Indian Journal of Geo Marine Sciences
Vol. 51 (02), February 2022, pp. 152-160

Bacterial and archaeal communities in near-surface marine sediments from İzmir Bay (Eastern Aegean Sea), using Quantitative PCR

A Kacar[a,*] & B Omuzbuken[b]

[a]Dokuz Eylul University, Institute of Marine Sciences and Technology, Inciralti-İzmir – 35340, Turkey

[b]Dokuz Eylul University, The Graduate School of Natural and Applied Sciences, İzmir – 35390, Turkey

*[E-mail: asli.kacar@deu.edu.tr]

Received 02 February 2021; revised 27 January 2022

Marine sediments are good sources of both subsistent and anthropogenic ingredients. Recently, an assessment of phylogenetic analysis at the genotypic and molecular levels has revealed the phylogenetic relationships of the prokaryotes. In our study, samples were collected from the surface of sediments in the İzmir Bay, located in the eastern Aegean Sea and surrounded by the coastal city of İzmir. Total genomic DNA was extracted from 0.25 g sediment of each sample and used in real-time qPCR to detect 16S rDNA genes of archaea, bacteria, sulfate-reducing bacteria, *Desulfobulbus*, and *Desulfosarcina-Desulfococcus*. Our results showed that the maximum number of all samples was found in bacterial primers (1.24×10^9 DNA copy numbers/g), while the minimum number was observed in *Desulfosarcina-Desulfococcus* primers (1.17×10^2 DNA copy numbers/g). Consequently, genetic studies should focus on investigating the abundance levels of seasonal and annual microbial groups in future studies.

[**Keywords**: Archaea, Bacteria, *Desulfobulbus*, *Desulfosarcina-Desulfococcus*, Sulfate-reducing bacteria]

Introduction

The subterranean surroundings are considered to be the most extensive biomass storage in nature[1,2]. Marine sediments compose the indigenous habitat for approximately 5.39×10^{29} bacteria[3] and archaea[4]. Until recently, the assessment of microbial diversity was not possible due to the lack of a suitable methodology[5].

Studies on molecular-phylogenetic identity reveal the individualism of population components. Non-cultured tests have greatly expanded the overall phylogenetic diversity of known microbial species[4,5].

Marine sediments are good sources of both subsistent and anthropogenic ingredients[6]. Although microbial activity is characterized by testing the conversion of these ingredients and the impact of the processes in seawater, relatively little is known about the contribution to the population[7]. Comparing diversity with microbial communities can help solve numerous ecological problems. It is essential to complete surveys of changes in the populations of the microbial groups, especially the primary constituent groups, in response to anthropogenic, natural and seasonal changes, as well as to define keystone species[8]. A recent evaluation of the phylogenetic analysis, which provides a large number of recognizable criteria at the genotypic and molecular degree[9], allows determining the phylogenetic affiliation of prokaryotes[10]. Several studies have reported an unexpected abundance or a predominance of microbial communities that were previously unclear or considered relatively unusual[11-13].

Sulfur is one of the abundant elements in nature. It mainly exists as gypsum ($CaSO_4$) or pyrite (FeS_2) in sediments and as sulfate in marine sediments. Microorganisms play a vital role in the conversion of sulfur. Sulfur in the form of sulfate is received by an organism as a nutrient and then is reduced to sulfide, which becomes part of the sulfur-containing amino acids and enzymes[14]. Sulfate reduction is a crucial microbiological process in marine sediments and seriously affects the activity and distribution of Sulfate-Reducing Bacteria (SRB). SRB plays a central role in the biogeochemical cycle of sulfur and carbon in marine sediments[15]. In addition, archaea have long been considered a small part of the microbial communities in marine sediments. Knowledge about the structure and distribution of archaea in marine sediments is limited and mainly focused on “extreme” habitats such as cold seeps and hydrothermal vents[16]. Current research suggests that the abundance of the archaeal community may be equivalent to the plethora

of the bacterial community or even prevail not only in the deep sea waters below the 1000 m but also in underground sediments[17-21].

Quantitative real-time PCR (qPCR) is a highly effective method that can be employed for quantifying microbial communities. Several primer sets were used[22] to amplify 16S rRNA gene fragments obtained from various groups of microorganisms belonging to the genera *Desulfobulbus*, *Desulfobacter*, *Desulfovibrio*, and *Desulfosarcina–Desulfococcus*[14].

This study aims to obtain more information about the number of microbial communities on the seabed. To investigate this, we created an SYBR Green-based qPCR assay to predict the number of cells of archaea, bacteria, sulfate-reducing bacteria, *Desulfobulbus*, and *Desulfosarcina-Desulfococcus* present in marine sediments. Our research is the first study of quantitative molecular phylogenetic analysis determined by using the microbial primer sets of marine sediments in İzmir Bay.

Materials and Methods

Site description and sampling

İzmir Bay is located in the eastern part of the Aegean Sea (Turkey) and is surrounded by the coastal city of İzmir. The physical characteristics of the İzmir Bay area are described by a seawater column with a volume of 11.5 billion m^3, a surface area of more than 500 km^2, and a coastline length of 64 km. It is divided into inner and mid-outer, in addition to being open to the Aegean Sea from the northwest side. The depth of the water column in the mid-outer part ranges from 20 to 70 m and decreases towards the inner part. The inner part contains numerous industrial and domestic wastewater discharge points from the city of İzmir compared to the other parts of the bay[23]. The water circulation in İzmir Bay changes according to the prevailing winds[24]. Tides (range from 20 to 50 cm) are not expected to cause currents strong enough to strongly mix the various water masses in İzmir Bay[25]. There are three different water masses in the bay; Aegean Sea water, İzmir Bay water and İzmir Bay inner water. Usually, Aegean Sea water (vertically homogenous) enters the bay from the north (near Karaburun in winter), and in summer, horizontally homogeneous inlets of the Aegean Sea occur above the pycnocline along an entire vertical section of the Foça-Karaburun. The outflow consists of a subsurface and a bottom layer near the Foça in winter, while in summer, it flows out under the pycnocline[26].

The sediment samples were collected from sediment surfaces using a Van Veen Grab Sampler from various parts of the İzmir Bay during a cruise on the R/V Koca Piri Reis in 2017. Samples range from 0 – 2 cm below the seabed with 15 sampling stations (Fig. 1).

DNA extraction

Total genomic DNA was extracted from 0.25 g sediment of each sample using the DNeasy PowerSoil DNA Isolation (Qiagen) kit according to the protocol supplied with some minor modifications. Extracted genomic DNA samples were stored at -20 °C. The concentration of total genomic DNA was determined by A260nm/A280nm ratio measurements using a Take3 plate with Synergy HTX- multimode reader (BioTek Instruments, Inc).

Selection of primer sets

Selection criteria were used for individual primers, which are characterized by high domain specificity and the ability to bind to a large number of accepted 16S rRNA genes. The characteristics of primer groups such as Archaea (ARC), Bacteria (BAC), Sulfate-Reducing Bacteria (SRB2), *Desulfobulbus* (DBB), and *Desulfosarcina-Desulfococcus* (DSS) which were used in the sampling area represented in this study (Table 1).

Real-time qPCR

The real-time qPCR was performed using the LightCycler 480 SYBR Green I Master Kit (Roche). Reactions were run on a LightCycler 96 (Roche) Q-PCR machine. Extracted genomic DNA was used in real-time qPCR to indicate 16S rRNA genes from archaea, bacteria, sulfate-reducing bacteria, *Desulfobulbus*, and *Desulfosarcina-Desulfococcus*.

16S rRNA genes of archaea, bacteria, sulfate-reducing bacteria, *Desulfobulbus*, and *Desulfosarcina-Desulfococcus* were amplified using primers ARC, BAC, SRB2, DBB and DSS (Table 1), respectively, with the following thermal program: pre-incubation 95 °C for 300 s, 45 cycles of 3 step amplification; denaturing (10 s at 95 °C), annealing (15 s at 50 °C), extension (15 s at 72 °C), 1 cycle of melting; denaturing (5 s at 95 °C), annealing (60 s at 65 °C), extension (1 s at 97 °C) and 1 cycle of cooling (10 s at 40 °C). A tenfold dilution series of known genomic DNA was used to constitute a standard curve. The calculations were performed using the LightCycler 96 (Roche) software.

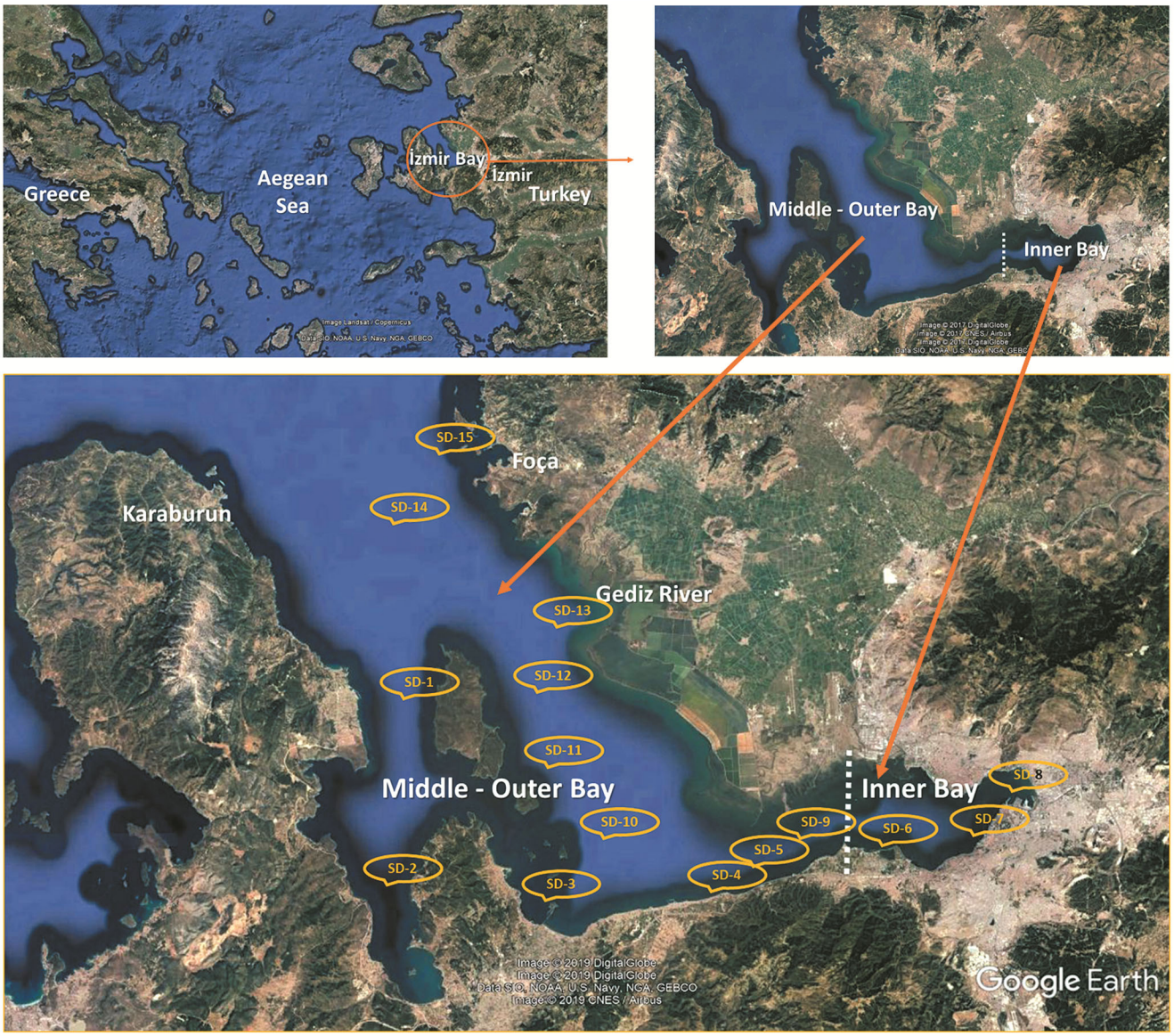

Fig. 1 — Location of İzmir Bay and sampling stations

Results

Abundance of bacterial and archaeal groups

The depth, types of surficial sediment and coordinates of stations at which the total genomic DNA was obtained are shown in Table 2. The average value of genomic DNA for all sediment samples is 102.5 ng/g dry sediment. The maximum value was estimated as 146.05 ng/g at station SD-9 located in the middle part of İzmir Bay. The minimum value was determined as 62.02 ng/g at station SD-14 located in the outer part of İzmir Bay. The depths of the stations (from the sea surface to bottom sediment) vary from 11.0 m to 70.0 m.

Real-time qPCR

As shown in Table 3, the maximum abundance of 16S rRNA gene of archaeal, bacterial, sulfate-reducing bacteria, *Desulfobulbus* group, and *Desulfosarcinales/Desulfococcales* group in sediments ranges from 1.24×10^{9} to 1.17×10^{2} DNA copy numbers/g dry sediment for qPCR. In addition, the quantitative values of all sediment samples obtained from real-time PCR are shown in Figures 2 – 4.

According to the results, microbial groups of ARC (4.83×10^{8} DNA copy numbers/g), BAC (1.24×10^{9} DNA copy numbers/g), DBB (9.82×10^{6} DNA copy numbers/g) and DSS (2.65×10^{7} DNA copy numbers/g) are the largest in number in SD-8 station,

Table 1 — The designed primers for extracted DNA samples of marine sediments

No	Name	Target group	Function	Sequence (5 → 3')	Amplicon size (bp)	Annealing temperature	References
1	ARC - 787F	Archaea	Q-PCR - Forward primer	ATT-AGA-TAC-CCS-BGT-AGT-CC	273	50.0 °C	27, 28
2	ARC - 1059R	Archaea	Q-PCR - Reverse primer	GCC-ATG-CAC-CWC-CTC-T			
3	BACT - 1369F	Bacteria	Q-PCR - Forward primer	CGG-TGA-ATA-CGT-TCY-CGG	142	50.0 °C	28, 29
4	BACT - 1492R	Bacteria	Q-PCR - Reverse primer	GGW-TAC-CTT-GTT-ACG-ACT-T			
5	SRB2 - 649F	SEEP-SRB2	Q-PCR - Forward primer	ACT-TGA-GTA-CCG-GAG-AGG-GA	180	53.0 °C	30
6	SRB2 - 808R	SEEP-SRB2	Q-PCR - Reverse primer	CCT-AGT-GCC-CAT-CGT-TTA-GG			
7	DBB - 649F	*Desulfobulbus* group	Q-PCR - Forward primer	GCT-TGA-GTA-TGG-GAG-GGG-A	180	53.0 °C	31
8	DBB - 808R	*Desulfobulbus* group	Q-PCR - Reverse primer	CAC-CTA-GTT-CTC-ATC-GTT-TAC-AGC			
9	DSS - 649F	Desulfosarcinales/ Desulfococcales group	Q-PCR - Forward primer	ACT-TGA-GTA-TGG-GAG-AGG-GAA-G	180	53.0 °C	31
10	DSS - 808R	Desulfosarcinales/ Desulfococcales group	Q-PCR - Reverse primer	ACC-TAG-TGT-TCA-CCG-TTT-ACT-GC			

Table 2 — The depth, types of surficial sediment and coordinate of sampling stations

Station Name	Depth of water column (m)	Types of surficial sediment (Duman *et al.*[25])
SD-1	39.5	Sandy Silt
SD-2	30.3	Sandy Silt
SD-3	26.3	Sandy Silt
SD-4	29.5	Silt
SD-5	30.3	Silt
SD-6	11.0	Sandy Silt
SD-7	17.4	Sandy Silt
SD-8	11.0	Sandy Silt
SD-9	23.9	Silt
SD-10	55.0	Sandy Silt
SD-11	52.5	Silt
SD-12	40.5	Silt
SD-13	38.0	Silt
SD-14	70.0	Silt
SD-15	58.0	Mud

Table 3 — The maximum abundance of archaeal, bacterial, sulfate-reducing bacteria, *Desulfobulbus* group, and *Desulfosarcinales/ Desulfococcales* group

Primers	Minimum	Maximum	Mean	Coefficient of variation
ARC	1.35×10^5	4.83×10^8	4.30×10^7	288.04
BAC	7.77×10^6	1.24×10^9	2.62×10^8	113.78
SRB2	4.30×10^2	2.06×10^7	1.82×10^6	290.40
DBB	7.31×10^2	9.82×10^6	1.70×10^6	144.83
DSS	1.17×10^2	2.65×10^7	4.54×10^6	147.70

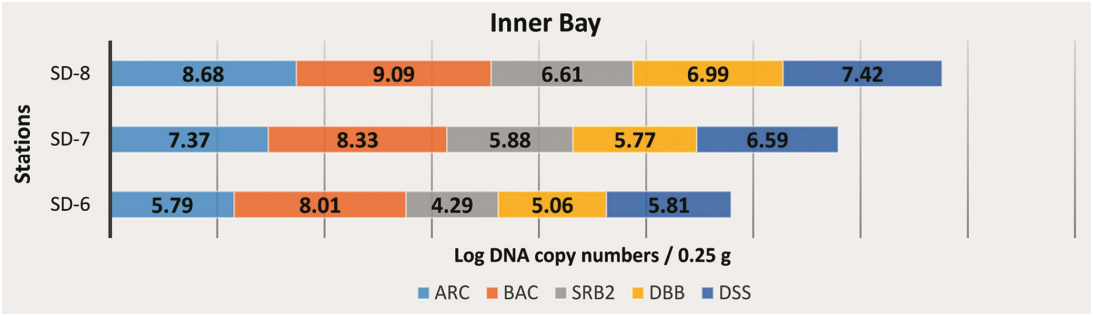

Fig. 2 — DNA copy numbers of Archaea, Bacteria, Sulfate-Reducing Bacteria, *Desulfobulbus* and *Desulfosarcina-Desulfococcus* groups in the inner bay

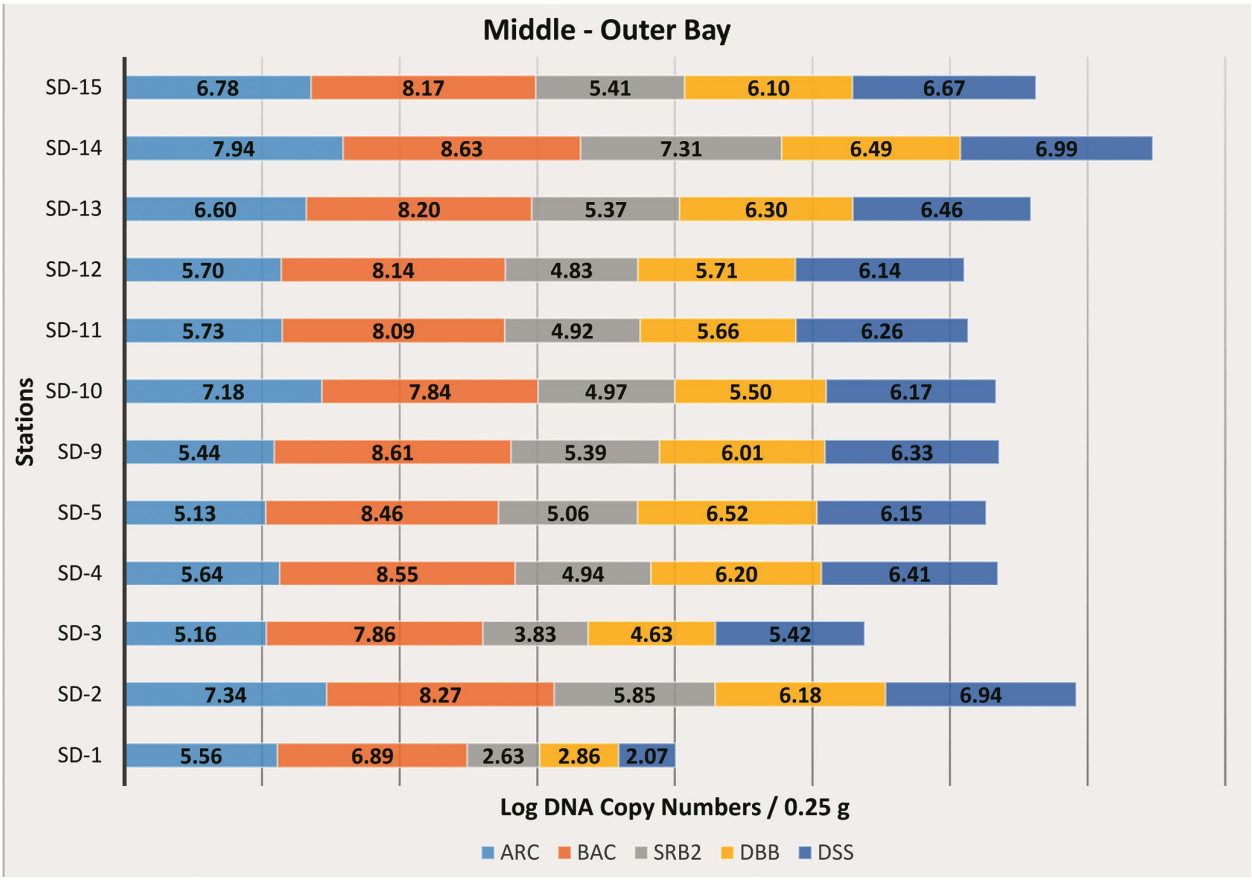

Fig. 3 — DNA copy numbers of Archaea, Bacteria, Sulfate-Reducing Bacteria, *Desulfobulbus* and *Desulfosarcina-Desulfococcus* groups in middle-outer Bay

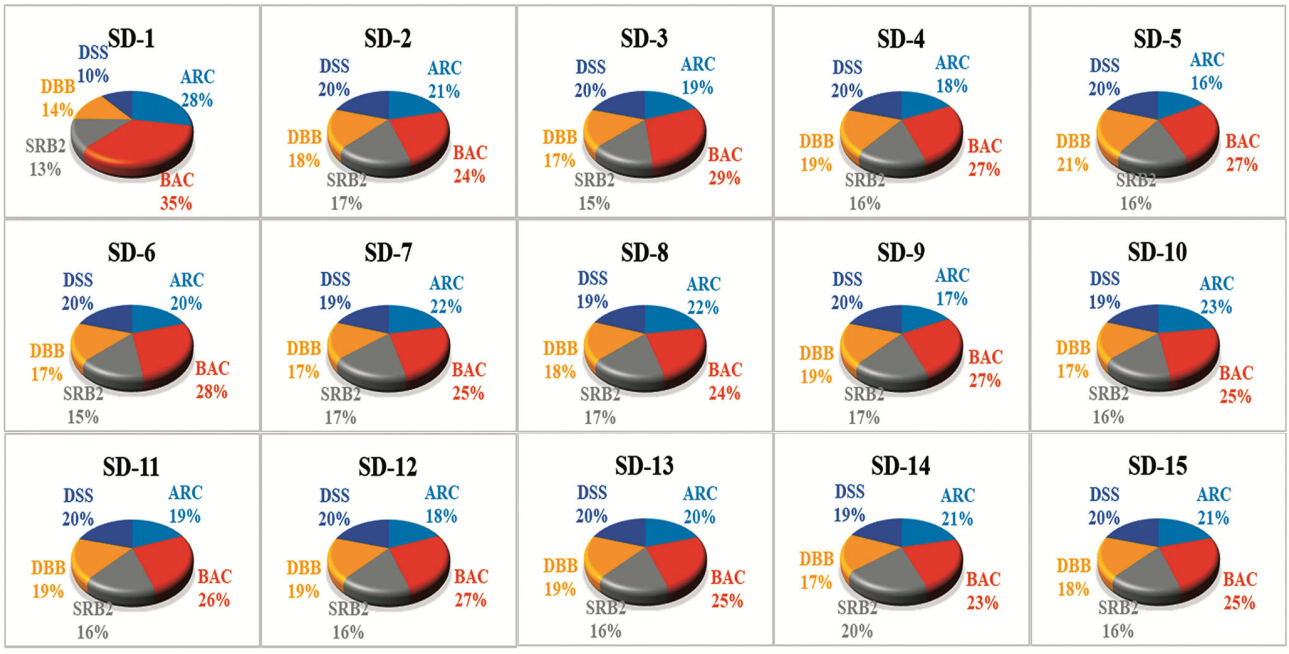

Fig. 4 — Percentages of Archaea, Bacteria, Sulfate-Reducing Bacteria, *Desulfobulbus* and *Desulfosarcina-Desulfococcus* groups from all stations

which is located in the inner bay with abundant organic pollutants. This station is located in the harbour of İzmir and it is known that the sediment pollutants are constantly being added, such as wastewater discharge. The SRB2 group primers still have high results (4.06×10^6 DNA copy numbers/g) at the SD-8 station, whereas the highest number (2.06×10^7 DNA copy numbers/g) of SRB2 groups was recorded for the SD-14 station. It is assumed that these are intense sulfate-reducing bacterial groups because the water masses from the bay pass through the station. SD-1 station, where BAC (7.77×10^6 DNA copy numbers/g), SRB2 (4.30×10^2 DNA copy numbers/g), DBB (7.31×10^2 DNA copy numbers/g) and DSS (1.17×10^2 DNA copy numbers/g) groups are determined with the lowest density, at the SD-1 station sediments are exposed to the clean water of the Aegean Sea from the north of the bay. Therefore, it is expected that microbial abundance in the sediment could be lower. This station expresses low results for ARC (3.67×10^5 DNA copy numbers/g) however, the lowest archaeal abundance (1.35×10^5 DNA copy numbers/g) was detected at the SD-5 station. The results show that pollution, especially in the inner part of the İzmir Bay, is caused by the discharge of domestic wastewater and untreated human and animal waste continuously.

Discussion

Characterization of microbial diversity models is imperative for understanding the biological foundations of an ecosystem. This is especially critical for coastal microbiological communities since they play a vital role in the arrangement of biogeochemical cycling at the sediment interface[32-34]. Sediment microorganisms are the keystone in organic depletion, immobilization of heavy metals and nutrient cycling[35]. Contamination with heavy metals and nutrients was found to cause changes in bacterial diversity, function and biomass[35-37]. Accordingly, it was necessary to determine the spatial and temporal changes in microbiological diversity and knowledge of the relationship with the biogeochemical cycle as well[34,38,39].

Marine sediments contain most of all prokaryotes in nature. However, the phylogenetic estimate of the abundance of microbial groups for deep and shallow marine environments remains a mystery. Our study focused on additional information on the number of microbial communities in marine sediments of a coastal city that is heavily polluted. The abundance of microbial communities was estimated by a real-time PCR using samples collected from 0 – 2 cm of the sediment surface. Cell numbers of archaea, bacteria, sulfate-reducing bacteria, *Desulfobulbus*, and *Desulfosarcina-Desulfococcus* were detected by SYBR Green-based qPCR in this study. The results depicted more sediment samples in the inner bay than in the mid-outer bay. The maximum number of all station samples was found in bacterial primers (1.24×10^9 DNA copy numbers/g), while the minimum number was observed in DSS primers (1.17×10^2 DNA copy numbers/g).

The bay has a fine-grained sediment area, and the surface sediments of the İzmir Bay can be divided into seven zones according to the grain size[25]. The outer bay is covered with silty and muddy sand from the western side while the eastern part is buried with silt and mud. The main part of the inner bay is covered with sandy silt, and the middle bay is covered with silt, sand and sandy silt. The surface sediment properties of our sampling stations are shown in Table 2. Of the seven sediment types, six are sandy silt, and one is silt and mud.

Vigneron *et al.*[28] published their study on the primers of archaea and bacteria and the primers of *Desulfobulbus*, and *Desulfosarcina-Desulfococcus*[31] with sediment push core samples in cold seep sediments. They collected sediment samples from the Sonora Margin, Gulf of California (USA). Archaeal and bacterial abundance results, by q-PCR, were established in two sediment layers (0 – 2 cmbsf); Bacteria (~ 5×10^8 and ~ 9×10^9 copies per g of sediment) were nearly 20-fold more numerous than archaea. Moreover, 16S rDNA copies per gram never exceeded 2.2×10^9 and 2.9×10^8 for bacteria and archaea in the entire sediment core at investigated stations, respectively. The abundance of DBB, DSS, SEEP SRB-2 was measured every 2 cm from the water-sediment interface to 15 cmbsf (core length). DSS concentrations were more abundant copies of SRB-associated 16S rDNA in sediment samples (4.52×10^9 16S rDNA copies/g) where sulfate concentrations were high. They indicated that the abundance of DSS, DBB and SEEP SRB2 decreased with increasing core depth. The primers used in their study were identical in sequence and amplicon length to our ARC, BAC, DSS and DBB primers. Marine sediments of the Peruvian continental margin

analyzed by real-time PCR were identified as a comparative quantitative microbial community[19]. This study was conducted in shallow and deeply buried sediments, and real-time qPCR was used to quantify various groups of microbial primers. Copy number of 16S rRNA gene for prokaryotes, bacteria, Geobacteraceae and sulfate-reducing prokaryotes (dissimilatory (bi)sulfite reductase gene - dsrA) was also investigated. This study shows that the number of all genes in the sediment decreased with increasing depth. The gene numbers of prokaryotes and bacteria showed similar results with a maximum of $10^8 – 10^{10}$ cm^{-3} gene copies in shallow sediments. The number of copies of the genes of Geobacteraceae and the sulfate-reducing prokaryotes was $10^6 – 10^8$ cm^{-3} gene copies in the sediments. Unlike the present study, Schippers and Neretin[19] examined sulfate-reducing bacteria and common bacteria according to different genes, besides the abundance of Geobacteraceae and prokaryotes. The results of our study were similar in the number of DNA copies of sulfate-reducing bacteria and common bacteria[19].

SYBR Green real-time q-PCR analysis was performed by Einen *et al.*[40] to quantify and detect archaea and bacteria existing in the glass shell of seabed basalts of different ages and water depths. Results of this study showed that the total number of cells present in the basalt ranged from 6×10^5 to 4×10^6 cells per gram of basalt glass. The samples were taken from different ages and water depths, however, no significant differences were found in the concentrations or relative abundance of archaea and bacteria. In contrast to our study, Einen *et al.*[40] worked with common primers for archaea and bacteria, and the study was based on the number of microbes on the surface of basalts. The present study revealed more differences in the number of microbial communities due to the fact that they were collected directly from the surface of sediment in İzmir Bay.

Conclusion

Sulfate is abundant in coastal marine sediments, SRB may contribute 50 % of the mineralization of organic matter[41,42]. Sulfate reduction plays a significant role in the modification of Dissolved Organic Matter (DOM) in coastal and estuarine sediments[43]. Thus, the primers DSS, DBB and SRB2 used in our study indicate the presence of sulfate-reducing bacterial groups in the sampling area. In addition, the abundance levels of archaea and bacteria were determined, and the results showed that the number of microbial groups can not be cultured. It is suggested that genetic studies should focus on the abundance levels of seasonal and annual microbial groups in future studies.

Acknowledgements

The authors would like to thank the scientist and crew of the R/V Koca Piri Reis during the cruises. Thanks are also due to Dr Lamiya Abasova and Dr Christopher Sorlien for editing the English grammar of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

The authors conceived the analysis together. AK and BO collected the sediment samples, performed the analysis, and drated the paper.

References

1 Whitman W B, Coleman D C & Wiebe W J, Prokaryotes: the unseen majority, *Proc Natl Acad Sci*, 95 (1998) 6578-6583. doi: 10.1073/pnas.95.12.6578

2 Inagaki F, Suzuki M K, Takai H, Oida T, Sakamoto K, *et al.*, Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk, *Appl Environ Microb*, 69 (12) (2003) 7224-7235. doi: 10.1128/AEM.69.12.7224–7235.2003

3 Parkes R J, Cragg B, Roussel E, Webster G, Weightman A, *et al.*, A review of prokaryotic populations and processes in sub-seafloor sediments, including biosphere: geosphere interactions, *Mar Geol*, 352 (2014) 409-425. doi:10.1016/j.margeo.2014.02.009

4 DeLong E F & Pace N R, Environmental diversity of bacteria and archaea, *Syst Biol*, 50 (4) (2001) 470-478.

5 Pace N R, A molecular view of microbial diversity and the biosphere, *Science*, 276 (1997) 734-740. doi: 10.1126/science.276.5313.734

6 Capone D G & Kiene R P, Comparison of microbial dynamics in marine and freshwater sediments: contrasts in anaerobic carbon catabolism, *Limnol Oceanogr*, 33 (1988) 725-749. doi:10.4319/lo.1988.33.4part2.0725

7 MacGregor B J, Moser D P, Alm E W, Nealson K H & Stahl D A, Crenarchaeota in Lake Michigan sediment, *Appl Environ Microbiol*, 63 (3) (1997) 1178-1181.

8 Daily G, *Nature's services—societal dependence on natural ecosystems*, (Island Press: Washington, D.C.), 1997, pp. 37–38.

9 Ludwig W & Schleifer K H, Bacterial phylogeny based on 16s and 23s rRNA sequence analysis, *FEMS Microbiol Rev*, 15 (1994) 155-173. doi:10.1111/j.1574-6976.1994.tb00132.x

10 Manz W, Amann R, Ludwig W, Vancanneyt M & Schleifer K H, Application of a suite of 16s rRNA-specific oligonucleotide probes designed to investigate bacteria of the

phylum cytophaga-flavobacter-bacteroides in the natural environment, *Microbiology*, 142 (1996) 1097-1106.

11 Fuhrman J A & Davis A A, Widespread Archaea and novel Bacteria from the deep sea as shown by 16S rRNA gene sequences, *Mar Ecol Prog Ser*, 150 (1997) 275-285.

12 Massana R, DeLong E F & Pedro A C, A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces, *Appl Environ Microbiol*, 66 (2000) 1777-1787. doi:10.1128/aem.66.5.1777-1787.2000

13 Kemp P F & Aller J Y, Bacterial diversity in aquatic and other environments: what 16S rDNA libraries can tell us, *FEMS Microbiol Ecol*, 47 (2004) 161-177. doi:10.1016/S0168-6496(03)00257-5

14 Muyzer G & Stams A J M, The ecology and biotechnology of sulphate-reducing bacteria, *Nat Rev Microbiol*, 6 (2008) 441-454. doi:10.1038/nrmicro1892

15 Kacar A, Aliağa (İzmir) denizel ortamındaki sülfat indirgeyen bakterilerin dağılımının en-muhtemel-sayı metodu (EMS) ile araştırılması (in Turkish), *Maku Febed*, 5 (2) (2014) 5-12.

16 Jørgensen B B & Boetius A, Feast and famine—microbial life in the deep-sea bed, *Nat Rev Microbiol*, 5 (2007) 770-781. doi:10.1038/nrmicro1745

17 Karner M B, DeLong E F & Karl D M, Archaeal dominance in the mesopelagic zone of the Pacific Ocean, *Nature*, 409 (2001) 507–510. doi:10.1038/35054051

18 Biddle J F, Lipp J S, Lever M A, Lloyd K G, Sørensen K B, *et al.*, Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru, *Proc Natl Acad Sci USA*, 103 (2006) 3846-3851. doi:10.1073/pnas.0600035103

19 Schippers A & Neretin L N, Quantification of microbial communities in near-surface and deeply buried marine sediments on the Peru continental margin using real-time PCR, *Environ Microbiol*, 8 (7) (2006) 1251-1260. doi:10.1111/j.1462-2920.2006.01019.x

20 Lipp J S, Morono Y, Inagaki F & Hinrichs K U, Significant contribution of Archaea to extant biomass in marine subsurface sediments, *Nature*, 454 (2008) 991-994. doi:10.1038/nature07174

21 Corinaldesi C, New perspectives in benthic deep-sea microbial ecology, *Front Mar Sci*, 2 (2015) p. 17. doi:10.3389/fmars.2015.00017

22 Daly K, Sharp R J & McCarthy A J, Development of oligonucleotide probes and PCR primers for detecting phylogenetic subgroups of sulfate-reducing bacteria, *Microbiology*, 146 (2000) 1693-1705. doi:10.1099/00221287-146-7-1693

23 Kacar A & Omuzbuken B, Assessing the seawater quality of a coastal city using fecal indicators and environmental variables (eastern Aegean Sea), *Mar Pollut Bull*, 123 (2017) 400-403. doi:10.1016/j.marpolbul.2017.08.052

24 Izdar E, *İzmir Korfezi oşinografi ve deniz deşarjı yöreleri batimetrik ve jeolojik etüdü. (Geological investigation of marine outfall areas and oceanography of İzmir Bay-in Turkish)*, Institute of Marine Science and Technology, Dokuz Eylül University, İzmir, Project No. DBTE 014; 1981.

25 Duman M, Avcı M, Duman S, Demirkurt E & Düzbastılar M K, Surficial sediment distribution and net sediment transport pattern in Izmir Bay, western Turkey, *Cont Shelf Res*, 24 (2004) 965-981. doi: 10.1016/j.csr.2004.03.001

26 Sayin E, Pazi İ & Eronat C, Investigation of water masses in İzmir Bay, western Turkey, *Turkish J Earth Sci*, 15 (2006) 343-372.

27 Yu Y, Lee C, Kim J & Hwang S, Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction, *Biotechnol Bioeng*, 89 (6) (2005) 670-679. doi:10.1002/bit.20347

28 Vigneron A, Cruaud P, Pignet P, Caprais J C, Cambon-Bonavita M A, *et al.*, Archaeal and anaerobic methane oxidizer communities in the Sonora Margin cold seeps, Guaymas Basin (Gulf of California), *ISME J*, 7 (2013) 1595-1608.

29 Suzuki M T, Taylor L T & DeLong E F, Quantitative analysis of small-subunit rRNA genes in mixed 50- microbial populations via nuclease assays, *Appl Environ Microbiol*, 66 (2000) 4605-4614.

30 Timmers P H A, Gieteling J, Widjaja-Greefkes H C A, Plugge C M, Stams A J M, *et al.*, Growth of anaerobic methane-oxidizing archaea and sulfate-reducing bacteria in a high-pressure membrane capsule bioreactor, *Appl Environ Microbiol*, 82 (4) (2015) 1286-1296.

31 Vigneron A, Cruaud P, Pignet P, Caprais J C, Gayet N, *et al.*, Bacterial communities and syntrophic associations involved in anaerobic oxidation of methane process of the Sonora Margin cold seeps, Guaymas Basin, *Environ Microbiol*, 16 (9) (2014) 2777-2790. doi:10.1111/1462-2920.12324

32 Bauer J E, Cai W J, Raymond P A, Bianchi T S, Hopkinson C S, *et al.*, The changing carbon cycle of the coastal ocean, *Nature*, 504 (2013) 61-70, doi10.1038/nature12857

33 Wallenstein M D, Myrold D D, Firestone M & Voytek M, Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods, *Ecol Appl*, 16 (2006) 2143-2152. doi:10.1890/1051-0761(2006)016

34 Ul-Hasan S, Bowers R M, Figueroa-Montiel A, Licea-Navarro A F, Beman J M, *et al.*, Community ecology across bacteria, archaea and microbial eukaryotes in the sediment and seawater of coastal Puerto Nuevo, Baja California, *PLoS ONE*, 14 (2) (2019) e0212355. doi:10.1371/journal.pone.0212355

35 Li J L, Lin S Y & Song Q, Characteristics of sediment bacterial community in response to environmental impacts in a sewage polluted river, *J Coastal Res*, 74 (2016) 196-206. doi:10.2112/SI74-017.1

36 Freese H, Karsten U & Schumann R, Bacterial abundance, activity, and viability in the eutrophic river Warnow, Northeast Germany, *Microb Ecol*, 51 (1) (2006) 117-127. doi:10.1007/s00248-005-0091-5

37 Sandaa R A, Torsvik V, Enger Ø, Daae F L, Castberg T, *et al.*, Analysis of bacterial communities in heavy metal-contaminated soils at different levels of communities in heavy metal-contaminated soils at different levels of resolution, *FEMS Microbiol Ecol*, 30 (3) (1999) 237-251. doi:10.1111/j.1574-6941.1999.tb00652.x

38 Haskell W Z, Prokopenko M G, Hammond D E, Stanley R H R & Sandwith Z O, Annual cyclicity in export efficiency in the inner Southern California Bight, *Glob Biogeochem Cycles*, 31 (2017) 357-376. doi:10.1002/2016GB005561

39 Whitton B A & Potts M, *The ecology of Cyanobacteria: their diversity in time and space*, (Springer Science & Business Media), 2007.

40 Einen J, Thorseth I H & Øvreas L, Enumeration of Archaea and Bacteria in seafloor basalt using real-time quantitative PCR and fluorescence microscopy, *FEMS Microbiol Lett*, 282 (2008) 182-187. doi:10.1111/j.1574-6968.2008.01119.x

41 Jørgensen B B, Mineralization of organic matter in the sea bed-the role of sulphate reduction, *Nature*, 296 (1982) 643-645. doi: 10.1038/296643a0

42 Zouch H, Karray F, Armougom F, Chifflet S, Hirschler-Réa A, *et al.*, Microbial diversity in sulfate-reducing marine sediment enrichment cultures associated with anaerobic biotransformation of coastal stockpiled phosphogypsum (Sfax, Tunisia), *Front Microbiol*, 8 (2017) p. 1583. doi: 10.3389/fmicb.2017.01583

43 Luek J L, Thompson K E, Larsen R K, Heyes A & Gonsior M, Sulfate reduction in sediments produces high levels of chromophoric dissolved organic matter, *Nat Sci Rep*, 7 (2017) p. 8829. https://doi.org/10.1038/s41598-017-09223-z