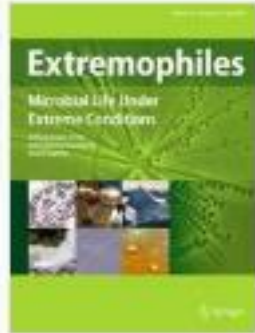
The background features a central light green cloud-like shape containing the title. Surrounding this are various abstract elements: blue and yellow organic shapes, black and grey hatched circles, and small solid dots in yellow, cyan, and black. The overall style is modern and artistic.

# 读书报告

牛铭铭

2018.8.4



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[Extremophiles](#)

pp 1-13 | [Cite as](#)

# Abundance and diversity of prokaryotes in ephemeral hypersaline lake Chott El Jerid using Illumina Miseq sequencing, DGGE and qPCR assays

[Authors](#)

[Authors and affiliations](#)

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Original Paper

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# 目录

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Introduction



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Results and Discussion



Conclusion



# PART 01

## Introduction



# Chott El Jerid



“

## 吉利特盐湖（ Chott El Jerid ）

Chott El Jerid是撒哈拉沙漠中最大的盐湖，长250公里，宽20公里，总面积5360平方公里，盐度高于33%NaCl。它是一个短暂的盐湖，位于一个10,500平方公里的干旱带封闭盆地中，其底部位于海平面以下仅17米处

（Gueddari等，1983）。这个陆地环境的年平均温度是21° C，年平均降雨量在80到140毫米之间，蒸发率约为1500毫米，产生强烈的负水平衡。是地球上最类似火星地貌的地方之一。

”



## Research Background



“

Our previous study explored the prokaryotic diversity during the dry season (Ben Abdallah et al. 2016)

However, the diversity of prokaryotes inhabiting Chott El Jerid during the wet season has so far not been studied.





# Aim



调查雨季时期Chott El Jerid的原核微生物群落（细菌，古细菌）。



研究雨季中硫酸盐还原和产甲烷种群的多样性和丰度。



比较Chott El Jerid与其他高盐生态系统中的微生物群落组成。

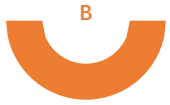




## PART 02

# Materials and Methods

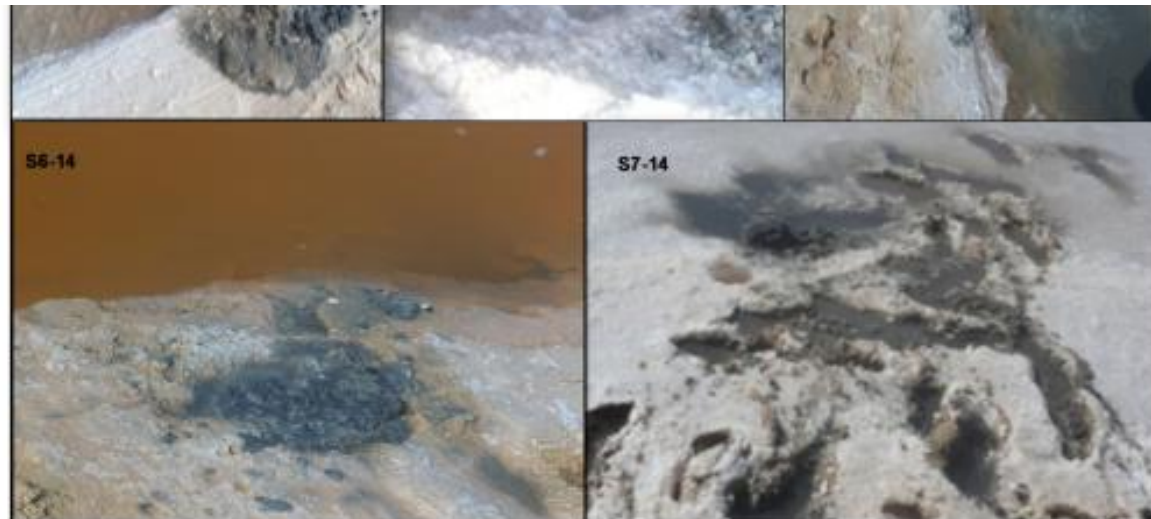


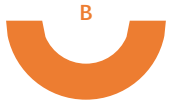


## Site description and sample collection



Sample	Site	Latitude	Longitude	Salinity (‰)	pH	Temperature (°C)
S3-14	Site 1	33°54'42.21"N	8°31'7.98"E	33.8	7.22	19
S4-14		33°54'42.21"N	8°31'7.98"E	33.8	7.22	
S5-14		33°54'42.21"N	8°31'7.98"E	33.8	7.22	
S6-14	Site 2	33°54'44.15"N	8°31'9.01"E	27.6	7.61	
S7-14		33°54'44.13"N	8°31'8.98"E	35.5	7.09	





# Illumina Miseq sequencing

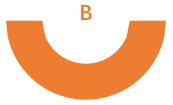
引物对

*Bacteria* : Pro341F/Pro805R

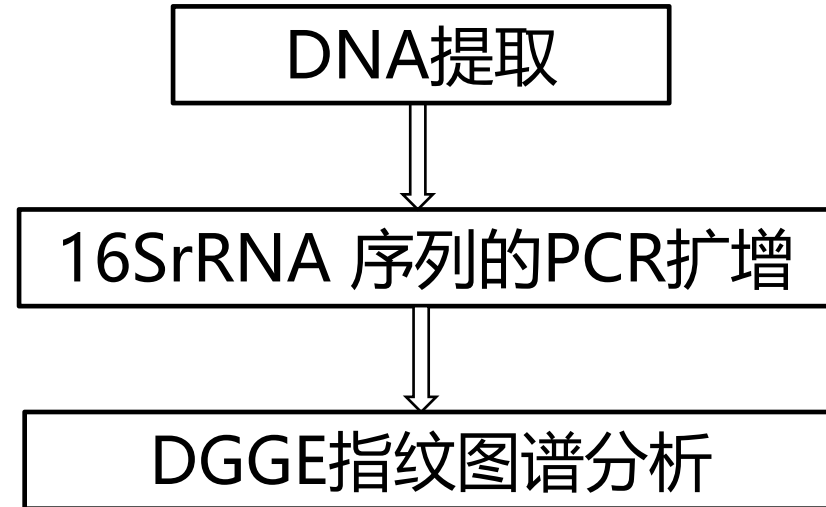
*Archaea* : Marc344F/March806R

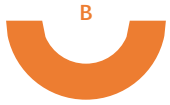
## 反应程序

94°C	4min	
94°C	30s	} 30×
55°C	30s	
72°C	45s	
72°C	10min	



# DGGE analysis of 16S rRNA, dsrB and mcrA gene fragments and sequencing





# DGGE analysis of 16S rRNA, dsrB and mcrA gene fragments and sequencing

除16S rRNA标记外， dsrB和mcrA基因分别用于硫酸盐还原菌和产甲烷群落的分类学分析。

## dsrB基因扩增

dsrAB: 1.9 kb

引物对: DSR1F and DSR4R

95°C 5min

95°C 40s

55°C 1min } 30×

72°C 3min

72°C 10min



dsrB: 350-bp

引物对: DSRp2060F-GC and DSR4R

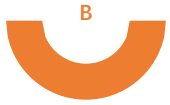
95°C 5min

95°C 1min

57°C 1min } 30×

72°C 1min

72°C 10min



# DGGE analysis of 16S rRNA, dsrB and mcrA gene fragments and sequencing

## mcrA 基因扩增

mcrA: 778 bp

第一次扩增:

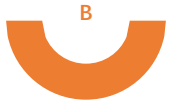
引物对: ME1 and ME2

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94°C	40s	
50°C	1min30s	
72°C	3min	
72°C	10min	

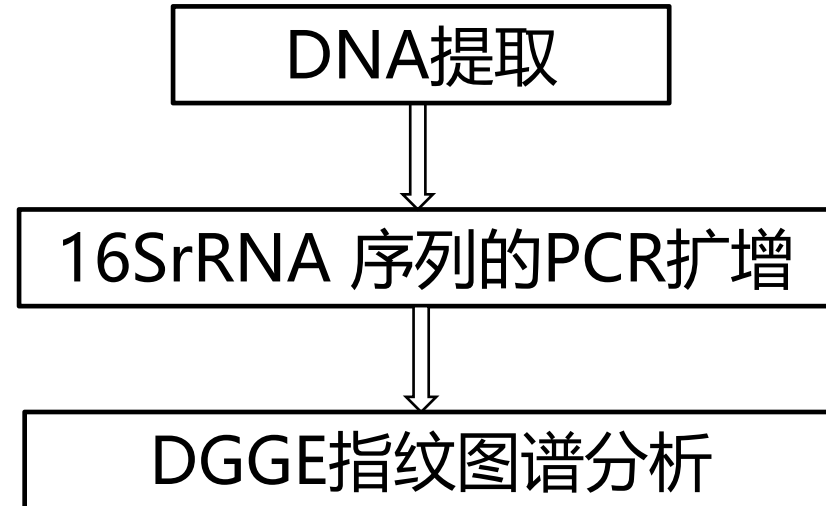
第二次扩增:

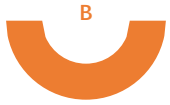
引物对: mcrAF-GC and mcrAR

95°C	5min	} 30×
95°C	45s	
54°C	45s	
72°C	2min	
72°C	10min	
4°C	∞	



# DGGE analysis of 16S rRNA, dsrB and mcrA gene fragments and sequencing



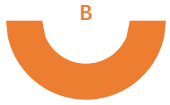


# DGGE analysis of 16S rRNA, dsrB and mcrA gene fragments and sequencing

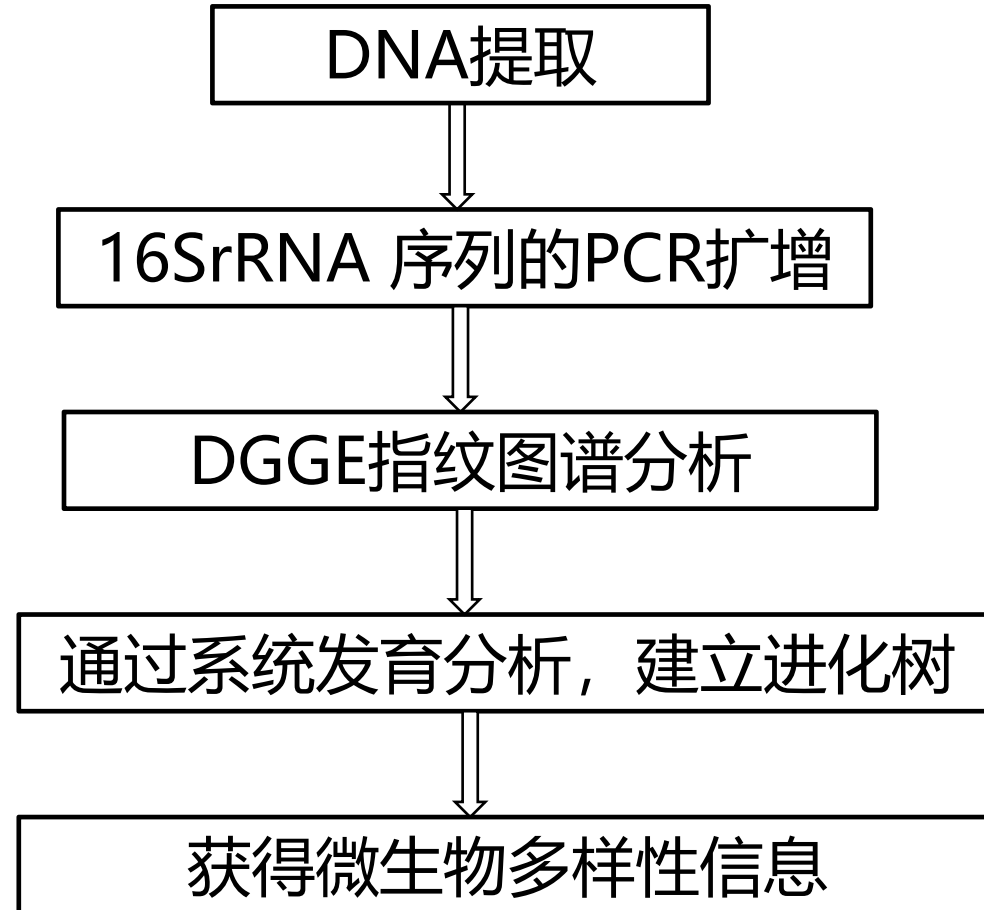
## 变性梯度凝胶



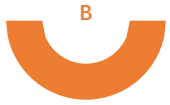
将dsrB和mcrA扩增子分别应用于含有35-65和20-80%变性梯度的6% (w / v) 聚丙烯酰胺凝胶, 基因片段在60V下跑16小时。



# DGGE analysis of 16S rRNA, dsrB and mcrA gene fragments and sequencing







# Quantification of 16S rRNA, dsrB and mcrA genes by quantitative real-time PCR (qPCR)

通过qPCR评估每个样品古菌和细菌群落的丰富程度

## 反应条件

sulfate-reducing Bacteria

95°C	30s	} 40x
95°C	5s	
54°C	30s	

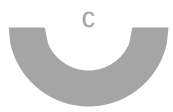
methanogens

95°C	10min	
95°C	5s	} 40x
55°C	30s	
72°C	30s	



## PART 03

# Results and Discussion

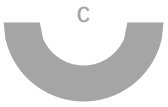


# Illumina Miseq-based analysis of microbial diversity using the prokaryotic universal primers

**Table 2** Richness and diversity estimation of microbial communities in Chott El Jerid samples

	Sample	Sequences	Chao1 richness estimator	Observed species	Shannon diversity index	Simpson diversity index	PD whole-tree indices
Prokaryote 16S rRNA gene universal primers	S3-14	47,573	246	226	3.19	0.67	19.87
	S4-14	55,824	100.07	95	1.66	0.49	9.26
	S5-14	67,717	138	129	1.83	0.50	11.41
	S6-14	38,621	125	118	1.67	0.47	11.11
	S7-14	35,820	115	100	1.69	0.49	9.53
<i>Archaea</i> 16S rRNA gene-specific primers	S3-14	38,473	53.66	52	2.49	0.73	2.73
	S4-14	16,653	39.80	38	1.69	0.58	1.98
	S5-14	62,695	50	47	2.20	0.73	2.23
	S6-14	80,400	36.50	34	0.82	0.22	1.99
	S7-14	103,145	56.66	55	2.40	0.76	2.38

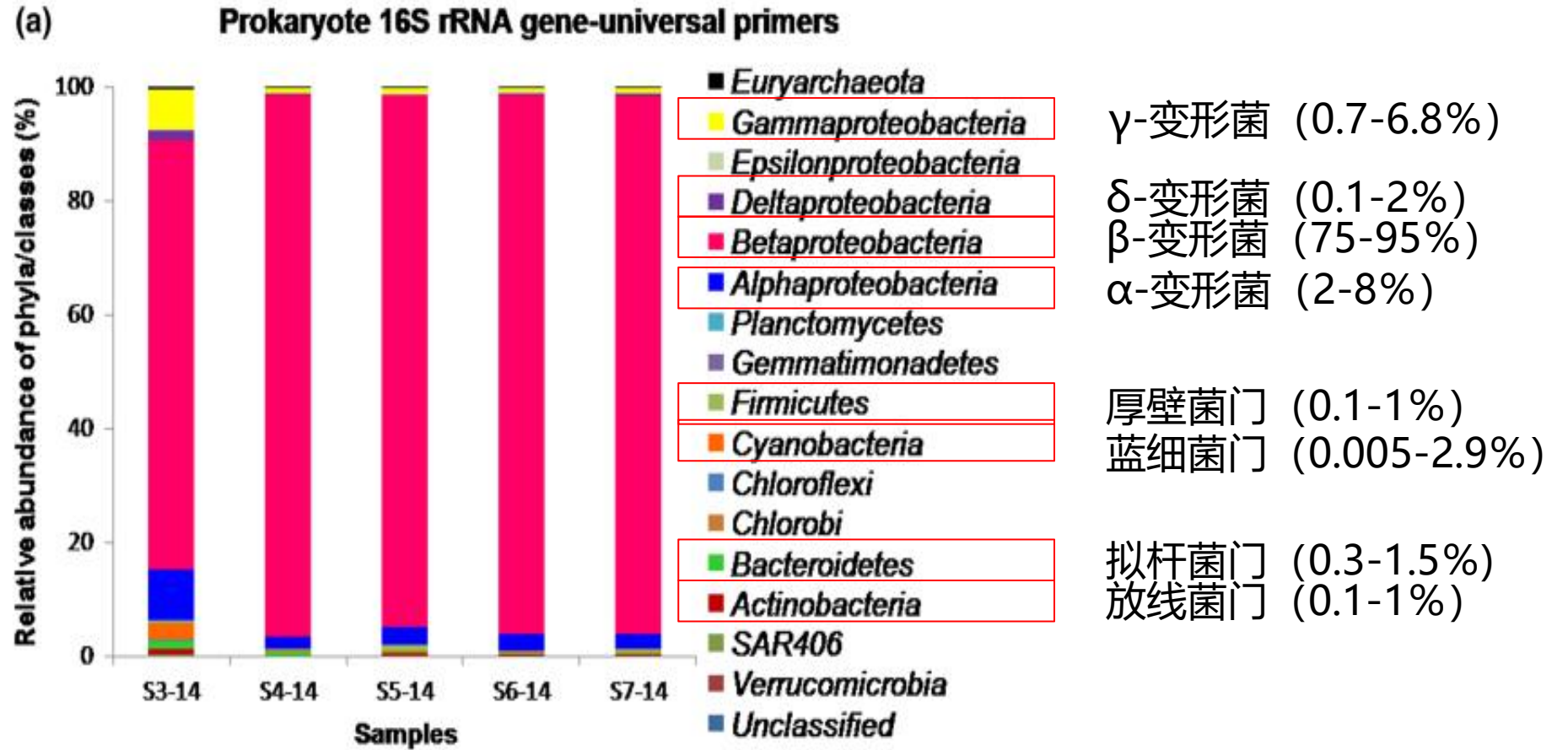
The analyses were based on Illumina Miseq sequencing data targeting the 16S rRNA genes

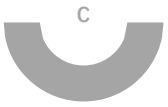


# Illumina Miseq-based analysis of microbial diversity using the prokaryotic universal primers

*Proteobacteria*变形菌门 (93-98.8%)

Fig. 2 Relative abundance of different prokaryotic taxa in each sample of the Chott El Jerid





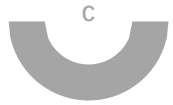
# llumina Miseq-based analysis of microbial diversity using the prokaryotic universal primers

**Table 3** Phylogenetic affiliation of the dominant bacterial OTUs obtained from Illumina Miseq sequencing analysis of Chott El Jerid samples targeting the 16S rRNA gene

OTU no. [GenBank number]	Sequences per sample (%)					Closest related microorganism retrieved from NCBI nucleotide database		
	S3-14	S4-14	S5-14	S6-14	S7-14	Taxonomy (phylum; class; order)	Species (accession number)	Similarity (%)
557211 [MG856364]	1.13	0	0.01	0.02	0	Cyanobacteria; Synechococcales	<i>Prochlorococcus marinus</i> subsp. <i>Pastoris</i> ATCC 700925 (AF180967)	96
1016465 [MG856365]	2.22	0.01	0	0.05	0.01	Proteobacteria; Alphaproteobacteria; Pelagibacterales	<i>Candidatus Pelagibacter</i> ubiquus HTCC1062 (NR_074224)	99
965129 [MG856366]	1.42	1.18	1.16	1.61	1.59	Proteobacteria; Alphaproteobacteria; Sphingomonadales	<i>Sphingomonas oligophenolica</i> JCM 12082 (AB018439)	99
20 [MG856367]	17.59	23.85	21.53	20.19	22.14	Proteobacteria; Betaproteobacteria; Burkholderiales	<i>Burkholderia vietnamiensis</i> LMG 10929 (AF097534)	99
177991 [MG856368]	53.56	66.58	67.03	69.46	67.55	Proteobacteria; Betaproteobacteria; Burkholderiales	<i>Ralstonia pickettii</i> ATCC 27511 (AY741342)	99
37 [MG856369]	1.11	1.26	1.17	1.45	1.32	Proteobacteria; Betaproteobacteria; Burkholderiales	<i>Ralstonia pickettii</i> ATCC 27511 (AY741342)	99
759916 [MG856370]	1	1.25	1.25	1.17	1.11	Proteobacteria; Betaproteobacteria; Burkholderiales	<i>Ralstonia pickettii</i> ATCC 27511 (AY741342)	99
3526876 [MG856371]	1.10	0.06	0.13	0.23	0.45	Proteobacteria; Deltaproteobacteria; Desulfobibrionales	<i>Desulfohalobium retbaense</i> DSM 5692 (NR_074907)	99
782953 [MG856372]	3.84	0.15	0.15	0.18	0.07	Proteobacteria; Gammaproteobacteria; Enterobacterales	<i>Pectobacterium carotovorum</i> ATCC 15713 (NR_116341)	99

83-94%

罗尔斯顿属

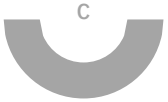


# Illumina Miseq-based analysis using the archaeal 16S rRNA gene-specific primers

**Table 2** Richness and diversity estimation of microbial communities in Chott El Jerid samples

	Sample	Sequences	Chao1 richness estimator	Observed species	Shannon diversity index	Simpson diversity index	PD whole-tree indices
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	S5-14	67,717	138	129	1.83	0.50	11.41
	S6-14	38,621	125	118	1.67	0.47	11.11
	S7-14	35,820	115	100	1.69	0.49	9.53
<i>Archaea</i> 16S rRNA gene-specific primers	S3-14	38,473	53.66	52	2.49	0.73	2.73
	S4-14	16,653	39.80	38	1.69	0.58	1.98
	S5-14	62,695	50	47	2.20	0.73	2.23
	S6-14	80,400	36.50	34	0.82	0.22	1.99
	S7-14	103,145	56.66	55	2.40	0.76	2.38

The analyses were based on Illumina Miseq sequencing data targeting the 16S rRNA genes

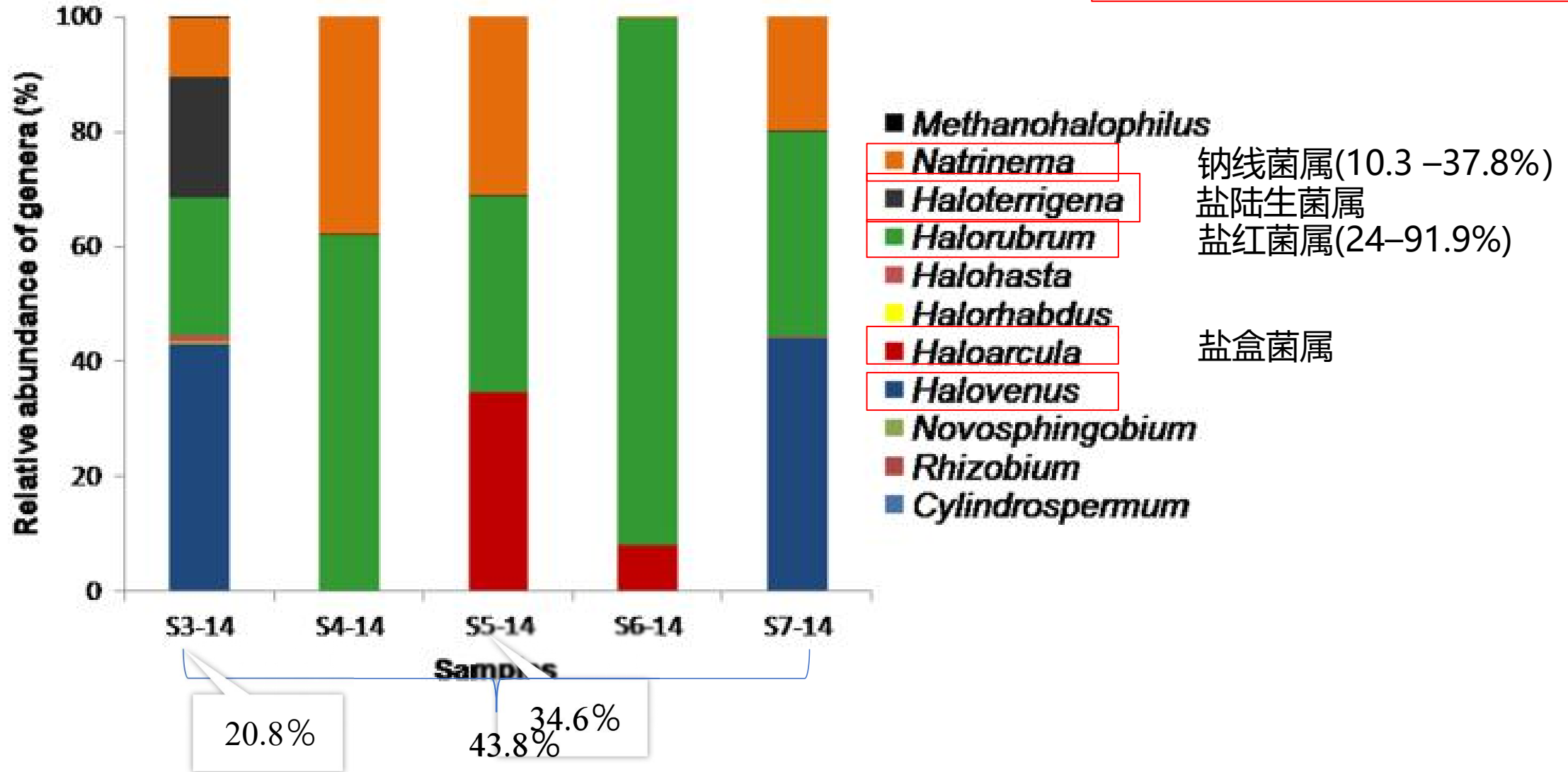


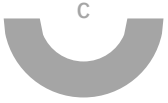
# Illumina Miseq-based analysis using the archaeal 16S rRNA gene-specific primers

*Euryarchaeota* 广古菌门(99.9%)  
*Halobacteria* 盐杆菌纲

(b)

### Archaea 16S rRNA gene-specific primers





# Illumina Miseq-based analysis using the archaeal 16S rRNA gene-specific primers

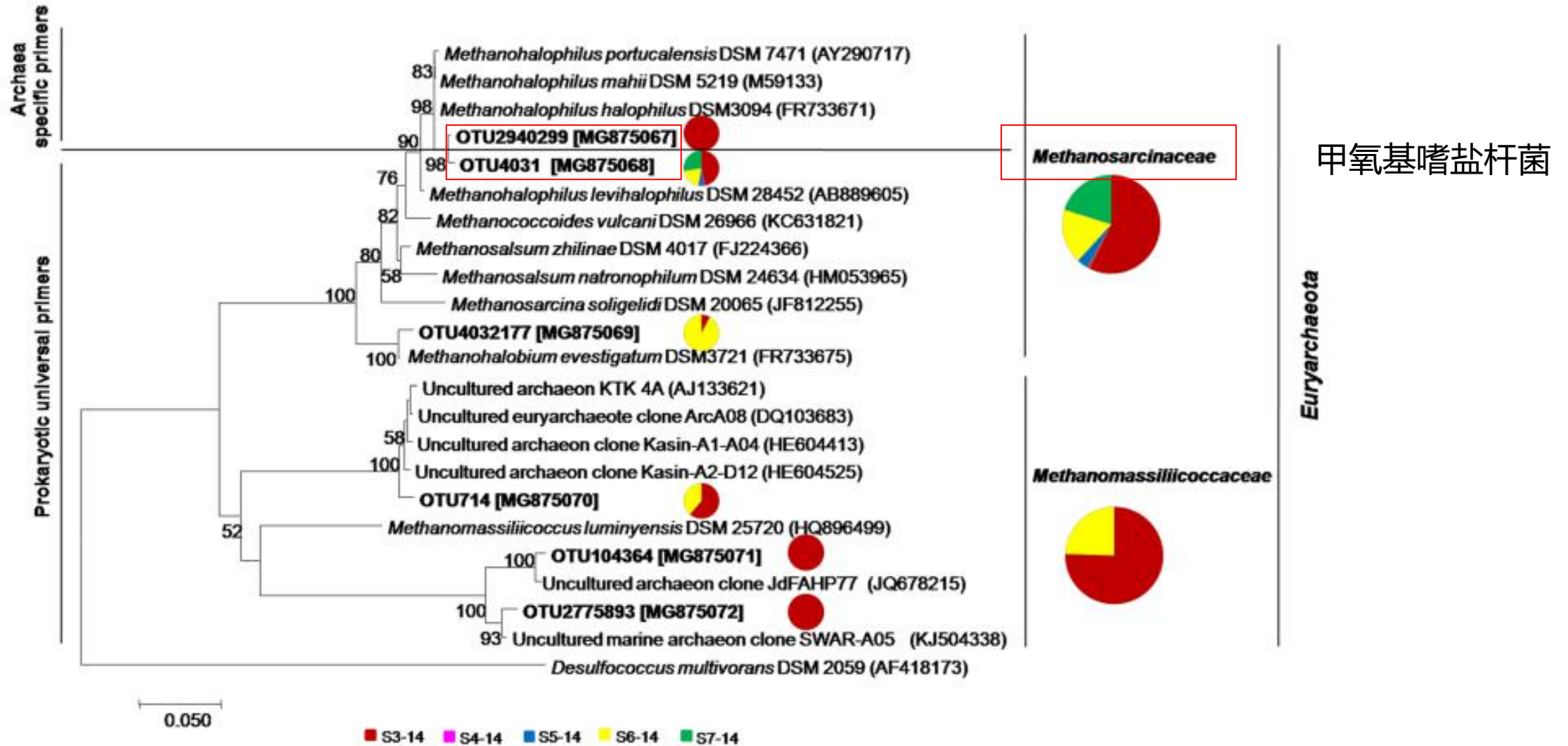
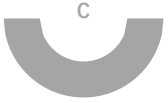


Fig. 3 Phylogenetic tree of the methanogenic archaeal 16S rRNA sequences originated from Chott El Jerid. The tree is based on the neighbor-joining method. The scale bar represents 5% sequence divergence

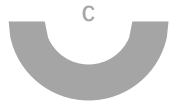




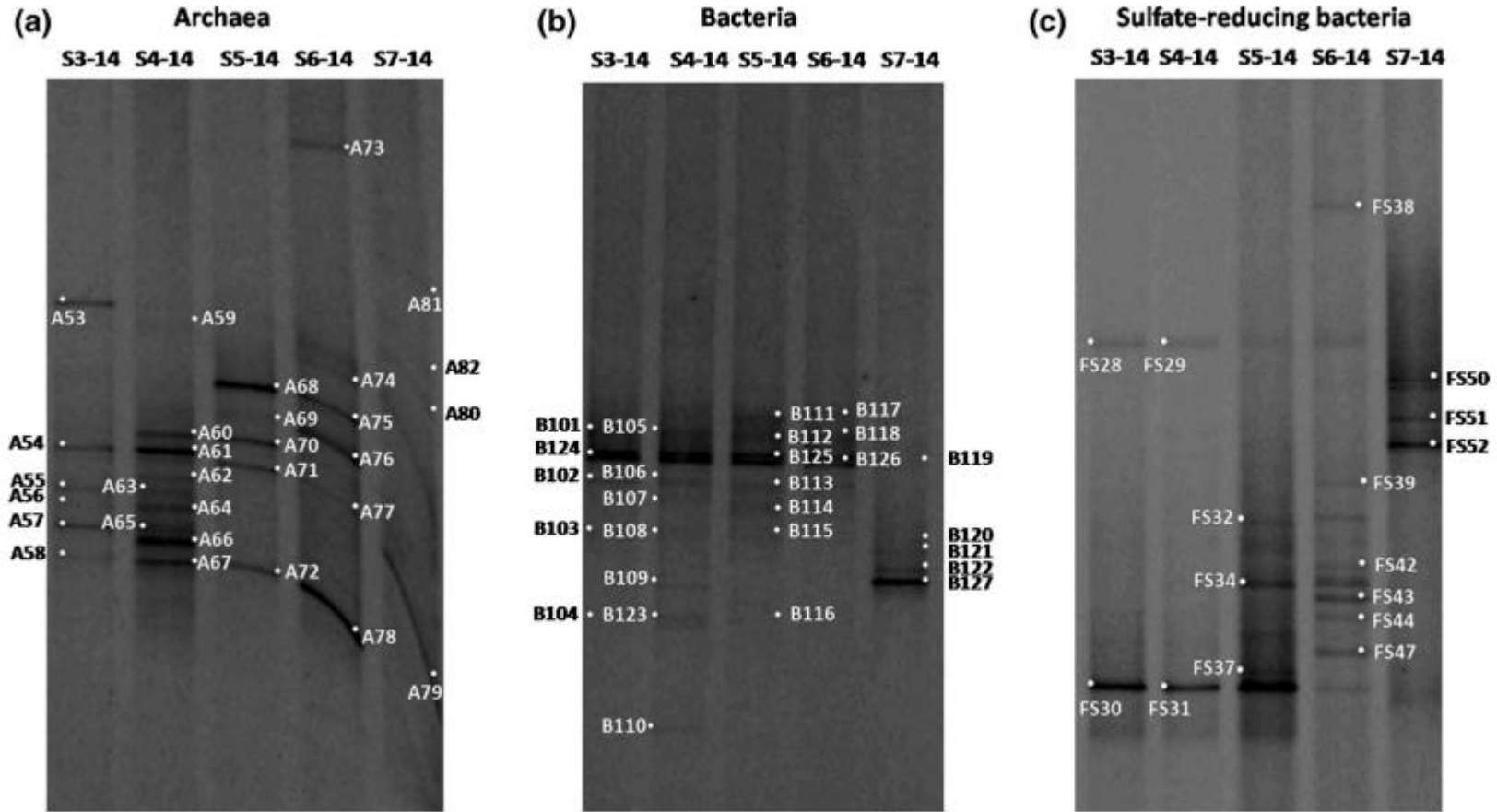
# Illumina Miseq-based analysis using the archaeal 16S rRNA gene-specific primers

**Table 4** Phylogenetic affiliation of the dominant archaeal OTUs obtained from Illumina Miseq sequencing analysis of Chott El Jerid samples targeting the 16S rRNA gene

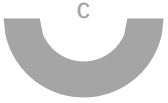
OTU no. [GenBank number]	Sequences per sample (%)					Closest cultivated relatives retrieved from NCBI nucleotide database			
	S3-14	S4-14	S5-14	S6-14	S7-14	Taxonomy (phylum; class; order) (%)	Species (accession number)	Similarity (%)	
304972 [MG875054]	0.03	0	0.01	0	27.21	<i>Euryarchaeota; Halobacteria; Halobacteriales</i>	<i>Halovenus salina</i> JCM 30072 (KJ661744)	95	盐红菌属
609811 [MG875055]	42.80	0	0.01	0.03	15.92	<i>Euryarchaeota; Halobacteria; Halobacteriales</i>	<i>Halovenus rubra</i> JCM 17269 (HM159605)	95	
240020 [MG875056]	0.01	0	34.22	0.01	0.02	<i>Euryarchaeota; Halobacteria; Halobacteriales</i>	<i>Haloarcula quadrata</i> JCM 11048 (AB010964)	99	
132088 [MG875057]	0	0	0	5.75	0	<i>Euryarchaeota; Halobacteria; Halobacteriales</i>	<i>Haloarcula marismortui</i> JCM 8966 (EF645693)	99	盐盒菌属
563669 [MG875058]	0	0	0	1.66	0	<i>Euryarchaeota; Halobacteria; Halobacteriales</i>	<i>Haloarcula marismortui</i> JCM 8966 (EF645693)	99	
559445 [MG875059]	1.06	0	0	0	0	<i>Euryarchaeota; Halobacteria; Haloferacales</i>	<i>Halohasta litorea</i> JCM 17270 (HM159607)	96	仅在样本S3-14中检测到
574209 [MG875060]	22.64	58.66	31.69	87.56	32.9	<i>Euryarchaeota; Halobacteria; Haloferacales</i>	<i>Halorubrum ezzemoulense</i> DSM 17463 (DQ118426)	99	伊兹梅尔盐红菌
553907 [MG875061]	1.05	2.19	1.47	3.52	1.44	<i>Euryarchaeota; Halobacteria; Haloferacales</i>	<i>Halorubrum coriense</i> DSM 10284 (L00922)	99	盐红菌属
551685 [MG875062]	10.76	0.01	0	0.01	0.01	<i>Euryarchaeota; Halobacteria; Natrionalbales</i>	<i>Haloterrigena jeotgali</i> DSM 18794 (EF077633)	99	
548723 [MG875063]	8.33	0.02	0.04	0.02	0.01	<i>Euryarchaeota; Halobacteria; Natrionalbales</i>	<i>Haloterrigena jeotgali</i> DSM 18794 (EF077633)	99	盐陆生菌属
4999 [MG875064]	1.05	0	0	0.01	0	<i>Euryarchaeota; Halobacteria; Natrionalbales</i>	<i>Haloterrigena jeotgali</i> DSM 18794 (EF077633)	98	
74122 [MG875065]	8.37	25.05	12.77	0.03	17.67	<i>Euryarchaeota; Halobacteria; Natrionalbales</i>	<i>Natrinema altunense</i> JCM 12890 (AY208972)	99	钠线菌属
3599 [MG875066]	1.43	11.13	17.39	0.03	1.33	<i>Euryarchaeota; Halobacteria; Natrionalbales</i>	<i>Natrinema altunense</i> JCM 12890 (AY208972)	99	



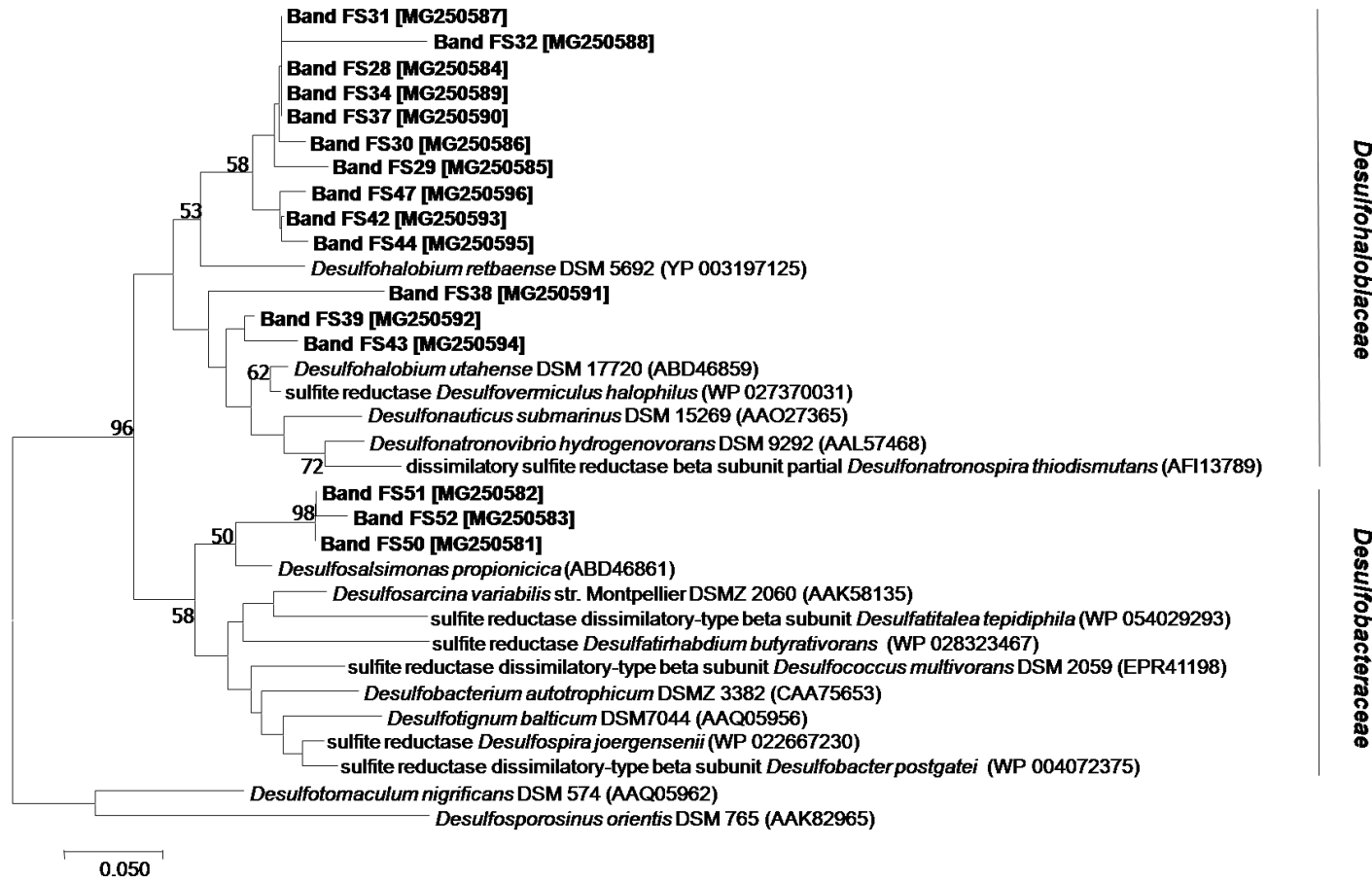
# DGGE-based analysis of archaeal and bacterial 16SrRNA gene



**Fig. 4** Denaturing gradient gel electrophoresis profiles of PCR-amplified archaeal 16S rRNA (a), bacterial 16S rRNA (b) and *dsrB* (c) gene fragments obtained from all samples analyzed



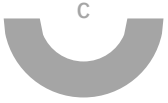
# Diversity of sulfate-reducing prokaryotes



硫代卤木科

杂硫杆菌科

Fig. S1 Phylogenetic tree based on amino acid sequences of the *dsrB* gene. The DGGE band number preceded by the fresh season (FS). Sequences of approximately 350 bp length were used to construct the tree. An out-group of the DsrB proteins of *Desulfotomaculum nigrificans* and *Desulfosporosinus orientis* was included to root the tree. Bootstrap values > 50% are indicated at nodes.



# Quantification of 16S rRNA and functional genes

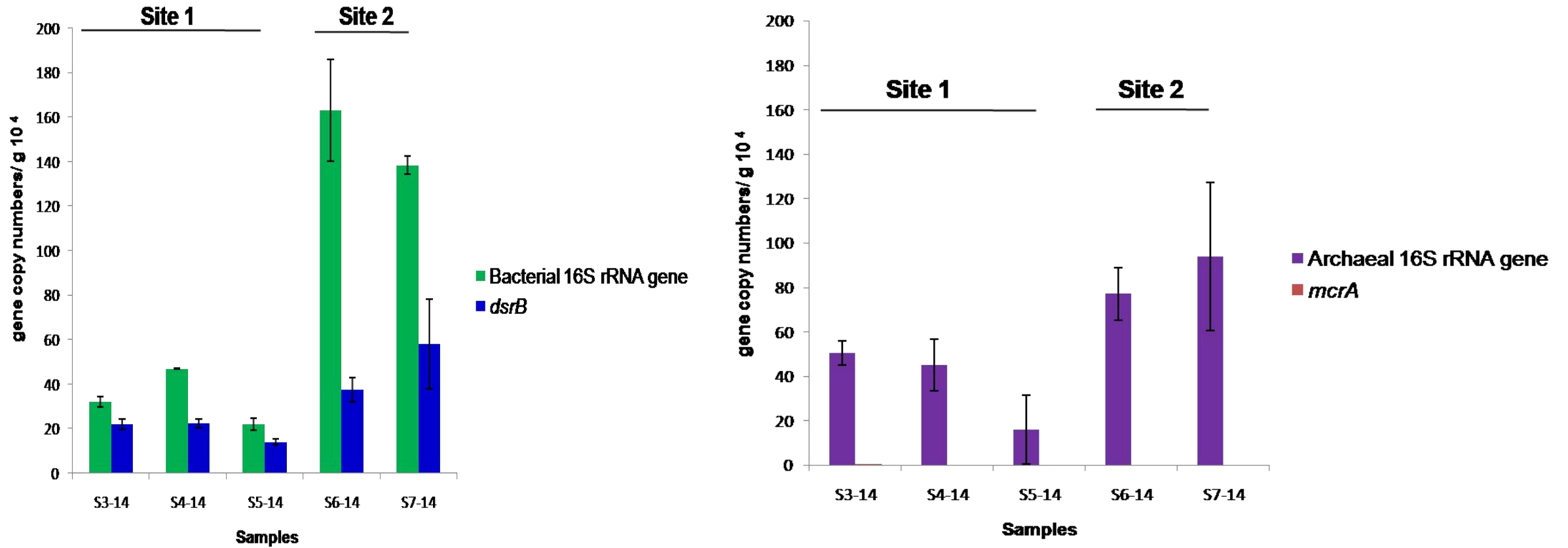
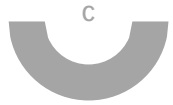
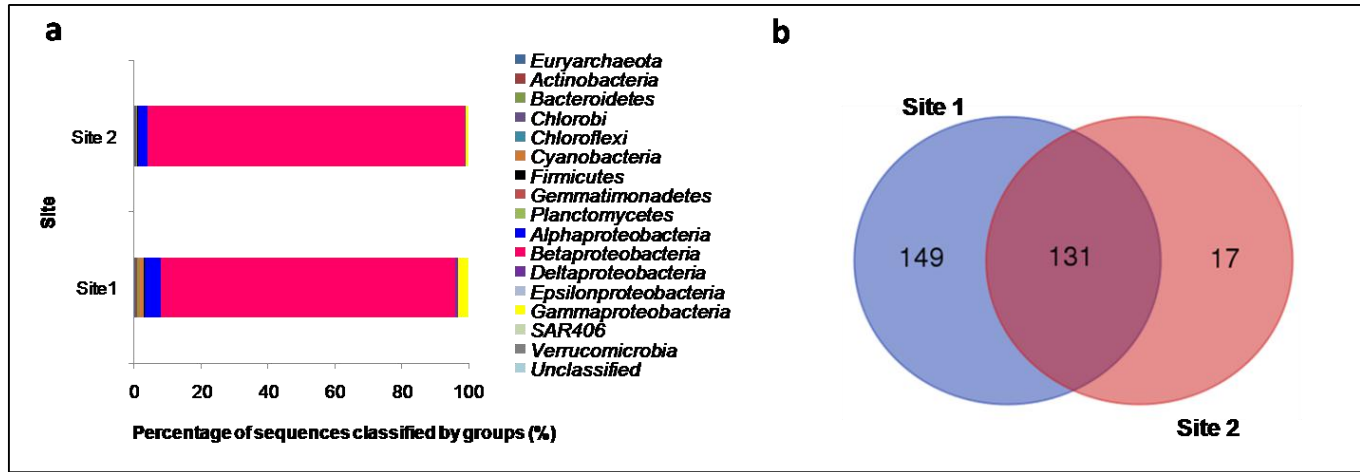


Fig. S2 Abundance of the total Bacteria, total Archaea, sulfate-reducing Bacteria and methanogenic Archaea determined by qPCR in the samples taken from Chott El Jerid.



# Microbial community abundance and structure between sites

## Prokaryote 16S rRNA gene- universal primers



## Archaea 16S rRNA gene- specific primers

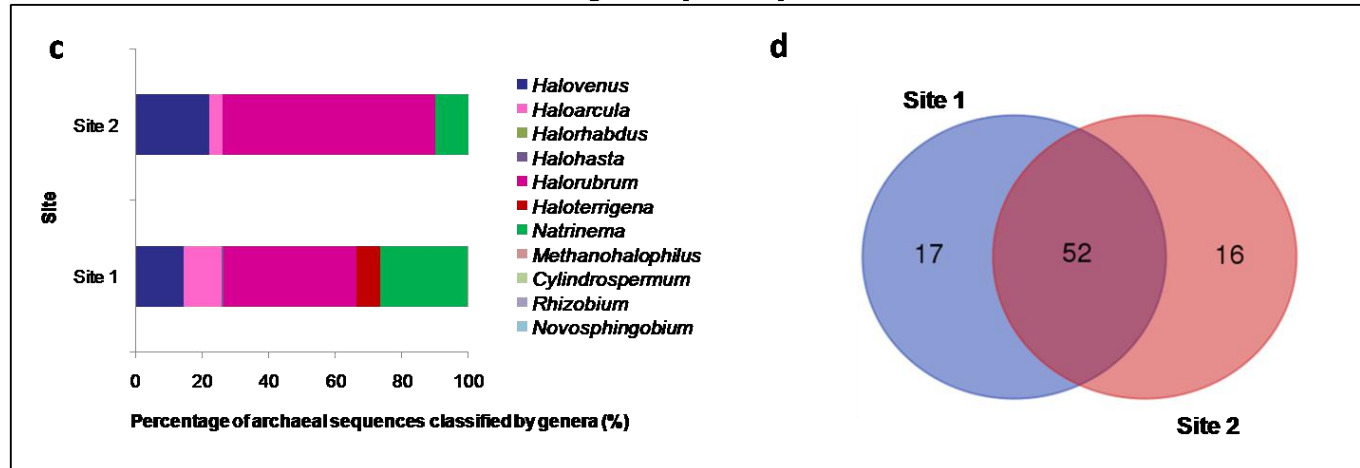
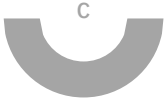


Fig. S3 Comparison of prokaryotic (a) and archaeal (c) communities obtained by Illumina Miseq sequencing. Venn diagrams demonstrating the numbers of unique and shared OTUs (3% distance level) of the samples studied. Site 1 represents S3-14; S4-14; S5-14 samples; Site 2 represents S6-14; S7-14. (b) Average OTU of prokaryotic sequences ; (d) Average OTU of archaeal sequences



# Microbial community abundance and structure between sites

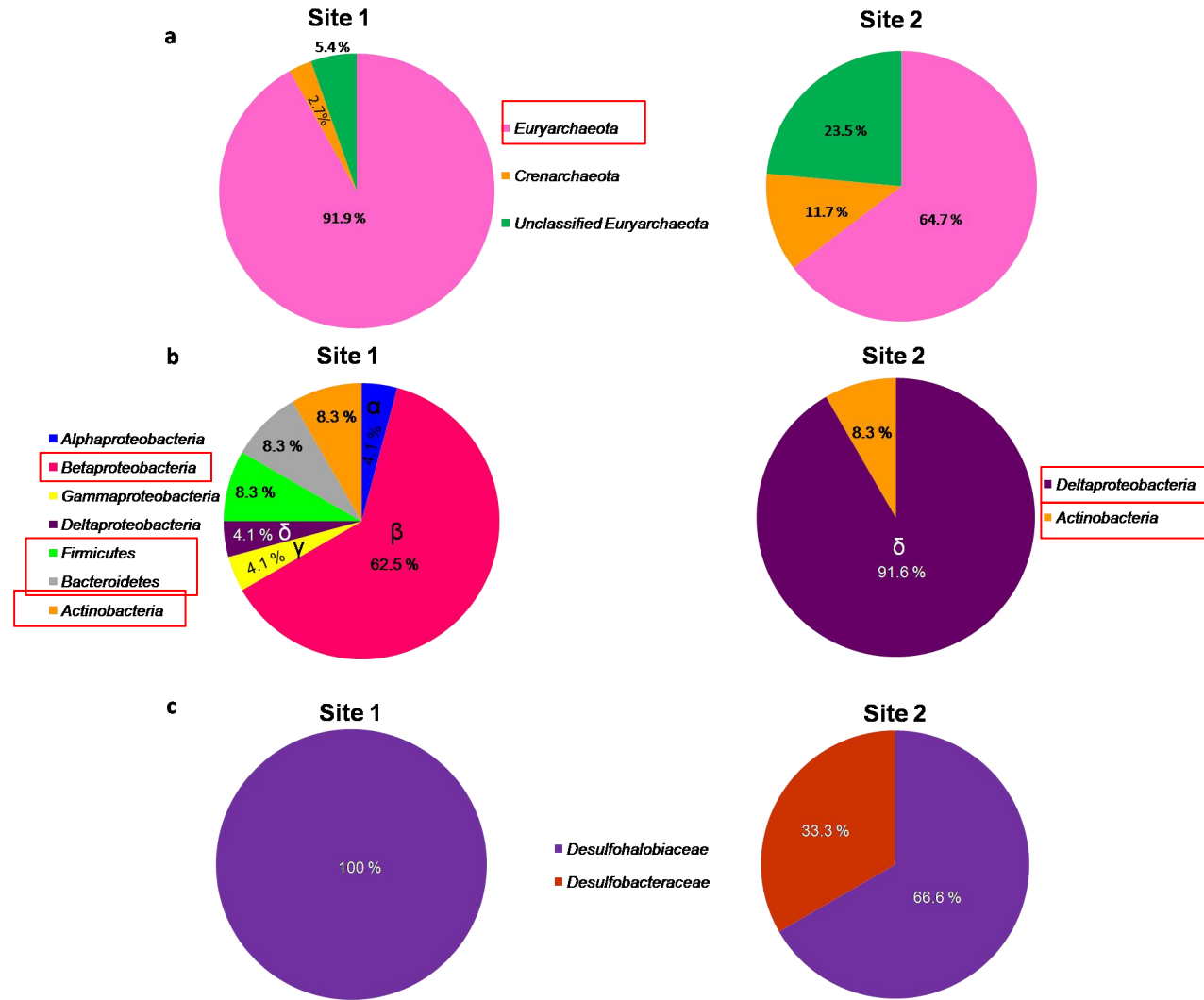


Fig. S4 Comparison of taxonomic archaeal (a); bacterial (b) profiles using 16S rRNA sequences and sulfate-reducing bacterial profiles using *dsrB* sequences (c) obtained by DGGE. The percentage was relative to the total number of sequences.



# PART 04

## Conclusion



## Conclusion



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本研究提供了有关栖息在高盐季节性湖泊Chott El Jerid的原核生物群落的有价值的信息。

使用Illumina Miseq的现代测序和传统的DGGE克隆来研究雨季期间的原核微生物。结果显示，在雨季期间，*betaproteobacterial* (*Ralstonia*物种) 和 *halobacterial* (*Halorubrum*物种) 分别是细菌和古细菌的主要组成部分。

在雨季期间也检测到厌氧群落，如产甲烷种群和硫酸盐还原菌，这些类型的嗜盐性群落的分离及其在不久的将来对生物技术应用的影响还有待研究。

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