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Diversity of culturable halophilic archaea isolated from Rambla Salada, Murcia (Spain)

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Abstract We have studied the diversity of culturable halophilic *Archaea* at Rambla Salada, Murcia (south-eastern Spain). We made 8 samplings at different places in this habitat during the years 2006 and 2007 and isolated a total of 49 strains, which were identified by means of phenotypic tests and the hypervariable V1–V3 region of the 16S rRNA gene sequences (around 500 bp). The ribosomal data showed that the isolates belonged to 12 genera within the *Halobacteriaceae* family, with *Haloferax* and *Natrinema* being the most abundant. Five strains showed less than 97% sequence identity with validly described species and may well represent new taxa. All the strains grew best with around 25% w/v salts, required high concentrations of NaCl and magnesium and produced red to pink colonies. They were facultative anaerobes with both respiratory and fermentative metabolisms. The diversity of the archaeal community was analysed with the MOTHUR package. We identified 14 OTUs at the 3% genetic distance level and found quite high diversity. Rarefaction curves of richness estimators and diversity indices demonstrated that our collection of isolates represented the archaeal community at Rambla Salada that can be isolated under the conditions used in this work. This is the first report to be published on the culturable archaea at Rambla Salada, an area of considerable ecological interest.

Keywords Biodiversity · Halophiles · Culturable archaea · Hypersaline habitat · Taxonomy · Rambla Salada

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Introduction

Rambla Salada is a saline "rambla" (a steep-sided river bed, normally dry but subject to flash flooding) located in Murcia (southeastern Spain). Rambla Salada has been declared a protected area by the Murcian regional government (BORM 10/09/1998), a place of community interest (LIC) by the European Union and a protected wildfowl zone (ZEPA). It is an athalassohaline habitat and includes areas of soil, water and sediments with different salt contents, deriving mainly from Miocene gypsiferous marls in the Fortuna basin (Muller and Hsü 1987). Nowadays, the habitat is seriously threatened by human activities that induce changes in the natural hydrology and salinity levels: inputs of freshwater, nutrients, pesticides and other pollutants are dramatically changing its biodiversity.

Velasco et al. (2006) first studied the primary producers and macro-invertebrates at Rambla Salada and demonstrated that their community composition was closely linked to salinity. Nevertheless, to our knowledge there have been no ecological studies so far describing the population of microorganisms inhabiting this rambla, although our group has in the past discovered two new halophilic bacterial species there: *Idiomarina ramblicola* (Martínez-Cánovas et al. 2004) and strain R53 of *Halomonas cerina* (González-Domenech et al. 2008). Thus, we

undertook an analysis of the community of prokaryotes that live in various environments in the different areas of the Rambla Salada with the aims: firstly, of quantifying the archaeal community; secondly, of isolating a significant number of archaeal strains of those that represent the community of these organisms; finally, of identifying them and ascertaining their diversity.

Materials and methods

Sample collection and physical–chemical determinations

We took samples from four different zones in Rambla Salada (Murcia, southeastern Spain) (Table 1): soil, sediment and water at the Finca de la Salina (site 1), water and sediment from a saline groundwater spring (site 2), soil from the Humedal de Derramadores (site 3) and water and sediment from the Tajo-Segura interconnecting canal (site 4). We collected a total of 32 samples over 2 years (February and June 2006, and February and November 2007). The samples were taken aseptically and stored at 4°C until study in the laboratory (always within 24 h). The soils and sediments were suspended in sterile 25% w/v NaCl solution (1 g in 9 ml), thoroughly homogenized by stirring and then serially diluted (up to 10^{-6}). Water samples were directly diluted in sterile 25% w/v NaCl solution. As much as 100 µl of dilutions 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were surface-plated on MY medium (Moraine and Rogovin 1966) supplemented with 30% w/v sea-salt solution (Rodríguez-Valera et al. 1985, 1981) and incubated at 41°C for 3 weeks.

pH and conductivity were measured at each sampling point. Conductivity was determined with an ECmeter (TetraConR 325), which automatically calculates salinity.

Counts and selection of the strains

Counts were made in those plates containing between 30 and 300 colonies. A collection of 50 colonies, chosen on the basis of their different appearances, were re-isolated by streaking on a fresh casamino acids medium (medium 2) following the recommendations of Oren (2006) and grown at the same temperature for the same length of time. The sites where each strain was isolated are shown in Table 2.

DNA extraction, PCR amplification of 16S rRNA genes and sequencing

Genomic DNA was extracted from log-phase cells according to Marmur (1961) with the modification developed by Martín-Platero et al. (2007). The hypervariable V1–V3 regions of the 16S rRNA gene sequences (around 500 bp) were then determined using the method described by Burns et al. (2004) with the specific primers for *Archaea*: F1 as forward primer (5′-ATTCCGGTTGATCC TGC-3′) (Ihara et al. 1997) and 1492R as reverse primer (5′-ACGGHTACCTTGTTACGACTT′-3′) (Grant et al. 1999). PCR amplifications were made using 50 µl reaction mixtures containing 20–100 ng of template DNA, 10 pmol

Table 1 Sampling sites in Rambla Salada

Sampling sites	Co-ordinates	Season	Physical–chemical parameters	
			Salinity (g L^{-1})	pH
Site 1: riverbed zone	38°07′34.44″N	February 2006	36.7–38.3	7.7–7.9
	1°07′11.13″W	June 2006	44.4–83	6.3–6.8
		February 2007	18.6–20.2	8.1–8.2
		November 2007	11.8–22.2	8.0–8.3
		February 2006	150	6.5
Site 2: upwelling zone	38°07′29.09″N	June 2006	140	7.1
	1°07′42.15″W	February 2007	157.6	6.7
		November 2007	151.2	7.2
		February 2006	25.2	8.4
Site 3: Humedal de Derramadores	38°10′24.96″N	June 2006	16	8.1–8.2
	1°05′38.73″W	February 2007	15	8.0–8.3
		November 2007	16	8.0–8.2
		February 2006	70	7.7
Site 4: transfer site	38°07′30.23″N	June 2006	62.1	8.7
	1°07′42.22″W	February 2007	34.1	8.1
		November 2007	29	8.3

Table 2 Affiliations of the archaeal 16S rRNA gene sequences on the basis of pairwise comparison by the EzTaxon server 2.1

Strain	Isolation site	Sample	Accession no.	% Identity[a]	Taxon (type strain)
M4-6a[b]	S3	Soil	HQ659168	93.3	*Haladaptatus paucihalophilus* DX253^T (DQ344974)
M4-6b[b]	S3	Soil	HQ659169	94.1	*Haladaptatus paucihalophilus* DX253^T (DQ344974)
M2-4b	S1	Soil	HQ659134	98.4	*Haloarcula argentinensis* arg-1^T (D50849)
M2-7a	S2	Sediment	HQ659136	98	*Haloarcula argentinensis* arg-1^T (D50849)
M2-4c	S1	Soil	HQ659144	99.1	*Haloarcula quadrata* 801030/1^T (AB010964)
M2-4d	S1	Soil	HQ659135	98.3	*Haloarcula quadrata* 801030/1^T (AB010964)
M3-2d	S1	Soil	HQ659158	98.6	*Halococcus hamelinensis* 100A6^T (DQ017835)
M3-8c	S2	Sediment	HQ659159	99.2	*Halococcus hamelinensis* 100A6^T (DQ017835)
M3-8d	S2	Sediment	HQ659155	98.4	*Halococcus hamelinensis* 100A6^T (DQ017835)
M2-2a	S1	Soil	HQ659130	98.3	*Haloferax mediterranei* R-4^T (D11107)
M3-2a	S1	Soil	HQ659150	98.7	*Haloferax mediterranei* R-4^T (D11107)
M3-4a	S1	Soil	HQ659151	98.6	*Haloferax mediterranei* R-4^T (D11107)
M4-4b	S1	Soil	HQ659161	98	*Haloferax mediterranei* R-4^T (D11107)
M4-4c	S1	Soil	HQ659164	98.8	*Haloferax mediterranei* R-4^T (D11107)
M4-4d	S1	Soil	HQ659163	98.4	*Haloferax mediterranei* R-4^T (D11107)
M4-1a	S1	Soil	HQ659160	97.9	*Haloferax mucosum* PA12^T (DQ860980)
M3-2b	S1	Soil	HQ659157	98.3	*Haloferax mucosum* PA12^T (DQ860980)
M3-8a	S2	Sediment	HQ659153	99.5	*Haloferax prahovense* TL6^T (AB258305)
M3-8b	S2	Sediment	HQ659154	99.1	*Haloferax prahovense* TL6^T (AB258305)
M2-4a	S1	Soil	HQ659133	99.2	*Halogeometricum borinquense* PR3^T (DQ853414)
M3-1c[b]	S1	Water	HQ659148	96.6	*Halomicrobium mukohataei* arg-2^T (EF645691)
M3-1d	S1	Water	HQ659149	97.7	*Halomicrobium mukohataei* arg-2^T (EF645691)
M2-2d[b]	S1	Soil	HQ659132	89.4	*Halorhabdus tiamatea* SARL4B^T (EF127229)
M1-10	S1	Soil	HQ659123	100	*Halorubrum aidingense* 31-hong^T (DQ355813)
M1-17	S1	Soil	HQ659125	99.8	*Halorubrum aidingense* 31-hong^T (DQ355813)
M1-20	S1	Soil	HQ659128	100	*Halorubrum aidingense* 31-hong^T (DQ355813)
M1-19	S2	Sediment	HQ659127	100	*Halorubrum aidingense* 31-hong^T (DQ355813)
M2-2b	S1	Soil	HQ659131	99	*Halostagnicola kamekurae* 194-10^T (AB489220)
M1-9	S3	Soil	HQ659122	98.3	*Halostagnicola kamekurae* 194-10^T (AB489220)
M2-11d	S3	Soil	HQ659141	97.7	*Halostagnicola kamekurae* 194-10^T (AB489220)
M1-13	S1	Soil	HQ659124	98.1	*Halostagnicola larsenii* XH-48^T (AM117571)
M2-11a	S3	Soil	HQ659140	97.8	*Halostagnicola larsenii* XH-48^T (AM117571)
M4-6c	S3	Soil	HQ659167	98.3	*Halostagnicola larsenii* XH-48^T (AM117571)
M1-23	S1	Soil	HQ659129	99.3	*Haloterrigena jeotgali* A29^T (EF077633)
M3-9b	S4	Water	HQ659156	99.1	*Haloterrigena jeotgali* A29^T (EF077633)
M3-7b	S2	Sediment	HQ659152	98.6	*Haloterrigena thermotolerans* PR5^T (AF115478)
M2-9b	S4	Water	HQ659139	99.3	*Haloterrigena thermotolerans* PR5^T (AF115478)
M1-7	S1	Soil	HQ659121	98.8	*Natrialba aegyptia* 40^T (AF251941)
M3-1a	S1	Soil	HQ659147	99.2	*Natrialba aegyptia* 40^T (AF251941)
M2-1d	S1	Soil	HQ659143	98	*Natrinema altunense* AJ2^T (AY208972)
M2-7c	S2	Sediment	HQ659146	98.6	*Natrinema altunense* AJ2^T (AY208972)
M2-9a	S4	Water	HQ659145	98.5	*Natrinema altunense* AJ2^T (AY208972)
M4-1b	S1	Soil	HQ659165	98.6	*Natrinema gari* HIS40-3^T (AB289741)
M2-1c	S1	Soil	HQ659142	97.5	*Natrinema pallidum* CIP 106292^T (AJ002949)
M4-1c	S1	Soil	HQ659166	99.3	*Natrinema pallidum* CIP 106292^T (AJ002949)
M4-4a	S1	Soil	HQ659162	98.7	*Natrinema pallidum* CIP 106292^T (AJ002949)
M1-18	S2	Sediment	HQ659126	98.7	*Natrinema pallidum* CIP 106292^T (AJ002949)

Table 2 continued

Strain	Isolation site	Sample	Accession no.	% Identity[a]	Taxon (type strain)
M2-8	S2	Sediment	HQ659138	98.1	*Natrinema pallidum* CIP 106292^T (AJ002949)
M2-7b[b]	S2	Sediment	HQ659137	96.3	*Natrinema pallidum* CIP 106292^T (AJ002949)

M1 February 2006; *M2* June 2006; *M3* February 2007; *M4* November 2007; *S1* site 1; *S2* site 2; *S3* site 3; *S4* site 4

[a] On the basis of pairwise comparison of the 16S rRNA gene sequences by the EzTaxon server 2.1

[b] Strain that probably constitutes a new taxon

of each primer (Sigma®), 0.2 mM of dNTP mix (Bioline®), 2 mM of $MgCl_2$, 5× PCR buffer (Bioline®) and 1.25 U of BioTaqTM DNA polymerase (Bioline®). Amplified PCR products were purified with the Illustra GFX DNA and Gel Band Purification kit (GE Healthcare®) and sequenced directly.

Sequence analysis

The sequences obtained were identified by a similarity-based search using the EzTaxon server 2.1. (http://147.47.212.35:8080/index.jsp) (Chun et al. 2007). Thereafter, the sequences were aligned using Clustal X (Thompson et al. 1997). To study the phylogenetic relationship among the isolates and other species of *Halobacteriaceae,* we applied neighbour-joining (NJ) and maximum parsimony (MP) criteria using the MEGA version 4 software (Tamura et al. 2007). Confidence levels for the phylogenetic trees were assessed by bootstrapping with 1000 replicates. The sequence of the type strain of *Methanospirillum hungatei* JF-1^T was used as outgroup.

Phenotypic characterization

We carried out the phenotypic tests described by Oren et al. (1997), which are the minimal standards for the description of new taxa in the order *Halobacteriales*.

Diversity measures and rarefaction analysis

Sequence alignments of the 16S rRNA genes allowed us to construct a distance matrix using MOTHUR (http://www.mothur.org/) (Schloss et al. 2009), a software package integrating an improved version of DOTUR (Schloss and Handelsman 2005). Once the matrix was generated, we conducted an OTU-based analysis to study archaeal diversity. The clustering algorithm was furthest neighbour. We carried out rarefaction studies taking the default value of 1000 as the number of randomizations. We also calculated the Shannon (H′) diversity, the reciprocal of Simpson's indexes (Simpson 1949; Magurran 1996) and Chao 1 and ACE species-richness estimators (Chao 1987).

Nucleotide sequence accession numbers

The sequences reported in this study have been submitted to the GenBank database under accession numbers HQ659121 to HQ659169.

Results

Physical–chemical measurements

Salinity in the different zones (sites 1–4) and samples (water, soil and sediment) taken at Rambla Salada ranged from 1.6 to 8.3% w/v in 2006 and from 1.5 to 3.4% w/v in 2007 with the exception of the water sampled at site 2 (natural groundwater spring), the salt content of which remained between 14 and 15.7% w/v. The pH ranged from 6.3 to 8.7 (Table 1).

Microbial counts and selection of the archaeal strains

Microbial counts (UFC/ml or UFC/g) revealed values of around 10^4 ($1.2–4.3 \times 10^4$) in February 2006 and around 10^6 ($1.2–2.6 \times 10^6$) in June 2006 and February and November 2007. We chose 50 isolates on the basis of the different appearances of their colonies (26 and 24 strains were chosen in 2006 and 2007, respectively). Phenotypic tests and ribosomal data (see below) proved that 49 of these 50 isolates were strains of archaea. This result suggested that the microbial counts could be attributed almost entirely to archaeal strains. The great majority of the colonies were red to pink, which is the norm among these microorganisms.

Phylogenetic analyses

The use of specific primers for the hypervariable region of the 16S rRNA gene of *Archaea* and subsequent sequencing of the PCR product allowed us to determine a preliminary phylogeny of the isolates. Both NJ and MP methods gave similar clusters, supported by bootstrap values of above 70% (Fig. 1). Phylogenetic analyses indicated that all the strains were related to different genera within the *Halobacteriaceae*, with *Haloferax* and *Natrinema* being

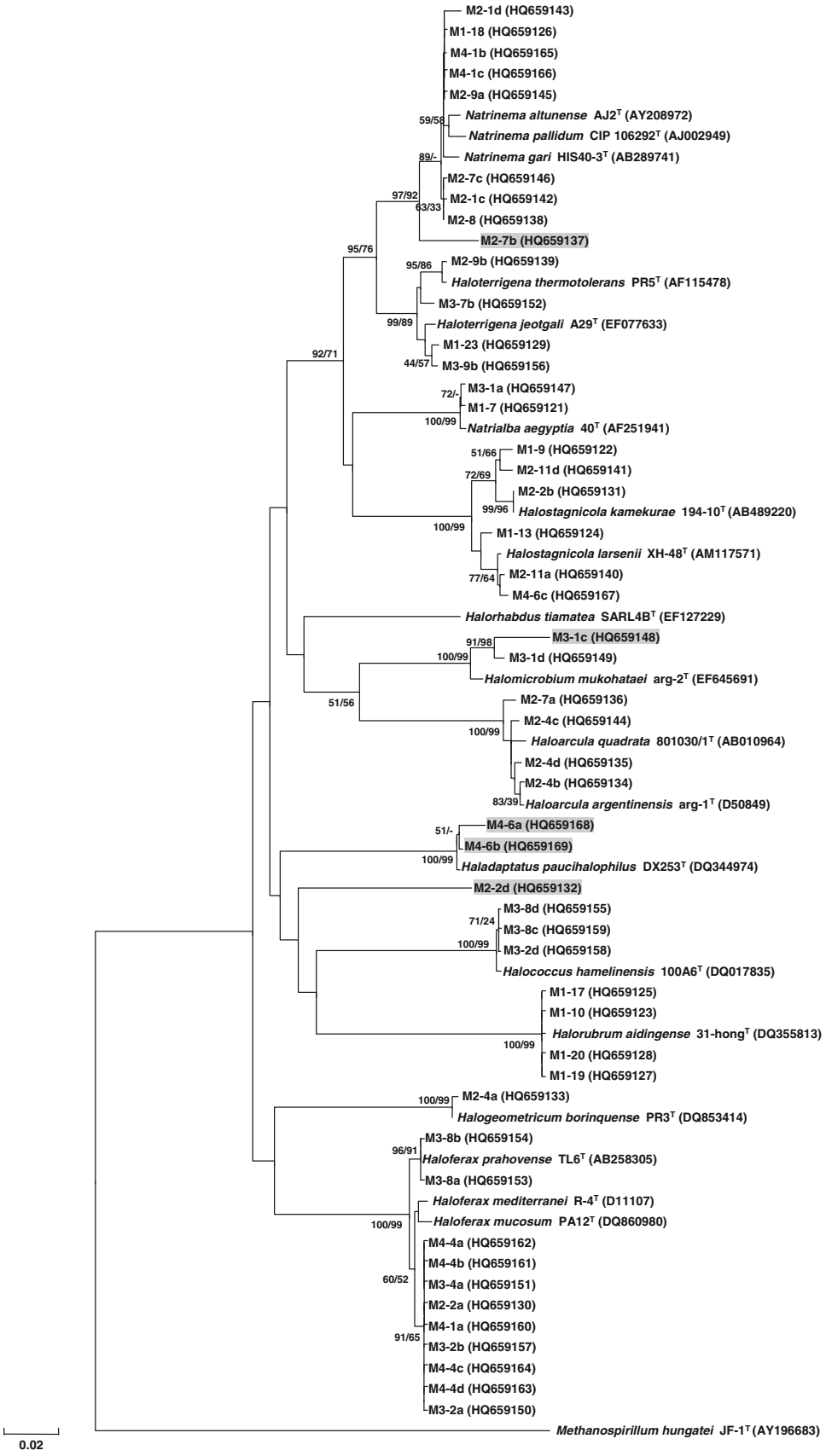

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of the archaeal isolates with respect to other members of the family *Halobacteriaceae*. The sequence of the type strain of *Methanospirillum hungatei* JF-1T was used as outgroup. *Bar* 1% sequence divergence. Common clusters resulting from both neighbour-joining and maximum-parsimony methods show bootstrap values at the corresponding nodes (in that order). The 5 strains with less than 97% similarity to validly described species are *shaded in grey*. GenBank/EMBL/DDBJ accession numbers are given in *parenthesis*

predominant with 10 representatives each. In addition, the 16S rRNA genes of strains M2-2d, M2-7b, M3-1c, M4-6a and M4-6b showed a similarity of less than 97% with other archaeal species, which leads us to surmise that they probably constitute new taxa (Table 2).

Phenotypic characterization

We used a total of 78 phenotypic tests to characterize the strains, in accordance with the minimal standards for the description of new taxa in the order *Halobacteriales* (Oren et al. 1997) (Table S1). All the strains were either Gram-negative rods or pleiomorphic, and extremely halophilic, growing best with 25% w/v sea salt. They required magnesium. They grew best between 37 and 41°C. Colonies ranged from pink to red in colour. They were facultative anaerobes. All fermented glucose and arginine and some of them respired with nitrate. They were resistant to ampicillin, chloramphenicol, erythromycin, nalidixic acid, penicillin and tetracycline.

Diversity measures and rarefaction analyses

Using the clustering algorithm implemented in the MOTHUR package, we identified 14 OTUs at the 3% distance level. We used rarefaction curves to compare the relative richness between the archaeal population from each sampling season, 2006 and 2007. The rarefaction analyses at 97% grouping stringency revealed that diversity was higher in 2006 than in 2007 (Fig. 2). Chao 1 and ACE rarefaction curves tend to be parallel to the *x*-axis, indicating a representative sampling under the conditions used (Fig. 3). The Chao 1 and ACE estimators predicted between 15 and 17 species at 97% grouping stringency: at a 95% confidence interval, the values for Chao 1 ranged between 14.18 and 26.47 and for ACE between 14.51 and 29.16, taking the whole sampling area into account (Fig. 3). Thus, the values for the predicted number of OTUs are quite close to the number of OTUs observed (see above). Furthermore, both the Chao 1 and ACE estimators were higher in 2006 (18.5 and 17.82) than in 2007 (8.2 and 11.3) (Table 3).

We also assessed diversity by means of Simpson's and Shannon's diversity indexes, using the MOTHUR programme and obtained values of 0.09 (reciprocal value of Simpson's index 10.05) and 2.35 for the total sampling area, respectively (Table 3). These values reflect quite high archaeal diversity. Both indices were also higher in 2006 (10 and 2.16) than in 2007 (5 and 1.74), as seen previously. In comparison to other studies of archaeal populations in hypersaline habitats (Clementino et al. 2008; Baati et al. 2008, 2010; Pasić et al. 2005), we found lower values for the richness estimators and similar values for diversity indices (Table 3).

Discussion

All extremely halophilic archaea, or haloarchaea, cultured to date belong to the *Halobacteriaceae* family within the order *Halobacteriales* in the phylum *Euryarchaeota*. They are found extensively in such saline environments as salt lakes and saltern crystallizer ponds and also in saline soils (Oren 1994; Burns et al. 2004; Grant et al. 2001; Maturrano et al. 2006; Pasić et al. 2005; Dave and Desai 2006). In recent years, some publications have also described their presence in medium-to-low-salinity environments or even non-saline habitats (Aller and Kemp 2008; Cambon-Bonavita et al. 2009). Furthermore, new molecular ecology

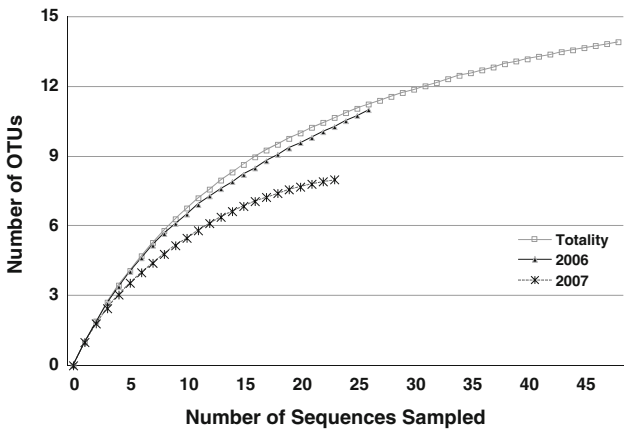

Fig. 2 Comparative rarefaction curves representing the observed archaeal diversity from the whole sampling area of Rambla Salada during each season with clustering stringency at 97%

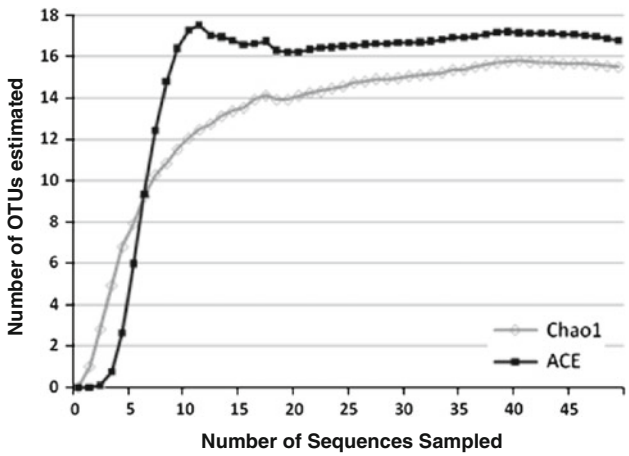

Fig. 3 Estimated OTU richness and diversity of archaea versus sample size from the whole sampling area in Rambla Salada. Estimated OTU richness is plotted for Chao 1 (*open diamonds*) and ACE (*filled squares*) estimators

Table 3 Comparison of estimated and observed richness for different populations of *Archaea* from several hypersaline habitats

Location	Salinity (% w/v NaCl)	Period (year)	No. of sequences	No. of OTUs[a]	Chao 1	ACE	H′	1/*D*	Reference
Rambla Salada Murcia (Spain)									
S1, S3, S4	1.6–8	2006	26	11	18.5	17.82	2.16	10.0	This study
S2	14–15								
S1, S3, S4	1.2–3.4	2007	23	8	8.2	11.3	1.74	5.0	
S2	15.1–15.7								
Total			49	14	15.5	16.78	2.35	10.05	
Araruama Lagoon Rio de Janeiro (Brazil)[b]									
P1	5	2004	53	28	75.5	95.7	2.98	–	Clementino et al. (2008)
P2	37		12	11	35.5	66.0	2.37	–	
Sfax (Tunisia)[b]									
M2	15	2005	36	8	23.0	–	1.16	2.17	Baati et al. (2008)
TS38	25		40	20	80.0	–	2.5	9.0	
S5	32		80	39	106.6	–	3.04	11.62	
Sfax (Tunisia)[b]									
M2	15	2006	35	13	25.0	–	2.34	10.0	Baati et al. (2010)
TS38	25		70	51	468.3	–	3.84	52.63	
Sečovlje Slovenia	ND	2003	120	15	27.0	20.32	2.23	7.14	Pasić et al. (2005)

Chao 1 richness estimator; *ACE* abundance-based coverage estimator; *H′* diversity, Shannon index; 1/*D* dominance, reciprocal of Simpson index (*D*); *ND* non-determinate

[a] According to MOTHUR results. OTUs and richness estimators were defined using a distance level of 3%. The number of OTUs and richness indexes from the whole area sampled was established at 97% identity. The sequence-assignment method was the furthest-neighbour approach

[b] Culture-independent study

techniques have found archaea belonging to the phylum *Crenarchaeota* in saline habitats, but they remain to be uncultured.

Our study has demonstrated that Rambla Salada is host to a substantial density and diversity of culturable halophilic archaea belonging to the *Halobacteriaceae*, even in zones of low and medium salinity (see Table 2) and that they represent a diverse group of taxa belonging to different genera and species.

As far as the total counts are concerned, they were high and quite similar to those obtained in other hypersaline habitats, such as solar salterns in Alicante (Spain) (Rodríguez-Valera et al. 1985, 1981) and in San Francisco (CA, USA) (Litchfield et al. 1999), where the values were 10^4 and 10^5–10^6 UFC/ml, respectively.

Salinity is one of the most important driving forces of diversity for both macro- and microorganisms (Auguet et al. 2010; Lozupone and Knight 2007; Tamames et al. 2010). According to Velasco et al. (2006), this is reflected in the composition of the communities of primary producers and macro-invertebrates at Rambla Salada. Our work has also demonstrated that the diversity of archaea is affected to some extent by this factor.

In our study, salinity was automatically calculated from the conductivity measurements made in situ during each sampling season. Generally, the salinity gradient reached its highest values in 2006. At site 2, a natural spring, the salinity values were practically constant throughout the sampling period, which might be expected from a permanent flow of saline groundwater. We found the highest biodiversity in 2006, which, according to the physical–chemical parameters measured, may well be related to higher salinity. Thus, in 2006 we isolated 12 genera (*Haladaptatus, Haloarcula, Halococcus, Haloferax, Halogeometricum, Halomicrobium, Halorhabdus, Halorubrum, Halostagnicola, Haloterrigena, Natrialba* and *Natrinema*), whilst in 2007 only 8 genera were identified (*Haladaptatus, Halococcus, Haloferax, Halomicrobium, Halostagnicola, Haloterrigena, Natrialba* and *Natrinema*). We found several taxa that were isolated only during one of the sampling periods. Thus, *Halorubrum aidingense, Haloarcula argentinensis, Haloarcula quadrata, Halogeometricum borinquense* and *Halorhabdus tiamatea* were isolated in 2006, whilst *Halomicrobium mukohataei, Haloferax prahovense, Halococcus hamelinensis* and *Haladaptatus paucihalophilus* were isolated in 2007.

On the other hand, it should be pointed out that in none of our samples did we find the extreme halophilic bacterium *Salinibacter*, the most significant features of which are similar to archaea (Antón et al. 2002).

As shown in Table 3, higher diversity was related to higher salinity, which, according to their diversity indices, is also the trend observed in hypersaline archaeal populations from other habitats (Clementino et al. 2008; Baati et al. 2008, 2010; Pasić et al. 2005).

In this study we did not detect all the archaea living in the habitat and the introduction of more isolation media, such as those mentioned by Burns et al. (2004), would probably allow the cultivation of a greater number of taxa, but rarefaction analyses using various richness estimators suggest that the total number of sequences studied within the area of Rambla Salada covers most of the culturable archaea under our chosen conditions. Moreover, although the Chao 1 and ACE estimators normally underestimate true richness when sample sizes are small (Hughes et al. 2001), we found in our study that the estimated value was quite similar to that observed.

In general terms, the diversity of haloarchaea at Rambla Salada was similar to that in other saline environments (Oren 2002; Burns et al. 2004; Baati et al. 2008, 2010; Clementino et al. 2008; Ozcan et al. 2007). Nevertheless, the predominant taxa were different. In solar salterns, one of the most thoroughly studied types of hypersaline habitat, the predominant population tends to be made up of strains belonging to the genera *Haloferax, Halorubrum, Halococcus, Haloterrigena, Haloarcula, Natrialba* and *Halobacterium* (Oren 2002). In saltern crystallizers, the predominant archaea are *Halorubrum* and *Haloquadratum* (Oren 2002). In Rambla Salada, however, we isolated more strains belonging to the genera *Natrinema* and *Haloferax*, this latter often being found in habitats with low salinity, although it grows in media containing 1.0–5.1 M NaCl and grows best at 2.5 M NaCl (Oren 2011). Nevertheless, these taxa were not dominant when the archaeal population was analysed by molecular methods. In fact, Oueragli (communication personal) found that *Haloarcula* is the most abundant taxon in Rambla Salada.

This study is the first to describe the culturable halophilic-archaeal community at Rambla Salada. Our results confirm the presence of a substantial biodiversity and density of archaea in this environment. We have in addition discovered a number of strains that may well constitute new taxa and are being subject to further scrutiny in our laboratory.

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