. Principal coordinate analysis of the DGGE patterns showed an important effect of incubation time on the archaeal communities. A total of 103 DGGE bands were identified, and phylogenetic analysis showed that 84.4 % and 15.5 % of these sequences were associated with the Euryarchaeota and Crenarchaeota groups, respectively. Most of the sequences obtained were related to uncultivated archaea. Using redundancy analysis, the response of archaeal communities to the type of hydrocarbon contamination used could also be observed in the hypersaline water-derived microcosms.

Keywords Archaeal communities · Petroleum hydrocarbons · Aquatic ecosystems · Microcosms

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1 Introduction

Natural environments worldwide are negatively affected by petroleum hydrocarbon spills (Atlas and Hazen 2011). In parallel, independent molecular analyses have revealed that Archaea are important members of the microbial community in many different environments (Teske and Sørensen 2008). However, the extent of the effects of petroleum hydrocarbon spills on archaeal communities is still a controversial issue (Redmond and Valentine 2012).

It has already been demonstrated that archaeal degradation of polycyclic aromatic hydrocarbons (PAHs) occurs under anaerobic conditions (Anderson and Lovley 2000) and that the inhibition of methanogenesis affects the degradation rates of PAHs (Chang et al. 2006). Under aerobic conditions, some halophilic archaeal isolates, mainly from the Halobacteria group (homonymous with Haloarchaea), have been described as hydrocarbon degraders. Bonfá et al. (2011) showed that halophilic *Haloferax* sp. strains are able to degrade PAH, and Erdoğmuş et al. (2013) isolated strains belonging to *Halobacterium*, *Halorubrum*, *Haloarcula* and *Haloferax* genera with the ability to grow using different PAHs as their sole carbon sources. Strains of *Haloarcula* and *Haloferax* genera were also described as *n*-alkane (C17–C20) degraders (Tapilatu et al. 2010), and Al-Mailem et al. (2010) showed that strains of *Haloferax*, *Halobacterium* and *Halococcus* could grow using both PAHs and alkanes (from C8 to C34) as their sole carbon sources.

Conversely, the dynamics of archaeal communities in petroleum hydrocarbon-contaminated environments is not well understood. Positive, negative and neutral effects of petroleum hydrocarbons on archaeal communities have been reported in the literature (Al-Mailem et al. 2012; Chang et al. 2006; Redmond and Valentine 2012; Röling et al. 2004; Taketani et al. 2010). Röling et al. (2004) showed that the abundance of archaea in beach sand-containing microcosms decreased in response to crude oil contamination. In contrast, Redmond and Valentine (2012) showed that the Deepwater Horizon Oil Spill in the Gulf of Mexico had no major effect on the archaeal community. However, relatively little information is available about the activity of the archaeal community at these contaminated sites. In fact, although the structure of archaeal communities has been analyzed in various studies using petroleum hydrocarbon-contaminated samples, more attention is typically given to bacterial communities (dos Santos et al. 2011; Taketani et al. 2010). Therefore, in this study, marine and hypersaline water-derived microcosms were established and treated with heptadecane or naphthalene (as models of the aliphatic or PAH fractions of petroleum hydrocarbons, respectively) and crude oil (as a complex mixture of hydrocarbons) to study the effects of these contaminants on the archaeal communities from these ecosystems. To achieve this goal, DNA and RNA extractions were performed from the different microcosms during their incubation period, and the samples obtained were amplified by PCR using archaeal 16S rRNA gene-based primers. The presence (DNA) and metabolic activity (RNA) of the archaeal communities were analyzed. The archaeal community profiles were then analyzed by genetic fingerprinting, and the sequencing of 16S rRNA revealed the main active archaea that were enriched in these petroleum hydrocarbon-contaminated microcosms.

2 Materials and Methods

2.1 Sample Sites and Establishment of Microcosms

Water samples (500 ml) from Massambaba Beach (a marine ecosystem) and from Vermelha Lagoon (a hypersaline ecosystem) were collected in triplicate from the Massambaba Environmental Protection Area located in Saquarema, Rio de Janeiro, Brazil. The proprieties of the water samples are described in Table 1. The microcosms were established in triplicate using 25 ml water samples and were grouped as follows (for both the marine and hypersaline samples): (1) microcosms contaminated with crude oil (1 % v/v), (2) microcosms contaminated with naphthalene as a representative PAH (1 % w/v), (3) microcosms contaminated with heptadecane as a representative aliphatic hydrocarbon (1 % v/v), and (4) non-contaminated microcosms as controls for microbial growth. Additionally, controls containing autoclaved water samplings contaminated with the different hydrocarbons were also performed. All microcosms were treated with N, K and P in a proportion of 10:1:1 to facilitate microbial growth. The microcosms were incubated in the dark for up to 32 days at room temperature (28 °C).

2.2 DNA and RNA Extraction

The archaeal community was analyzed using molecular methods before (T1) and 4 days (T2), 12 days (T3) and 32 days (T4) after initiating the experiments. Total DNA was extracted from all of the samples, and RNA was also extracted after 12 and 32 days of microcosm incubation. A volume of 30 ml of the T1 samples and 1.5 ml of the T2, T3 and T4 samples was used for the nucleic acid extractions. For nucleic acid extraction, the microbial cells were lysed using a combination of lysozyme (1 mg ml^{-1}) and alkaline lysis buffer (FastDNA™ Spin Kit for soil; QBIOgene, Carlsbad, CA, USA). The DNA

Table 1 Physicochemical properties of the water samples (data from Jurelevicius et al. 2013)

Samples[a]	Air temperature	Water temperature	pH	Salinity (%)	Total NaCl (%)	Organic matter (mg l^{-1})	TPH in sediment (μg g^{-1})
MAS	30 °C	24 °C	8.00	4.0	1.1	27	3.59
VER	30 °C	28 °C	8.05	5.0	1.5	54	17.49

[a]MAS and VER represent Massambaba Beach and Vermelha Lagoon, respectively

was purified according to the method of Pitcher et al. (1989) and subsequently eluted in 50 μl of TE buffer (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0); the total RNA extracted from the lysates was purified using an RNeasy kit (Qiagen, São Paulo, SP, Brazil) according to the manufacturer's protocol. The amount of DNA and RNA extracted from each sample was determined using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Suwanee, GA, USA). The extracted RNAs were then treated with RNase-free DNase I (Fermentas International Inc., Burlington, VT, USA), and cDNA was synthesized using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions.

2.3 PCR Amplification of the Genes Encoding Archaeal 16S rRNA

A nested PCR approach was used to amplify the archaeal 16S rRNA gene sequences from both the extracted DNA and the synthesized cDNAs. Each PCR reaction was performed in a 25-μl mixture containing 20 pmol of each primer, 0.2 mM of each dNTP, 5 μl of 5× PCR buffer (100 mM Tris–HCl, pH 9.0, and 500 mM KCl), 1.5 mM $MgCl_2$ and 1 U of *Taq* DNA polymerase (Promega, Madison, WI, USA). The primers Arch21f (5′-TTCYGGTTGATCCYGC CIGA-3′) and Arch958r (5′-YCCGGCGTTG AMTCCAATT-3′) were used for the first round of PCR. The primers Arch344f-GC (5′-CGCCCG CCGCGCCCCGCGCCCGTCC-3′) and Arch519r (5′-TTACCGCGGCKGCTG-3′) (Bano et al. 2004) were then used for the second-round amplification using a 1:100 dilution of the first-round PCR product as a template. The PCR reaction included an initial denaturation step at 95 °C for 5 min, 30 cycles of a denaturation step at 95 °C for 1 min, a primer annealing step at 57 °C for 1 min and an extension step at 72 °C for 1.5 min, followed by a final step of 72 °C for 10 min.

2.4 Archaeal Community Fingerprinting and Construction of Clone Libraries

Denaturing gradient gel electrophoresis (DGGE) analysis was carried out using a Bio-Rad DCode Universal Mutation Detection System (Bio-Rad Laboratories, Munich, Germany) as previously described in Jurelevicius et al. (2010). DGGE-generated profiles were analyzed using GelCompar II software (version 4.06; Applied Maths, Kortrijk, Belgium). The Pearson correlation coefficient was calculated based on the densitometric curves of the profile lanes as described by Hardoim et al. (2009). Dendrograms were constructed based on the Ward coefficient using the BioNumerics software (Applied Maths, Ghent, Belgium). The binary matrices generated from the DGGE lanes were also exported to PC-ORD software (McCune and Mefford 2011) to perform redundancy analysis (RDA) and principal coordinate analysis (PCoA).

Selected bands were retrieved from the DGGE gels, eluted in water, and PCR reamplified using the second-round PCR reaction as described above. These amplicons were used to construct clone libraries using the InsTAclone PCR Cloning Kit (Fermentas, Maryland, USA) according to the supplier's instructions. The insert-containing clones were sequenced using the forward primer M13F (5′-GTAAAACGACGGCCAG T-3′) of the vector pTZ57R/T in an ABI Prism 3100 automatic sequencer (Applied Biosystems Inc., California, USA) using the facilities of Macrogen, Inc. (South Korea).

2.5 Sequence Analysis

The electropherogram files generated by DNA sequencing were analyzed using the Phred program (Ewing and Green 1998) for base calling and trimming of vector and low-quality (<20) sequences. The further downstream analyses were performed using MOTHUR software (Schloss et al. 2009). All sequences were aligned using sequences from the archaeal Silva Database Project

(www.arb-silva.de) as templates, and the sequences were clustered considering 99 % similarity between them. The sequences were taxonomically assigned using the Ribosomal Database Project's Naïve Bayesian classifier (Wang et al. 2007) with a confidence of 80 %.

2.6 Total Petroleum Hydrocarbon (TPH) Determination

TPH values were determined in all hydrocarbon contaminated hypersaline water-containing microcosms and in their respective controls (autoclaved water samplings contaminated with the different hydrocarbons and incubated during 32 days) using a high-accuracy gas chromatography method with a modified EPA 8015D technique (EPA 2000).

3 Results

The physical, chemical and environmental proprieties of the water samples from Vermelha Lagoon and Massambaba Beach are shown in Table 1 and in Jurelevicius et al. (2013). The water samples from both ecosystems have slightly alkaline pH (varying from 8.00 to 8.05). The total salinity of the samples from Vermelha Lagoon (5 %) was higher than that of the samples collected from Massambaba Beach (4 %). The content of NaCl, organic matter (OM) and TPH was also higher in the samples from Vermelha Lagoon than in the samples from Massambaba Beach (Table 1).

Before the establishment of the different microcosms (T1), the presence of archaeal community members in the samples collected from both ecosystems was analyzed by the PCR amplification of the archaeal 16S rRNA-encoding gene. DGGE analysis showed that both ecosystems have different planktonic archaeal communities (Fig. 1). However, after the fourth day of incubation, archaea could not be detected in the DNA or RNA samples obtained from the hydrocarbon-contaminated marine microcosms. In contrast, archaea were detected in the DNA samples obtained from the hypersaline-derived microcosms after 4 days of incubation (T2) with heptadecane and crude oil and from the DNA and RNA samples obtained from all the hydrocarbon-contaminated hypersaline microcosms at the 12th (T3) and 32nd (T4) days of incubation. No archaea were detected in the microcosms that were used as controls (i.e., without the addition of hydrocarbons).

3.1 Archaeal Communities in the Hydrocarbon-Contaminated Hypersaline Microcosms

PCR-DGGE was used to study the response of the archaeal communities in hypersaline water from Vermelha Lagoon to contamination with different hydrocarbons. The microcosms established in triplicates exhibited similar archaeal communities, as shown in the DGGE dendrograms (Fig. 2a, b and c). The archaeal communities selected for by the addition of heptadecane, naphthalene or crude oil in the microcosms differed from the natural archaeal communities observed prior to hydrocarbon contamination (H_T1, N_T1 and O_T1; Fig. 2a, b and c, respectively). The profiles that represented the different incubation periods of the microcosms were well-separated in the dendrograms. Therefore, a strong influence of the hydrocarbon contaminant exposure time could be observed in the archaeal community found in Vermelha Lagoon. When DNA- or RNA-based profiles were compared by DGGE, we observed the presence of common bands in the profiles of each hydrocarbon used. However, it seems that the archaeal communities that predominated in the microcosms based on DNA analysis were not the same as those observed through RNA-based analysis (which measures active archaeal communities) in the hydrocarbon-contaminated microcosms after 12 and 32 days of incubation (Fig. 2a, b and c).

3.2 Structure of Archaeal Communities in Hypersaline Vermelha Lagoon

In total, 103 bands were retrieved from the DGGE gels (marked with black bullets in Fig. 2) and then reamplified and sequenced. These bands represented 81 unique sequences. Phylogenetic analysis showed that 15.5 % of these sequences were associated with archaeal Crenarchaeota, and 84.4 % of the sequences corresponded to the archaeal Euryarchaeota group. However, most of the sequences obtained here were associated with uncultivated archaea found in a variety of environments.

A total of 75 % of the DGGE band sequences retrieved from the T1 samples were affiliated with uncultivated Archaea of Marine Benthic Group D and DHVEG-1 Euryarchaeota (Table 2). The remaining 25 % were associated with the *Natronorubrum* and *Halopiger* genera belonging to the Halobacteria class (8.3 % each) and to the Marine Hydrothermal Vent Group (8.3 %).

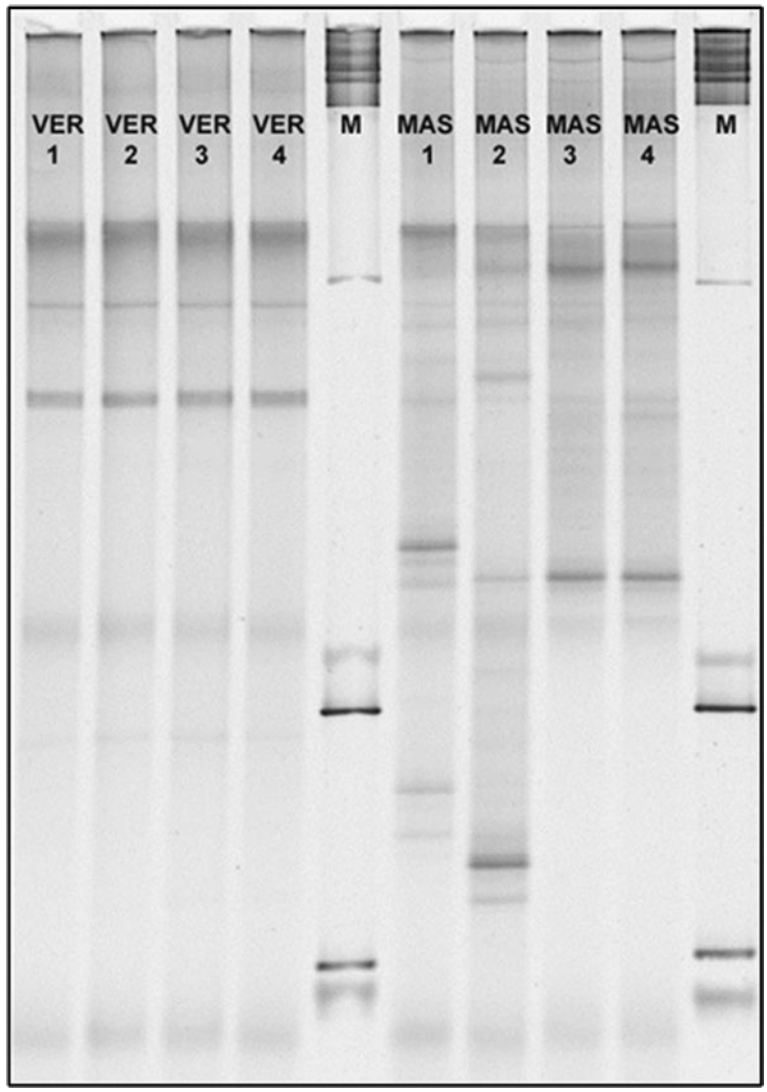

Fig. 1 Denaturing gradient gel electrophoresis (*DGGE*) fingerprints of archaeal 16S rRNA gene fragments amplified using extracted DNA obtained from Vermelha Lagoon (*VER*) and Massambaba Beach (*MAS*) as a template. The *lanes* marked with *M* correspond to a 1-kb ladder (Promega)

Archaeal communities in the heptadecane-, naphthalene- and crude oil-contaminated microcosms varied according to whether DNA- or RNA-based analyses were used. DNA-based analyses of the heptadecane-, naphthalene- and crude oil-contaminated microcosms showed that 84.6 %, 77.3 % and 77.8 % of the DGGE band sequences, respectively, were associated with Marine Benthic Group D and DHVEG-1 Euryarchaeota (Table 2). Based on the analysis of the RNA of the microcosms, the sequences affiliated with Marine Benthic Group D and DHVEG-1 Euryarchaeota represented only 9.1 % of the sequenced bands from the heptadecane- and crude oil-contaminated microcosms and 14.3 % of the sequenced bands from the naphthalene-contaminated microcosms. Moreover, sequences related to Halobacteria corresponded to 36.4 %, 14.3 % and 36.4 % of the sequenced bands from the RNA-based analysis of the heptadecane-, naphthalene- and crude oil-contaminated microcosms, respectively, and 45.5 %, 42.9 % and 27.3 % were associated with unclassified Crenarchaeota, respectively (Table 2). Only Crenarchaeota from group C3 were observed by analysis of the RNA from the naphthalene- and crude oil-contaminated microcosms. In the heptadecane-contaminated microcosms, 27.3 % of sequences were related to the pMC2A209 group based on RNA analysis (Table 2). Moreover, identical sequences related to the genus *Halorhabdus* were obtained from the RNA-based analysis of heptadecane- and naphthalene-contaminated microcosms.

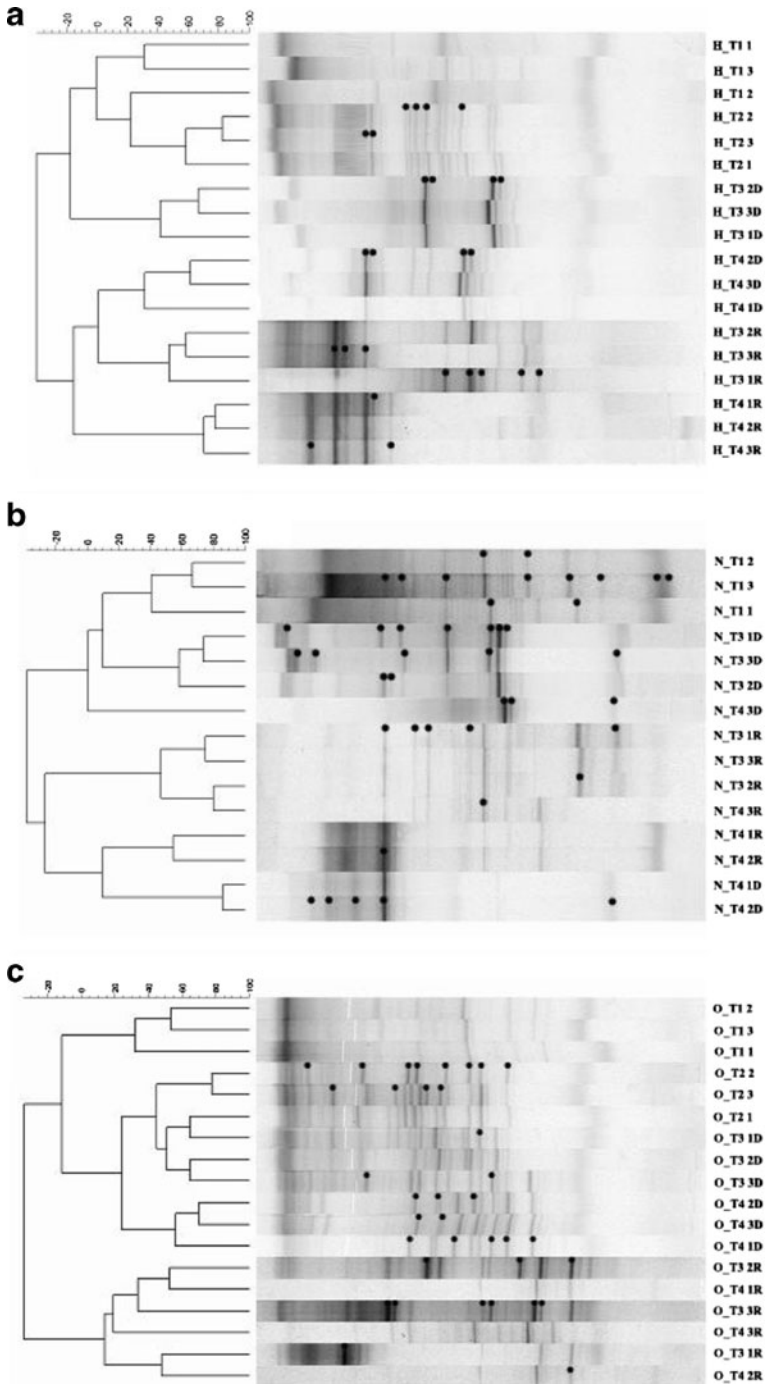

Fig. 2 Denaturing gradient gel electrophoresis (*DGGE*) fingerprints of archaeal 16S rRNA gene fragments amplified from the DNA and RNA templates obtained from **a** heptadecane-, **b** naphthalene- and **c** crude oil-contaminated microcosms, and the corresponding dendrograms obtained after cluster analysis based on the Pearson correlation coefficient using the Ward coefficient to compare the total archaeal 16S rRNA gene fragments amplified from each microcosm. The bands marked with black bullets were retrieved from the DGGEs, reamplified, and sequenced. The first letter (*H*, *N* and *O*) represents the hydrocarbon contamination (heptadecane, naphthalene and crude oil, respectively) and is followed by the incubation period of the microcosm (T1 — before the contamination, T2 — 4 days, T3 — 12 days, T4 — 32 days). The numbers 1 to 3 correspond to the replicates. D and R represent DNA- and RNA-based analyses, respectively

3.3 Multivariate Analyses of DGGE Patterns

Ordination of the DGGE profiles using PCoA supported the aforementioned effect of incubation time on the archaeal communities of heptadecane- and naphthalene-contaminated microcosms (Fig. 3a, b). However, this effect was less pronounced in the microcosms that were contaminated with crude oil, even though the RNA-extracted samples were separated from the remaining samples by axis 2 (Fig. 3c). DGGE-PCoA analyses also showed a relationship between the sequenced bands and the incubation periods (Fig. 3a, b

Table 2 Taxonomic identification and relative abundance of the sequenced bands

Tax code[a]	Phylum	Class	Genus/group	T1(DNA)	H(DNA)[b]	H(RNA)	N(DNA)	N(RNA)	O(DNA)	O(RNA)
a1	Euryarchaeota	Thermoplasmata	Marine Benthic Group D and DHVEG-1	75.0 %	84.6 %	9.1 %	77.3 %	14.3 %	77.8 %	9.1 %
a2	Euryarchaeota	Thermoplasmata	KTK 4A	0.0 %	0.0 %	9.1 %	0.0 %	28.6 %	0.0 %	9.1 %
a3	Euryarchaeota	Thermoplasmata	Marine Group II	0.0 %	0.0 %	0.0 %	18.2 %	0.0 %	0.0 %	0.0 %
a4	Euryarchaeota	Thermoplasmata	Marine Group III	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	3.7 %	0.0 %
a5	Euryarchaeota	Thermoplasmata	20c-4	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	18.2 %
a6	Euryarchaeota	Halobacteria	*Halopiger*	8.3 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %
a7	Euryarchaeota	Halobacteria	*Natronorubrum*	8.3 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %
a8	Euryarchaeota	Halobacteria	*Halorhabdus*	0.0 %	0.0 %	9.1 %	0.0 %	14.3 %	0.0 %	0.0 %
a9	Euryarchaeota	Halobacteria	Marine Hydrothermal Vent Group	8.3 %	0.0 %	27.3 %	0.0 %	0.0 %	0.0 %	27.3 %
a10	Euryarchaeota	Halobacteria	Deep Sea Hydrothermal Vent Group 6	0.0 %	7.7 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %
a11	Euryarchaeota	Halobacteria	Miscellaneous Euryarchaeotic Group	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	9.1 %
a12	Euryarchaeota	Methanomicrobia	AK5	0.0 %	7.7 %	0.0 %	0.0 %	0.0 %	3.7 %	0.0 %
a13	Crenarchaeota		Group C3	0.0 %	0.0 %	18.2 %	4.5 %	42.9 %	0.0 %	27.3 %
a14	Crenarchaeota		pMC2A209	0.0 %	0.0 %	27.3 %	0.0 %	0.0 %	0.0 %	0.0 %
a15	Crenarchaeota		Miscellaneous Crenarchaeotic Group	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	7.4 %	0.0 %
a16	Crenarchaeota		Marine Benthic Group B	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %	7.4 %	0.0 %
			Number of DGGE bands sequenced	12	13	11	22	7	27	11

[a] Code for each archaeal taxa. The same code was used in Fig. 3a, b and c

[b] Total bands obtained in T2 (4 days), T3 (12 days), and T4 (32 days). The letters H, N and O represent heptadecane, naphthalene and crude oil, respectively

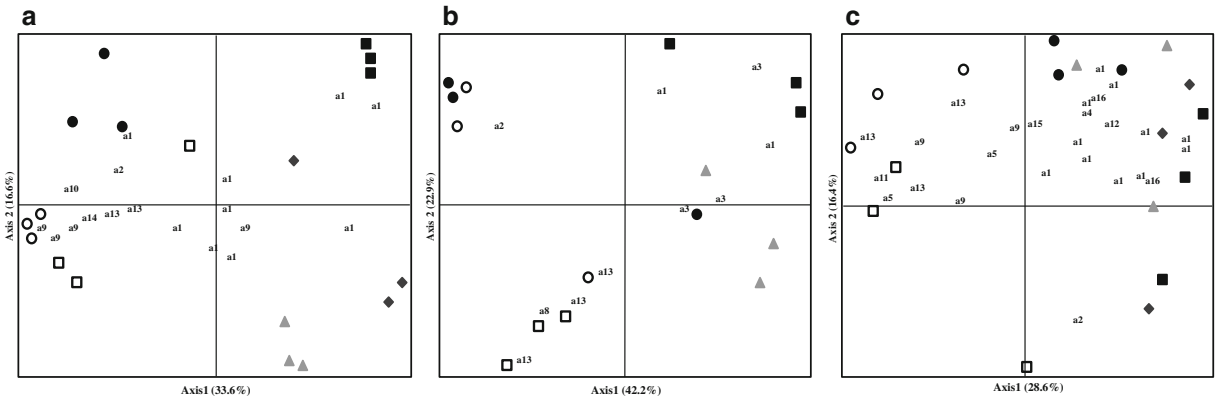

Fig. 3 Principal coordinate analysis (*PCoA*) ordination diagram of PCR-DGGE profiles obtained from **a** heptadecane-, **b** naphthalene- and **c** crude oil-contaminated microcosms. The symbols represent: (*filled triangle*) T1, (*filled diamond*) T2, (*filled square*) T3_DNA, (*empty square*) T3_RNA, (*filled circle*) T4_DNA and (*empty circle*) T4_RNA. The letter "a" followed by a number indicates the distribution of the most frequent archaeal taxa (Table 2) in the biplot axis

and c). For example, the bands affiliated with the Marine Benthic Group D and DHVEG-1 Euryarchaeota (coded as a1) were correlated with the samples from heptadecane-, naphthalene- and crude oil-contaminated microcosms that were analyzed on the basis of their DNA, as shown in Fig. 3a, b and c.

The similarity of the archaeal communities in the heptadecane-, naphthalene- or crude oil-contaminated microcosms was further assessed after the alignment of all DGGE profiles using the Bionumerix software (Fig. 4a). In addition, samples representing each incubation time and microcosm were loaded on a single DGGE gel for direct comparison (Fig. 4b). The dendrogram analyses using both comparison strategies were very similar (Fig. 4a and b), and again, the samples grouped mainly according to the incubation time. However, when the DGGE profiles were compared using RDA, the samples from the contaminated microcosms tended to cluster based on the time of incubation and the type of hydrocarbon used (heptadecane, naphthalene or crude oil; Fig. 5). Finally, the samples representing the archaeal communities before hydrocarbon contamination clustered separately from the hydrocarbon-contaminated samples (Fig. 5), as also observed in the dendrogram analyses (Fig. 4a and b).

3.4 TPH Degradation

TPH values ($\mu g\ l^{-1}$) obtained in the different controls (autoclaved water samplings contaminated with the different hydrocarbons and incubated during 32 days) were: (1) crude oil, 42.1 (±11.4); (2) heptadecane, 4.2 (±0.08); and (3) naphthalene, 28.3 (±0.1). In the hydrocarbon-contaminated microcosms, the TPH values observed corresponded to 54 % of crude oil degradation (19.2±2.8), 19 % of heptadecane degradation (3.4±0.4) and 23 % of naphthalene degradation (21.8±1.1).

4 Discussion

Archaeal communities from the marine Massambaba Beach ecosystem and the hypersaline Vermelha Lagoon ecosystem, both located in the Massambaba Environmental Protection Area, Rio de Janeiro, Brazil, were studied. Different archaeal communities were observed in these ecosystems. Although the salinity determined in this study varied from 4 % in samples collected from Massambaba Beach to 5 % in samples collected from Vermelha Lagoon, it has been previously reported that the salt content can vary annually from 3.5 up to 8 % in Vermelha Lagoon (Barbiére 1985). As suggested by Grant (2004), the higher ionic activity observed in hypersaline ecosystems compared to those of marine ecosystems may affect the microbial community, as was observed here.

After the establishment of the microcosms, the archaeal community was not detected in the hydrocarbon contaminated-marine microcosms. This result is in agreement with those presented by Röling et al. (2004), where archaea could not be detected in marine sand-containing microcosms after crude oil

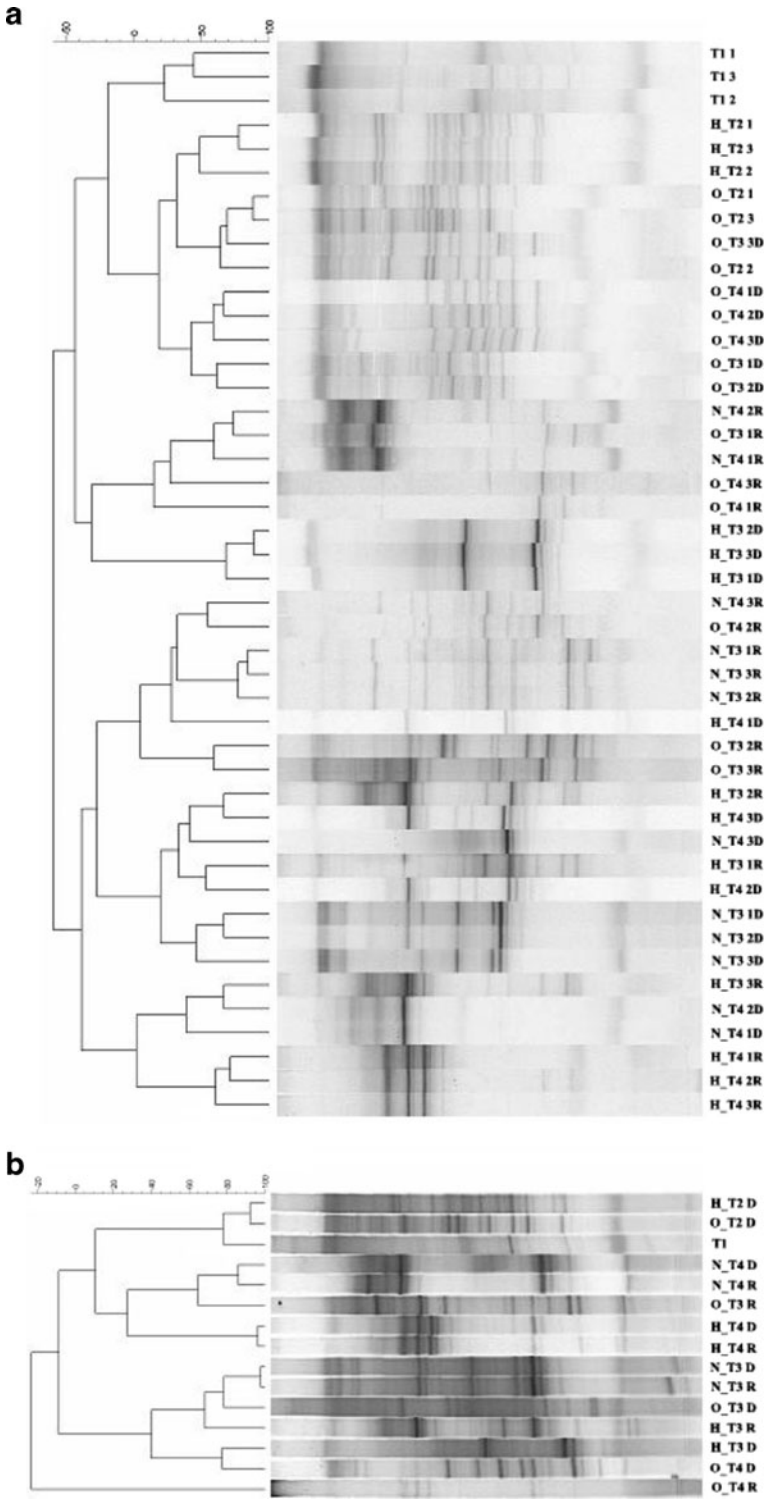

Fig. 4 Denaturing gradient gel electrophoresis (*DGGE*) fingerprints of archaeal 16S rRNA gene fragments amplified from DNA and RNA templates and the corresponding dendrograms, as described in Fig. 2. **a** The gels shown in Fig. 2a, b and c are combined here in a single figure. **b**) Samples representing each incubation time and the different microcosms loaded on a single DGGE gel to facilitate the comparison

contamination. Conversely, the presence of archaea has been demonstrated in natural marine ecosystems that were previously contaminated with petroleum hydrocarbons (Redmond and Valentine 2012; Taketani et al. 2010). The continuous influx of either natural seawater or invertebrates could have contributed to

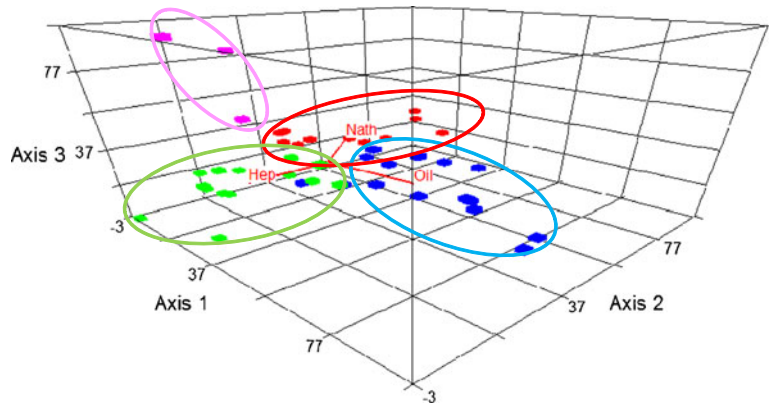

Fig. 5 Redundancy analysis (*RDA*) ordination diagram based on the genetic fingerprint data of the hydrocarbon-enriched archaeal communities with the hydrocarbons used to contaminate the microcosms (heptadecane, naphthalene and crude oil) as variables (*arrows*). Diagram corresponds to the genetic fingerprint pattern presented in Fig. 4a. The different colors represent the heptadecane-contaminated (*green*), naphthalene-contaminated (*red*), oil-contaminated (*blue*) and non-contaminated samples (*pink*) microcosm samples

archaea establishment in these environments, as suggested by Röling et al. (2004). Moreover, other microorganisms (such as bacteria and micro-eukaryotes) present in these water samples could have also influenced the archaeal communities in microcosms.

In this study, the contamination of the hypersaline water containing microcosms with different hydrocarbons was not deleterious to archaeal groups because these prokaryotes maintained their metabolic activity up to the 32nd day of incubation (as shown by DNA- and RNA-based analyses). Most aerobic hydrocarbon-degrading archaea described so far have been isolated from hypersaline ecosystems (Al-Mailem et al. 2010; Bonfá et al. 2011; Erdoğmuş et al. 2013; Tapilatu et al. 2010). Additionally, previous studies have suggested that archaea are the primary component of the microbial community in hypersaline environments (Andrei et al. 2012). However, to the best of our knowledge, this is the first time that the dynamics of archaeal communities in a hypersaline ecosystem contaminated with different types of petroleum hydrocarbons has been studied.

Variation in the composition of archaeal communities in response to exposure to the different hydrocarbons used was observed during the incubation of these microcosms. In addition, differences were also observed in DNA- and RNA based analyses. This fact may indicate that the most active archaea (RNA-based analysis) were not predominant in hypersaline microcosms (DNA-based analysis). Most of the sequences obtained here from DNA-based analysis of non-contaminated Vermelha Lagoon water and of all contaminated microcosms were associated with uncultivated archaea of Thermoplasmata Euryarchaeota. Likewise, archaeal OTUs related to the Thermoplasmata class from the Euryarchaeota phylum comprised more than 70 % of the archaeal communities obtained from DNA-based libraries for crude oil-polluted and unpolluted waters from the Gulf of Mexico after the Deepwater Horizon oil spill (Redmond and Valentine 2012). In the RNA-based analyses, we observed the presence of sequences related to the Halobacteria class from the Euryarchaeota phylum and to groups of the Crenarchaeota phylum in heptadecane-, naphthalene- and crude oil-contaminated microcosms. The Halobacteria class from the Euryarchaeota phylum has been previously described as harboring numerous hydrocarbon-degrading archaeal species (Al-Mailem et al. 2010; Bonfá et al. 2011; Erdoğmuş et al. 2013; Tapilatu et al. 2010). However, as far as we know, members of the phylum Crenarchaeota have never been described as hydrocarbon degraders and do not comprise the main archaeal phylum in hypersaline ecosystems (Auguet et al. 2010).

In addition to the marked response of the hydrocarbon-contaminated archaeal communities in Vermelha Lagoon to the incubation time, the communities were also selected based on the hydrocarbon source available. Although some hydrocarbon-degrading archaeal species described to date can utilize both alkanes and the PAH constituents of crude oil (Al-Mailem et al. 2010; Bonfá et al. 2011; Erdoğmuş et al. 2013; Tapilatu et al. 2010), they do not typically utilize both simultaneously. Moreover, these compounds are usually toxic to microbial communities

(Kim et al. 2012). The result of hydrocarbon contamination is therefore a selection for different archaeal communities that can either degrade or tolerate these toxic compounds.

A decrease in TPH after 32 days of microcosm incubation was observed in this study, suggesting the hydrocarbonoclastic potential of the archaeal communities. However, we cannot rest absolutely assured that the archaea in our analysis participated in the degradation of TPH, even though some authors have suggested that archaeal strains can attack different organic compounds no matter how complex they are (Le Borgne et al. 2008; Margesin and Schinner 2001). Other studies have shown that the minimal response to hydrocarbon contamination is consistent with the ecological behavior of archaea in chronically energy-stressed ecosystems (Valentine 2007). Therefore, further studies are still necessary to understand the impact of the enriched archaeal community on petroleum hydrocarbon degradation in hypersaline ecosystems such as Vermelha Lagoon.

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References

Al-Mailem, D. M., Sorkhoh, N. A., Al-Awadhi, H., Eliyas, M., & Radwan, S. S. (2010). Biodegradation of crude oil and pure hydrocarbons by extreme halophilic archaea from hypersaline coasts of the Arabian Gulf. *Extremophiles, 14*(3), 321–328.

Al-Mailem, D. M., Eliyas, M., & Radwan, S. S. (2012). Enhanced haloarchaeal oil removal in hypersaline environments via organic nitrogen fertilization and illumination. *Extremophiles, 16*(5), 751–758.

Anderson, R. T., & Lovley, D. R. (2000). Hexadecane decay by methanogenesis. *Nature, 404*(6779), 722–723.

Andrei, A. Ş., Banciu, H. L., & Oren, A. (2012). Living with salt: metabolic and phylogenetic diversity of archaea inhabiting saline ecosystems. *FEMS Microbiology Letters, 330*(1), 1–9.

Atlas, R. M., & Hazen, T. C. (2011). Oil biodegradation and bioremediation: a tale of the two worst spills in US history. *Environmental Science & Technology, 45*, 6709–6715.

Auguet, J. C., Barberan, A., & Casamayor, E. O. (2010). Global ecological patterns in uncultured Archaea. *ISME Journal, 4*(2), 182–190.

Bano, N., Ruffin, S., Ranson, B., & Hollibaught, J. T. (2004). Phylogenetic composition of arctic ocean archaeal assemblages and comparison with Antarctic assemblages. *Applied and Environmental Microbiology, 70*, 781–789.

Barbiére, E. B. (1985). *Condições climáticas dominantes na porção oriental da lagoa de Araruama (RJ) e suas implicações na diversidade do teor de salinidade. In Caderno de Ciências da Terra* (Vol. 59, 35 p). SP, Brazil: Universidade de São Paulo.

Bonfá, M. R., Grossman, M. J., Mellado, E., & Durrant, L. R. (2011). Biodegradation of aromatic hydrocarbons by Haloarchaea and their use for the reduction of the chemical oxygen demand of hypersaline petroleum produced water. *Chemosphere, 84*(11), 1671–1676.

Chang, W., Um, Y., & Holoman, T. R. (2006). Polycyclic aromatic hydrocarbon (PAH) degradation coupled to methanogenesis. *Biotechnology Letters, 28*(6), 425–430.

dos Santos, H. F., Cury, J. C., Do Carmo, F. L., Dos Santos, A. L., Tiedje, J., Van Elsas, J. D., et al. (2011). Mangrove bacterial diversity and the impact of oil contamination revealed by pyrosequencing: bacterial proxies for oil pollution. *PLoS One, 6*(3), e16943. doi:10.1371/journal.pone.0016943.

EPA 8015C. (2000). Nonhalogenated organics using GC/FID. EPA, revision 3, 33p.

Erdoğmuş, S. F., Mutlu, B., Korcan, S. E., Güven, K., & Konuk, M. (2013). Aromatic hydrocarbon degradation by halophilic Archaea isolated from Çamalt Saltern, Turkey. *Water Air and Soil Pollution, 224*, 1449. doi:10.1007/s11270-013-1449-9.

Ewing, B., & Green, P. (1998). Base-calling of automated sequencer traces using phred: II. Error probabilities. *Genome Research, 8*, 186–194.

Grant, W. D. (2004). Life at low water activity, review. *Philosophical Transactions of the Royal Society Biological Sciences, 359*(1448), 1249–1267.

Hardoim, C. C., Costa, R., Araújo, F. V., Hajdu, E., Peixoto, R., Lins, U., et al. (2009). Diversity of bacteria in the marine sponge *Aplysina fulva* in Brazilian coastal waters. *Applied and Environmental Microbiology, 75*(10), 3331–3343.

Jurelevicius, D., Korenblum, E., Casella, R., Vital, R. L., & Seldin, L. (2010). Polyphasic analysis of the bacterial community in the rhizosphere and roots of *Cyperus rotundus* L. grown in a petroleum-contaminated soil. *Journal of Microbiology and Biotechnology, 20*(5), 862–870.

Jurelevicius, D., Alvarez, V. M., Marques, J. M., Lima, L. R., Dias, F. D., & Seldin, L. (2013). Bacterial community response to petroleum hydrocarbon amendments in freshwater, marine and hypersaline water-containing microcosms. *Applied and Environmental Microbiology, 79*(19), 5927–5935.

Kim, J. N., Kim, B. S., Kim, S. J., & Cerniglia, C. E. (2012). Effects of crude oil, dispersant, and oil-dispersant mixtures on human fecal microbiota in an in vitro culture system. MBio doi:pii: e00376-12. 10.1128/mBio.00376-12.

Le Borgne, S., Paniagua, D., & Vazquez-Duhalt, R. (2008). Biodegradation of organic pollutants by halophilic Bacteria and Archaea. *Journal of Molecular Microbiology and Biotechnology, 15*, 74–92.

Margesin, R., & Schinner, F. (2001). Potential of halotolerant and halophilic microorganisms for biotechnology. *Extremophiles, 5*, 73–83.

McCune, B., & Mefford, M. J. (2011). PC-ORD Multivariate analysis of ecological data, Version 6.0 MjM Software, Gleneden Beach, Oregon, USA.

Pitcher, D. G., Saunders, N. A., & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Letters in Applied Microbiology, 8*(4), 151–156.

Redmond, M. C., & Valentine, D. L. (2012). Natural gas and temperature structured a microbial community response to the Deepwater Horizon oil spill. *Proceedings of the National Academy of Sciences of the United States of America, 109*(50), 20292–20297.

Röling, W. F., de Brito Couto, I. R., Swannell, R. P., & Head, I. M. (2004). Response of Archaeal communities in beach sediments to spilled oil and bioremediation. *Applied and Environmental Microbiology, 70*(5), 2614–2620.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology, 75*, 7537–7541.

Taketani, R. G., Franco, N. O., Rosado, A. S., & van Elsas, J. D. (2010). Microbial community response to a simulated hydrocarbon spill in mangrove sediments. *Journal of Microbiology, 48*(1), 7–15.

Tapilatu, Y. H., Grossi, V., Acquaviva, M., Militon, C., Bertrand, J. C., & Cuny, P. (2010). Isolation of hydrocarbon-degrading extremely halophilic archaea from an uncontaminated hypersaline pond (Camargue, France). *Extremophiles, 14*(2), 225–231.

Teske, A., & Sørensen, K. B. (2008). Uncultured archaea in deep marine subsurface sediments: have we caught them all? *ISME Journal, 2*(1), 3–18.

Valentine, D. L. (2007). Adaptations to energy stress dictate the ecology and evolution of the Archaea. *Nature Reviews Microbiology, 5*, 316–323.

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology, 73*, 5261–5267.