BACTERIAL PROFILES OF THE MUD FORMATIONS OBSERVED FROM A REMOTELY OPERATED VEHICLE (ROV) IN THE DEEP OF THE CANAKKALE STRAIT (DARDANELLES), TURKEY

Article *in* Fresenius Environmental Bulletin · September 2019

CITATION
1

READS
200

6 authors, including:

Gulsen Altug
Istanbul University
84 PUBLICATIONS **542** CITATIONS
SEE PROFILE

Pelin S. Çiftçi Türetken
Istanbul University
59 PUBLICATIONS **349** CITATIONS
SEE PROFILE

Gurun Sevan
Gensenta
30 PUBLICATIONS **156** CITATIONS
SEE PROFILE

Samet Kalkan
Recep Tayyip Erdoğan Üniversitesi
25 PUBLICATIONS **150** CITATIONS
SEE PROFILE

© by PSP Volume 28 – No. 9/2019 pages 6389-6399 Fresenius Environmental Bulletin

BACTERIAL PROFILES OF THE MUD FORMATIONS OBSERVED FROM A REMOTELY OPERATED VEHICLE (ROV) IN THE DEEP OF THE CANAKKALE STRAIT (DARDANELLES), TURKEY

Gulsen Altug[1,*], Pelin Saliha Ciftci Turetken[1], Sevan Gurun[1], Samet Kalkan[2], Yunus Emre Tasova[3], Mustafa Ozyalvac[3]

[1]Istanbul University, Faculty of Aquatic Sciences, Department of Marine Biology, Istanbul, Turkey
[2] Recep Tayyip Erdogan University, Fisheries Faculty, Rize, Turkey
[3]Denar Ocean Engineering, Istanbul, Turkey

ABSTRACT

The Çanakkale Strait, as a part of the Turkish Strait System (TTS), is an important water route in the world which connects the Mediterranean Sea to the Sea of Marmara and hence to the Black Sea via the Istanbul Strait. Due to its peculiar hydrodynamic characteristics, the area offers unique opportunities for researching profiles of bacteria under different, poorly described conditions. The samples of the mud formations, observed by "Remotely-Operated Vehicles" (ROV) with a diameter of roughly 120m and a height of 1.5-2m at a depth of 24m on the seabed of the Çanakkale Strait, were investigated regarding bacterial composition, metabolic characteristics of heterotrophic bacteria and environmental variables. Gram-negative fermenting and non-fermenting bacteria were the most common group in terms of species numbers, compared to Gram-positive cocci and non-spore-forming and spore-forming bacilli, both also found in the sample of mud formations and surface sediment around them. In the study, four species, *Micrococcus lylae, Lysinibacillus fusiformis, Bordetella trematum* and *Roseomonas gilardii* were recorded for the first time in the Turkish Seas. The results of the study contribute to an increasing knowledge on bacterial diversity and bacterial interactions regarding metabolically-talented bacteria and the environmental conditions of different texture on the floor of the seabed.

KEYWORDS:
Dardanelles, bacterial community, heterotrophic bacteria, mud hills, sediment.

INTRODUCTION

The discovery of the deep biosphere has shown that a major part of the microbial biosphere might be present in surface sediments [1-3]. Bacteria have a critical role in the decomposition of organic matter and recycling of nutrients in marine environments. Major biogeochemical processes in marine environments are related to the activities of heterotrophic microbes [4-6]. Since microbial communities play an important role in biogeochemical cycles, knowledge of bacterial diversity and the community structure of surface sediments is crucial for understanding marine ecosystem functioning.

When community profiles of bacteria have been linked to variable environmental factors, it is clear that influences of various and variable environmental and hydrographic conditions, shape peculiar sediment profiles of each micro-geographical marine area. In view of this, marine areas, which have unique hydrographical peculiarities, such as the Çanakkale Strait in Turkey, offer interesting opportunities for bacteriological studies.

In addition to common methods in detecting heterotrophic bacterial diversity, are culture-independent studies, where many studies report that cultured strains of marine bacteria can represent significant fractions of the bacterial biomass. Based on DNA-DNA hybridization of the genomic DNAs of isolates obtained by the traditional medium, against community DNA, it has been suggested that readily-cultivable bacteria are abundant in the marine water column [7-10]. Bacteria that are active in situ can be identified using molecular biological methods. However, these methods cannot reveal the whole spectrum of physiological capabilities that are essential to understanding the ecology of a single bacterial species. Therefore, for investigation of microbial adaptations to environmental conditions, pure cultures remain crucial [11].

In this study, mud formations, observed from a remotely-operated vehicle (ROV) and the surface sediment around them, were sampled from the bottom of the Çanakkale Strait in Turkey. The community profiles of mud-associated bacteria and the surface sediments around mud hills, regarding variable environmental parameters were investigated for the first time, with an aim to understanding the variances of bacterial community profiles and enzymatic reactions between mud formation and

the surface sediment of nearby mud hills. The metabolic–reactions of mud-associated bacteria were compared with the bacteria isolated from the surface sediments of nearby mud knolls. These samples, investigated for the first time, were to describe bacterial community profiles, metabolic peculiarities of mud-associated bacteria and potentials of the isolates for possible use in the industrial application.

MATERIALS AND METHODS

Study area and sampling. The terms of ″mud hill/mud knoll″ were used to describe the mud formations with a diameter of roughly 120 m and a height of 1.5-2 m at a depth of 24 meters on the seafloor of the Çanakkale Strait, Turkey. The mud formations were observed with Remotely Operated Vehicles (ROV) at 22 meters depth of the Çanakkale Strait, Turkey in spring 2014. The samples were collected both from the mud knolls and the normal surface sediments nearby the mud formations. The sampling area was shown in Figure 1.

View of the mud hills on the seafloor of the Çanakkale Strait, Turkey April 2014 was shown in Figure 2.

The sampling coordinates: *EC7 East: 496089.00 North 4487098.00, EN2 East: 495631.00 North 4488047.00, EC6 East: 495998.00 North 495998.00, ES2 East: 495496.00 North 4487982.00*

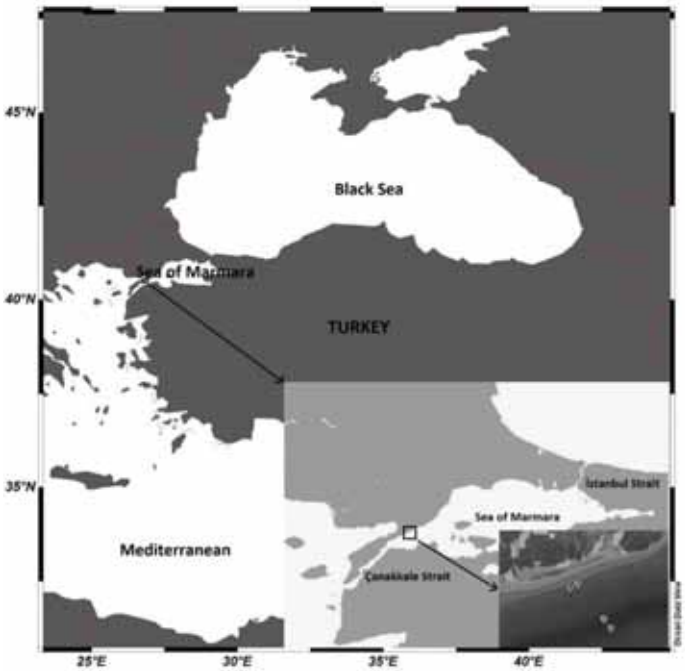

FIGURE 1
Sampling area; the Çanakkale Strait, Turkey

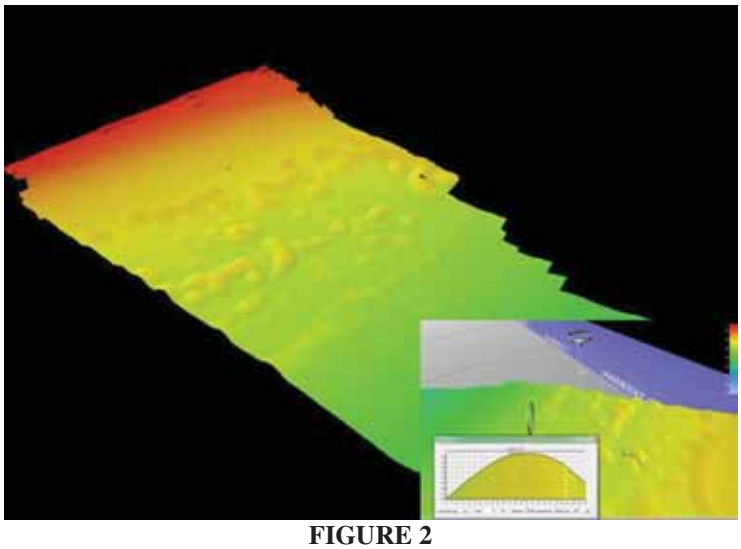

FIGURE 2
Views of the mud knolls at depth of 24 m on the seafloor of the Çanakkale Strait, Turkey June, 2014

Bacteriological analyses. The samples were collected, serial dilutions of 10^{-5} were prepared in 9-mL amounts of sterile seawater (artificial seawater, Sigma) and inoculated (0.2 mL) in duplicate on Marine Agar (Difco), and the plates were incubated for 5 days at 22 ± 0.1 °C (15). At the end of the incubation, colonies were counted and picked colonies were restreaked several times to obtain pure cultures.

Metabolic profiling of the isolates. The VITEK 2 Compact 30 (bioMérieux, France) automated micro identification system was used for detecting biochemical responses of the bacterial isolates against various substrates. The pure isolates were Gram-stained and then identified using GN (Gram-negative fermenting and nonfermenting bacilli), GP (Gram-positive cocci and nonspore-forming bacilli), and BCL (Gram-positive spore-forming bacilli) cards in the automated micro identification system VITEK 2 Compact 30 (bioMerieux, France). The identification cards are based on biochemical tests (46 tests for BCL, 43 tests for GP, 47 tests for GN) measuring carbon source utilization, enzymatic activities, inhibition, and resistance. Calculations are performed on raw data and compared to thresholds to determine reactions for each test. On the VITEK 2 Compact, test reaction results appear as "(–)" or "(+)". Reactions that appear in parentheses were evaluated as an indicator of weak reactions that are too close to the test threshold [12].

Hydrographic Parameters. Temperature, salinity and density values were measured *in situ* using the CTD (RBR Concerto) at the sampling areas.

FlowQuest (LinkQuest) acoustic current profiler was used to measure current speed (mm/sec, operation frequency 1000 kHz) of the sampling location. Directions of currents were measured using fixed RDCP (Recording Doppler Current Profiler 600).

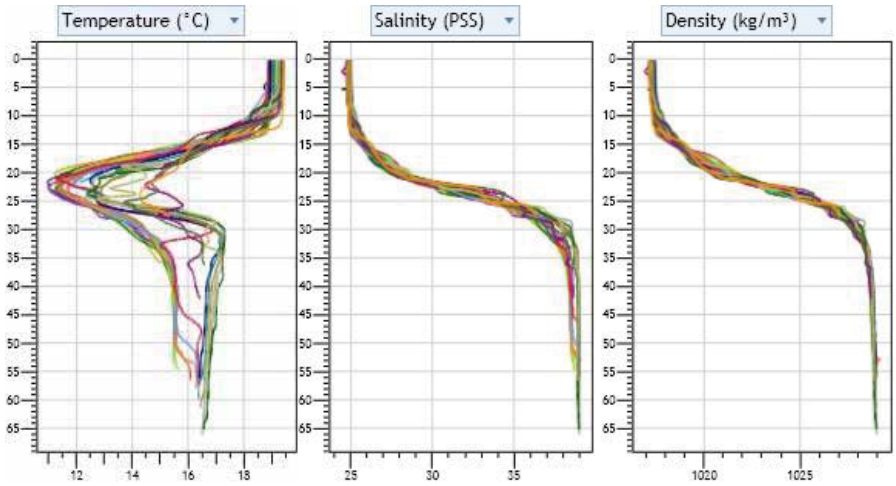

FIGURE 3
Temperature, salinity and density profiles of water column at sampling location

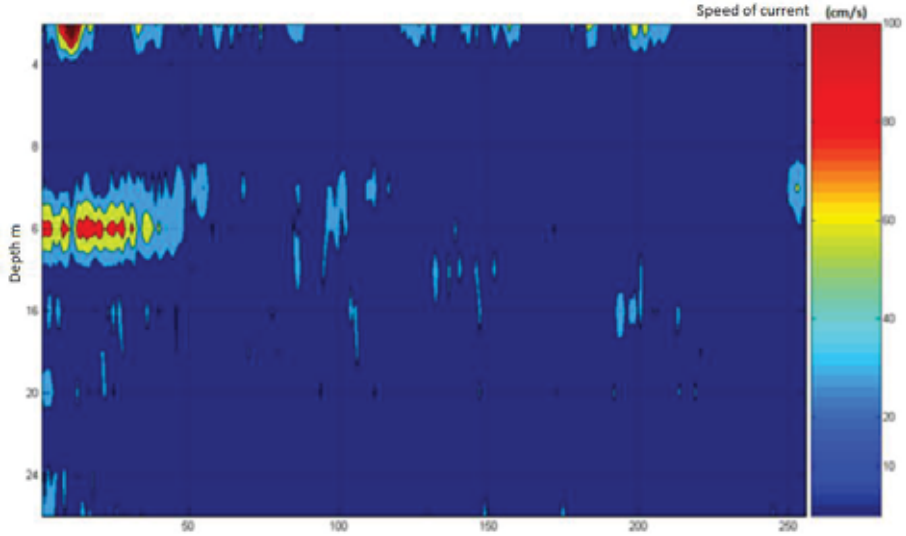

FIGURE 4
Flow Quest acoustic current profilers of the sampling location during the 24 hours at the sampling point, Çanakkale Strait, Turkey June 2014.

RESULTS

The recorded values of variable environmental parameters; temperature, salinity and density of the sampling areas were summarized in Figure 3. The recorded Flow Quest acoustic current profilers of the sampling location were shown in Figure 4. The recorded directions of currents in the sampling location were shown in Figure 5.

Heterotrophic aerobic bacteria count (HPC) /total colony forming unit (cfu/g) were shown in Table 1.

Heterotrophic plate count was found higher in the samples of the mud hills than the surface sediment around them.

Cultivable aerobic heterotrophic bacteria species isolated from the mud formations and the surface sediments in the deep of the Çanakkale Strait, Turkey were shown on the Table 2.

Total numbers of the identified isolates were found higher in the samples of the mud knolls than the surface sediment around them.

Gram-negative fermenting and non-fermenting bacteria were the most common group in terms of species number in comparison to Gram-positive cocci and non-spore-forming and spore forming bacilli in the samples both mud formations and the surface sediment around them.

The presence of four bacteria species; *Micrococcus lylae, Lysinibacillus fusiformis, Bordetella trematum* and *Roseomonas gilardii,* belonging to four different families from the mud knolls and surface sediments were reported for the first time in the Turkish Seas.

All of the Gram-negative isolates displayed positive reactions at various rates against tested substrates. However, the frequency of the positive reactions of the strains isolated from the mud samples was higher than the strains isolated from the surface sediments.

More than 50% of Gram-negative isolates that exhibit a positive reaction against substrates tested was summarized in Figure 6. Biochemical characteristics of the strains identified by using GN card in VITEK 2 Compact 30 were shown in Table 3. Biochemical characteristics of the strains identified by using BCL card in VITEK 2 Compact 30 were shown in Table 4. Biochemical characteristics of the strains identified by using GP card in VITEK 2 Compact 30 were shown in Table 5.

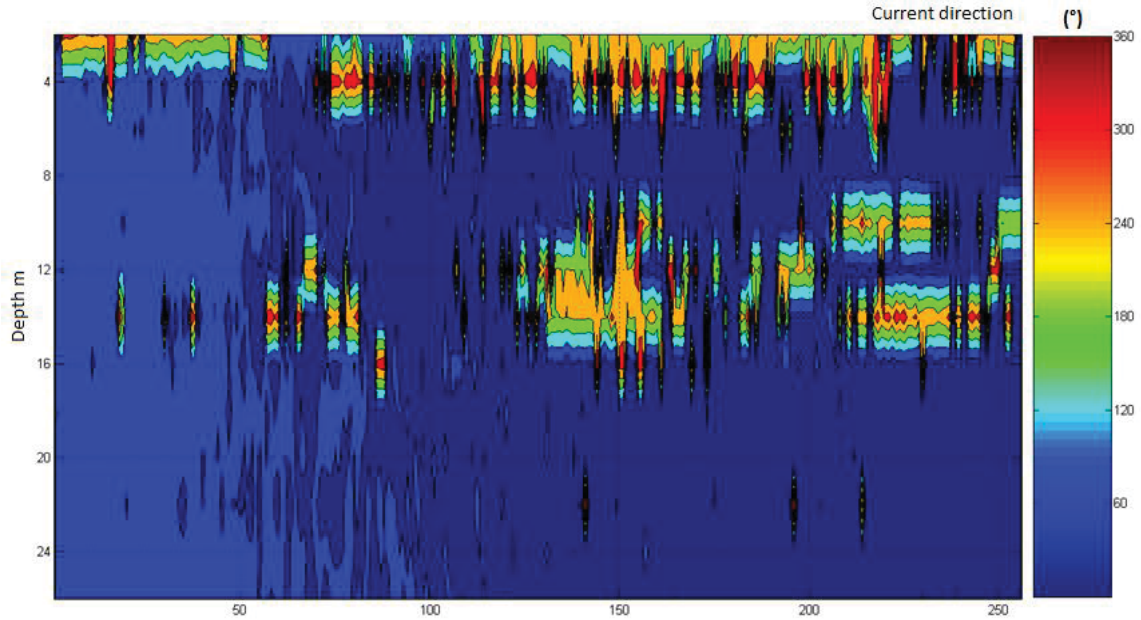

FIGURE 5
Directions of currents in the sampling location: Çanakkale Strait, Turkey, June 2014.

TABLE 1
The count of heterotrophic aerobic bacteria (cfu/g) in the samples taken from the mud hills and the surface sediment of deep of the Çanakkale Strait.

Sampling site	Heterotrophic plate count (cfu/g)	Mean
Mud knoll 1	$31x10^{11}$	
Mud knoll 2	$27x10^{11}$	$29\ x10^{11}$
Surface sediment1	$18x10^{10}$	
Surface sediment 2	$15x10^{10}$	$16x10^{10}$

TABLE 2
Bacteria species isolated from the mud formations and surface sediments on seafloor of the Çanakkale Strait, Turkey

Phylum/Class	Family	Species	Mud	Sedim	The areas that species isolated previously isolated
Actinobacteria/ Actinomycetales	Micrococcaceae	*Micrococcus luteus* Lehmann and Neumann, 1896	+	+	Aegean Sea [21], Istanbul Strait [27], Güllük Bay, Aegean Sea [28], Coastal areas of the Lebanon and Syria [29]
		M. lylae Kloos et al. 1974	+	+	***This study***
		Kocuria kristinae	+	-	Ballast waters [22], Güllük Bay, Aegean Sea [28]
Firmicutess/ Bacilli	Bacillaceae	*Bacillus cereus* Frankland and Frankland 1887	+	+	Istanbul Strait [27], Güllük Bay, Aegean Sea [28], Gökçeda Island, Aegean Sea [30]
		B. mycoides Flügge 1886	+	-	Istanbul Strait [27], Gökçeda Island, Aegean Sea [30]
		B. pumilus Meyer and Gottheil 1901	+	+	Istanbul Strait [27], Güllük Bay, Aegean Sea [28], Gökçeda Island, Aegean Sea [30]
		B. thuringiensis Berliner 1915	+	-	Istanbul Strait [27], Gökçeda Island, Aegean Sea [30]
		Lysinibacillus fusiformis (Ahmed et al., 2007)	+	+	***This study***
Proteobacteria/ α Proteobacteria	Sphingomonadaceae	*Sphingomonas paucimobilis* (Holmes et al. 1977), Yabuuchi et al. 1990	+	+	Ballast waters [22], Istanbul Strait [27], Güllük Bay, Aegean Sea [28], Aegean Sea [30].
	Brucellaceae	*Ochrobactrum anthropi* Holmes et al., 1988	+	+	Ballast waters [22]
Proteobacteria/ β Proteobacteria	Alcaligenaceae	*Achromobacter denitrificans* Roger and Tan (1983) Coenye et al., 2003	+	+	Ballast waters [22].
		A. xylosoxidans Roger and Tan (1983) Coenye et al., 2003	+	+	Ballast waters [22]
		Bordetella trematum	+	-	***This study***
	Burkholderiaceae	*Cupriavidus pauculus*	+	+	Gökçeda Island, Aegean Sea [30]
		Burkholderia mallei	-	+	Gökçeda Island, Aegean Sea [30]
	Neisseriaceae	*Chromobacterium violaceum* Bergonzini 1880	+	-	Güllük Bay [28], Coastal areas of the Lebanon and Syria [29]
	Comamonadaceae	*Delftia acidovorans* (den Dooren de Jong 1926) Wen et al. 1999	+	+	Gökçeda Island, Aegean Sea [30]
	Ralstoniaceae	*Roseomonas gilardii*	+	-	***This study***
Proteobacteria/ γ Proteobacteria	Pseudomonadaceae	*Pseudomonas luteola* Kodama et al., 1985) Holmes et al., 1987	+	+	The Sea of Marmara [21], Coastal areas of Lebanon, Syria [29], Aegean Sea [30],
	Xanthomonadaceae	*Stenotrophomonas maltophilia* Palleroni and Bradbury 1993	+	+	Ballast waters [22], Istanbul Strait [27], Güllük Bay [28], Gökçeda Island, Aegean Sea [30]
	Enterobacteriaceae	*Klebsiella oxytoca*	+	+	The Sea of Marmara [21], Ballast waters [22], Istanbul Strait [27]
		K. pneumoniae ssp pneumoniae	-	+	Ballast waters [22]
		Enterobacter cloacae	+	+	The Sea of Marmara [21], Ballast waters [22], Istanbul Strait [27]
		E. coli	+	+	The Sea of Marmara [21], Istanbul Strait [27]
Gram (-) fermenting and non-fermenting bacteria	60	50			
Gram (+) cocci and non-spore-forming bacilli	55	32			
The spore-forming Gram (+) bacilli	30	15			
Total numbers of the isolates	145	97			
Total numbers of the species	22	18			
First records species	4	2			

TABLE 3
The positive reaction percentage of the Gram-negative isolates against tested substrates.

Test Substrates	Representation	Positive Reaction %	
		Mud	Sediment
ILATk	L-LACTATE alkalinisation	88,9	20
SUCT	SUCCINATE alkalinisation	87,3	20
ProA	L-Proline ARYLAMIDASE	63,5	10
TyrA	Tyrosine ARYLAMIDASE	63,5	15
ELLM	ELLMAN	61,9	20
PyrA	Pyrrolydonyl-ARYLAMIDASE	58,7	15
GGT	GAMMA-GLUTAMYL-TRANSFERASE	58,7	10
PHOS	PHOSPHATASE	57,1	5
CIT	CITRATE (SODIUM)	55,6	10
URE	UREASE	52,4	10
CMT	COUMARATE	47,6	10
APPA	Ala-Phe-Pro ARYLAMIDASE	46,0	5
BGLU	*BETA-GLUCOSIDASE*	46,0	0
LIP	*LIPASE*	36,5	5
GGAA	*Glu-Gyl-Arg-ARYLAMIDASE*	34,9	10
AGLTp	*Glutamyl Arylamidase pNA*	31,7	10
dGLU	*D-GLUCOSE*	31,7	5
AGLU	*ALPHA-GLUCOSIDASE*	28,6	10
dMAL	*D-MALTOSE*	27,0	0
O129R	*O/129 RESISTANCE*	27,0	0
BNAG	*BETA-ACETYL-GLUCOSAMINIDASE*	23,8	0
IMLTa	*L-MALATE assimilation*	15,9	0
dTRE	*D-TRHALOSE*	14,3	0
SAC	*SACCHAROSE/SUCROSE*	12,7	0
MNT	*MALONATE*	12,7	0
Dcel	*D-CELLOBIOSE*	7,9	0
dMNE	*D-MANNOSE*	7,9	0
dMAN	*D-MANNITOL*	6,3	0
BXYL	*BETA-XYLOSIDASE*	6,3	0
BGAL	*BETA-GALACTOSIDASE*	4,8	0
PLE	*PALATINOSE*	4,8	0
IHISa	*L-HISTIDINE assimilation*	4,8	0
GlyA	*Glycine ARYLAMIDASE*	3,2	0
BAlap	*BETA-Alanine arylamidase pNA*	1,6	0
dTAG	*D-TAGATOSE*	1,6	0
AGAL	*ALPHA-GALACTOSIDASE*	1,6	0
ILATa	*L-LACTATE assimilation*	1,6	0
ADO	*ADONITOL*	0	0
IARL	*L-ARABITOL*	0	0
H2S	*H2S PRODUCTION*	0	0
OFF	*FERMENTATION/GLUCOSE*	0	0
dSOR	*D-SORBITOL*	0	0
5KG	*5-KETO-D-GLUCONATE*	0	0
NAGA	*Beta-N-NCETYL-GALACTOSAMINIDASE*	0	0
ODC	*ORNITHINE DECARBOXYLASE*	0	0
LDC	*LYSINE DECARBOXYLASE*	0	0
BGUR	*BETA-GLUCORONIDASE*	0	0

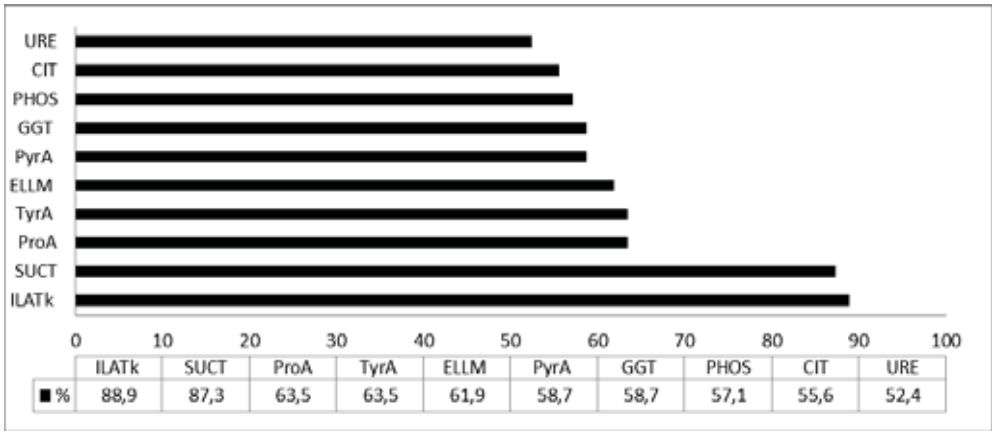

FIGURE 6
The positive reactions percentages against tested substrates of the Gram-negative bacteria isolated from the mud hills
(The percentage of the strains displayed positive values more than 50% were shown).

TABLE 4
The positive reaction percentage of the bacilli isolates against tested substrates.

Test Substrates	Representation	Positive Reaction %	
		Mud	Sediment
LeuA	*Leucine-ARYLAMIDASE*	100	20
APPA	*Ala-Phe-Pro ARYLAMIDASE*	100	20
ESC	*Esculin hydrolysis*	100	20
PyrA	*L-Pyrrolydonyl- ARYLAMIDASE*	87,5	10
AlaA	*Alanine ARYLAMIDASE*	87,5	10
PheA	*Phenylalanine ARYLAMIDASE*	75	10
PVATE	*PYRUVATE*	75	10
dTRE	*D-TREHALOSE*	75	20
dGLU	*D-GLUCOSE*	75	20
dRIB	*D-RIBOSE*	75	10
NaCI 6.5%	*GROWTH IN 6.5% NaCl*	75	20
POLYB_R	*POLYMIXIN_B RESISTANCE*	75	10
MTE	*MALTOTRIOSE*	62,5	10
NAG	*N-ACETYL-D-GLUCOSAMINE*	62,5	10
TTZ	*TETRAZOLIUM RED*	62,5	10
dMNE	*D-MANNOSE*	50	10
TyrA	*Tyrosine ARYLAMIDASE*	37,5	10
BNAG	*BETA-N-ACETYL GLUCOSAMINIDASE*	37,5	5
PHC	*PHOSPHORYL CHOLINE*	37,5	10
OLD	*OLEANDOMYCIN RESISTANCE*	37,5	5
ProA	*L- Proline ARYLAMIDASE*	25	10
MdG	*METHYL-A-D-GLUCOPYRANOSIDE acidification*	25	50
ELLM	*ELLMAN*	25	0
BXYL	*BETA-XYLOSIDASE*	12,5	0
AspA	*L-Aspartate ARYLAMIDASE*	12,5	0
BGAL	*BETA-GALACTOSIDASE*	12,5	0
AGAL	*ALPHA-GALACTOSIDASE*	12,5	0
AMAN	*ALPHA- MANNOSIDASE*	12,5	0
GlyA	*Glycine ARYLAMIDASE*	12,5	0
dMAN	*D-MANNITOL*	12,5	0
BGLU	*BETA-GLUCOSIDASE*	12,5	0
dTAG	*D-TAGATOSE*	12,5	0
LysA	*L-Lysine-ARYLAMIDASE*	0	0
CDEX	*CYCLODEXTRIN*	0	0
dGAL	*D-GALACTOSE*	0	0
GLYG	*GLYCOGEN*	0	0
INO	*Myo-INOSITOL*	0	0
MdX	*METHYL-D-XYLOSIDE*	0	0
dMLZ	*D-MELEZITOSE*	0	0
PLE	*PALATINOSE*	0	0
IRHA	*L-RHAMNOSE*	0	0
BMAN	*BETA-MANNOSIDASE*	0	0
AGLU	*ALPHA-GLUCOSIDASE*	0	0
INU	*INULIN*	0	0
PSCNa	*PUTRESCINE assimilation*	0	0
KAN	*KANAMYCIN RESISTANCE*	0	0

DISCUSSION

In the present study, ″unusual mud formations″, observed via a "Remotely Operated Vehicle" (ROV) at the bottom of the Çanakkale Strait, Turkey were investigated regarding their bacterial community profile and metabolic response of the isolated bacteria with regard to enzyme expression capability against tested substrates. The mud formations and normal surface sediment around them were compared in order to understand bacterial differences regarding analyses of the counts of cultivable aerobic heterotrophic bacteria, bacterial enzyme expression capacity, diversity and composition.

The Çanakkale strait, as a study area for observing the mud formations, has a counter-current system, formed as a result of the less saline waters (17 psu) of the Black Sea (upper currents) and the concentrated saline waters (38 psu) of the Mediterranean Sea (undercurrents) (Fig. 2). Additionally, this current system was described as an important constituent in chemical oceanographic structure, ecological states and the productivity of the Sea of Marmara and the Turkish Strait System.

The Çanakkale and Istanbul Strait is prone to biological and chemical pollution due to the environmental pollution from the Sea of Marmara [13]. [14], using the accumulation rate of labile organic C in the sediments, showed that changes in ecosystem functioning can increase the efficiency of heterotrophic prokaryotes in transforming organic detritus pools into biomass. However, there is no available data on opposite currents, system-related particle distribution, and abundance of particle-associated

TABLE 5
The positive reaction percentage of the Gram-positive isolates against tested substrates.

Test Substrates	Representation	Positive Reaction %	
		Mud	Sediment
ADH1	*ARGININE DIHYDROLASE 1*	100	30
LeuA	*Leucine-ARYLAMIDASE*	100	30
AlaA	*Alanine ARYLAMIDASE*	100	20
TyrA	*Tyrosine ARYLAMIDASE*	90	20
ILATk	*L-LACTATE alkalinisation*	90	30
AGLU	*ALPHA-GLUCOSIDASE*	80	10
ProA	*L-Proline ARYLAMIDASE*	80	10
URE	*UREASE*	70	20
ADH2s	*ARGININE DIHYDROLASE 2*	60	10
PyrA	*Pyrrolydonyl-ARYLAMIDASE*	50	20
APPA	*Ala-Phe-Pro- ARYLAMIDASE*	30	10
PHOS	*PHOSPHATASE*	30	10
dMAN	*D-MANNITOL*	30	0
dMNE	*D-MANNOSE*	30	0
SAL	*SALICIN*	20	0
SAC	*SACCHAROSE/SUCROSE*	20	0
OPTO	*OPTOCHIN RESISTANCE*	20	0
BGAL	*BETA-GALACTOSIDASE*	10	0
AspA	*L-Aspartate ARYLAMIDASE*	10	0
AMAN	*ALPHA- MANNOSIDASE*	10	0
dSOR	*D-SORBITOL*	10	0
dGAL	*D-GALACTOSE*	10	0
dTRE	*D-TRHALOSE*	10	0
AMY	*D-AMYGDALIN*	0	0
PIPLC	*PHOSPHATIDYLINOSITOL PHOSPHOLIPASE C*	0	0
dXYL	*D-XYLOSE*	0	0
CDEX	*CYCLODEXTRIN*	0	0
BGAR	*BETA GALACTOPYRANOSIDASE*	0	0
BGURr	*BETA GLUCURONIDASE*	0	0
AGAL	*ALPHA-GALACTOSIDASE*	0	0
BGUR	*BETA-GLUCORONIDASE*	0	0
POLYB	*POLYMIXIN RESISTANCE*	0	0
dRIB	*D-RIBOSE*	0	0
LAC	*LACTOSE*	0	0
NAG	*N-ACETYL-D-GLUCOSAMINE*	0	0
dMAL	*D-MALTOSE*	0	0
BACI	*BACITRACIN RESISTANCE*	0	0
NOVO	*NOVOBIOCIN RESISTANCE*	0	0
NC6.5	*GROWTH IN 6.5% NaCl*	0	0
MBdG	*METHYL-BD-GLUCOPYRANISIDE*	0	0
PUL	*PULLULAN*	0	0
dRAF	*D-RAFFINOSE*	0	0
O129R	*O/129 RESISTANCE*	0	0

and free-living bacteria at the bottom of the Çanakkale Strait. It is known, however, that particle-associated bacteria levels are higher than free-living bacteria in marine environments. High levels of heterotrophic bacteria detected in mud formations, rather than the surface sediments around the mud knolls, showed that these formations are appropriate environments in which to induce bacterial heterotrophic activity and growth.

Additionally, the initial detection of high bacteria counts and enzymatic dynamics in mud hills may be related to the tendency of bacteria to attach themselves to suspended particles at the bottom of the sea. It is known that when pollutants arrive at natural water environments, their most common accumulation site is within sediments. High epibacteria levels detected in the mud knoll samples, allow us to suggest that these formations may occur as a result of an accumulation of organic and inorganic substances via water movements such as undercurrents (Figure 3, 4), at certain points at the bottom of the sea.

It is known that decomposition of organic substance occurs by reactions of the bacteria to specific adaptations. For investigation of microbial adaptations to environmental conditions and to understand the ecology of a single bacterial species, description of physiological capabilities of pure cultures remain crucial [11].

In the present study, bacterial isolates were compared between the samples taken from the mud formations and natural sediment samples, with an aim to describing the metabolic response types of bacteria regarding two different habitats. A total of 242 cultivable bacterial isolates were characterized by an automated micro identification system, based on biochemical tests measuring carbon source utilization, enzymatic activities and inhibition. Metabolic response rates, against tested substrates, regarding positive reaction frequency of the isolated bacteria from the mud hills, were higher than surface sediment samples.

While the community profiles of the cultivated bacteria were markedly different (as a result of the abundance rates of the bacterial species), 72% of the bacterial species was similar in both the sediment and the mud formation samples. As an example, the *Sphingomonas paucimobilis*, aerobic Gram-negative bacillus, both belonging to the family Sphingomonadaceae and Alpha Proteobacteria class, were found to be the most common species in the samples of mud formations. *Sphingomonas* has been found in aquatic environments, both freshwater and seawater, and also in terrestrial habitats [15].

S. paucimobilis was reported to be the most abundant surface-associated bacteria in the sponges taken from the northern part of the Aegean Sea in Turkey [16]. It was also reported that the *Sphingomonas* species was predominant in all biofilms [17, 18] and has a high production capacity for extracellular polymeric substances and strong adhesion properties [19]. In addition, due to their' metabolic diversity, they have been considered a potential microbial agent for biological remediation studies. *S. paucimobilis* was also reported as able to degrade lignin-related biphenyl chemical compounds [20].

Independent from this study, we also tested oil hydrocarbon degradation capacity of *S. paucimobilis* isolated from mud formations. At the end of a three week incubation period of individual bacteria with oil hydrocarbon in experiments, the degradation rate, regarding the GC-MS analyses, were recorded to be over 80% (data not shown). In this study, some bacterial species isolated from the mud formation, as in *S. paucimobilis*, offered some signs for possible use as a candidate species for further study.

Due to the fact that bacterial communities are all different and dynamic and relate to various environmental factors, each marine environment offers unique opportunities for understanding bacterial roles in marine ecosystem functioning. However, it is still unknown how a bacterial community responds to its environmental changes regarding pollution factors at the bottom of the seas. For instance, in this study, members of the phylum Firmicutes and Proteobacteria, including nitrogen-fixing bacteria and various pathogenic bacteria, were recorded to be the dominant group in the mud-hill samples.

The most common group in terms of species number, in both mud formations and surface sediment around them, were Gram-negative pathogenic bacteria. Detection of high pathogenic bacteria consisting of Gram-negative fermenting and non-fermenting bacteria belonging to the Enterobacteriaceae family, and multi-drug resistant isolates (data not shown), allowed us to conclude that the bottom of the Çanakkale Strait indicates some evidence of human-source–pollution. For instance, isolation of the multi-drug resistant *Stenotrophomonas maltophilia,* as the most common species in normal surface sediments and its occurrence in mud formations also support this.

The majority of bacteria present in surface sediments and the mud knoll samples were recorded as saprophyte bacteria of fecal or terrestrial origin and also pathogenic bacteria. The detected bacterial species implied that the study region under anthropogenic stress, related to terrestrial-sourced and non-pointed pollution. The occurrence of human-source bacterial pollution in this region was also reported in our previous studies [21-25].

CONCLUSION

In this study, the bacterial analysis was appropriate for detecting compositional differences in bacterial communities inhabiting extraordinary formations such as mud knolls and normal surface sediments, from samples taken simultaneously at the bottom of the Çanakkale Strait. Variations in bacterial community composition between two different samples were recorded, to correspond with differences in habitat characteristics. Our data suggest that detected differences between natural surface sediment and mud formations, regarding composition and metabolic response of the bacteria, may relate to environmental conditions where pollution exists at the bottom of the sea.

Extracellular enzymes, produced by sediment bacteria, play an important role in accumulated and buried organic matter decomposition, nutrient recycling, and earth element transformation and mobilization [26]. As such, our hypothesis is that the interactions between hydrographical processes and variable environmental conditions, accumulation of organic-inorganic substances, including heterotrophic activity may induce a "sludging" tendency and produce unusual occurrences such as the mud knolls at the bottom of the sea.

The comparative analysis of bacterial communities in the samples provided, increase our knowledge about bacterial diversity and composition, in poorly described conditions at the bottom of the Çanakkale Strait. This data will allow us to go forward with studies in which the effects of environmental pollution on bacterial communities and their functions on the seabed of the Çanakkale Strait, will be evaluated. In addition, this study offers us a knowledge with which to compare exoenzyme activities of cultivable bacteria, and for a better understanding of their biochemical roles and the biomaterials to be used as a source for further biotechnological studies.

ACKNOWLEDGEMENTS

The authors wish to thank to Istanbul University Scientific Research Projects Unit (Project number: 20928) for their financial support.

REFERENCES

[1] Parkes, R.J., Cragg, B.A., Bale, S., Getliff, J.M., Goodman, K., Rochelle, P.A., Fry, J.C., Weightman, A.J. and Harvey, S.M. (1994) Deep bacterial biosphere in Pacific Ocean sediments. Nature. 371, 410–413.

[2] Whitman, W.B., Coleman, D.C., and Wiebe, W.J. (1998) Prokaryotes: the unseen majority. Proceedings of the National Academy of Sciences of the United States of America. 95, 6578–6583.

[3] Köpke, B., Wilms, R., Engelen, B., Cypionka, H. and Sass, H. (2005) Microbial Diversity in Coastal Subsurface Sediments: A Cultivation Approach Using Various Electron Acceptors and Substrate Gradients. Applied and Environmental Microbiology. 71(12), 7819–7830

[4] Pomeroy, L.R. (1980) Microbial roles in aquatic food webs. In: Colwell, R.R. (ed.) Aquatic microbial ecology. Univ. Maryland Press, College Park. 85-109.

[5] Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A. and Thingstad, F. (1983) The ecological role of water column microbes in the sea. Marine Ecology Progress Series. 10, 257-263.

[6] Azam, F. and Fuhrman, J.A. (1985) Measurement of bacterioplankton growth in the sea and its regulation by environmental conditions. In: Hobbie, J.E., Williams, P.J. (eds.) Heterotrophic activity in the sea. Plenum. New York, 179-196.

[7] Rehnstam, A.S, Backman, S., Smith, D.C., Azam, F., Hagström, Å. (1993) Blooms of sequence-specific culturable bacteria in the sea. FEMS Microbiology Letter. 102, 161-166.

[8] González, J.M., Whitman, W.B., Hodson, R.E. and Moran, A.M. (1996) Identifying numerically abundant culturable bacteria from complex communities: an example from a lignin enrichment culture. Applied and Environmental Microbiology.62, 4433-4440.

[9] Pinhassi, J., Zweifel, U.L. and Hagström, A. (1997) Dominant marine bacterioplankton species found among colony-forming bacteria. Applied and Environmental Microbiology. 63, 3359-3366.

[10] González, J.M., Moran, M.A. (1997) Numerical dominance of a group of marine bacteria in the subclass of the class Proteobacteria in coastal seawater. Applied and Environmental Microbiology. 63, 4237-4242.

[11] Fry, J.C. (2000) Bacterial diversity and "unculturables". Microbiology Today. 27, 186–188.

[12] Pincus, D.H. (2005) Microbial identification using the bioMerieux VITEK® 2 System. In: Miller, M.J. (Ed.) Encyclopedia of Rapid Microbiological Methods, PDA/DHI. 1, 1–32.

[13] Turkoglu, M., Yenici, E., Ismen, A., Kaya, S. (2004) Variations of nutrient and chlorophyll-a in the Çanakkale Strait (Dardanelles). Ege Journal of Fisheries and Aquatic Sciences. 21(1-2), 93-98.

[14] Danovaro, R. and Pusceddu, A. (2007) Biodiversity and ecosystem functioning in coastal lagoons: Does microbial diversity play any role? Estuarine, Coastal and Shelf Science. 75(1-2), 5982-5989.

[15] Balkwill, D.L., Fredrickson, J.K. and Romine, M.F. (2006) Sphingomonas and Related Genera. In: Dworkin, M. (Ed.) The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community. Springer-Verlag, New York.

[16] Altug, G., Topaloglu, B., Ciftci Turetken, P.S., Gurun, S., Kalkan, S., Kabaraciyan, M. (2013b) Investigation of Anti-Bacterial Activity of Marine Sponge Extracts from Gökçeada Island, Aegean Sea, Turkey. The Scientific and Technological Research Council of Turkey. TUBITAK 112Y236 Technical Report.

[17] Jang, H.J., Choi, Y.J. and Ka, J.O. (2011) Effects of diverse water pipe materials on bacterial communities and water quality in the annular reactor. J. Microbiol. Biotechnol. 21(2), 115- 123

[18] Simoes, L.C., Simoes, M. and Vieora, M.J. (2010) Adhesion and biofilm formation on polystyrene by drinking water-isolated bacteria. Antonie Van Leeuwenhoek. 98(3), 317-329

[19] Kurrisery, S.R., Kanavillil, N., Leung, K.T., Chen, A., Davey, L. and Schraft, H. (2010) Electrochemical and Microbiological Characterization of Paper Mill Biofilms. Biofouling. 26(7), 799-808

[20] Masai, E., Katayama, Y., Nishikawa, S. and Fukuda, M. (1999) Characterization of Sphingomonas paucimobilis SYK-6 genes involved in degradation of lignin-related compounds. Journal of Industrial Microbiology and Biotechnology. 23(4), 364-373.

[21] Altug, G., Aktan Turan, Y., Oral, M., Topaloglu, B., Dede, A., Keskin, Ç., Isinibilir Okyar, M., Cardak, M. and Ciftci, P.S. (2011) Biodiversity of the Northern Aegean Sea and Southern Part of the Sea of Marmara, Turkey. Marine Biodiversity Records. 4, 1-17.

[22] Altug, G., Gurun, S., Cardak Ciftci, P.S., Kalkan, S. (2012) Occurrence of Pathogenic Bacteria in Some Ships' Ballast Water Incoming from Various Marine Regions to The Sea of Marmara, Turkey. Marine Environmental Research. 81, 35-42.
[23] Altug, G., Cardak, M., Ciftci, P.S. and Gurun, S. (2013) First Records and Microgeographical Variations of Culturable Heterotrophic Bacteria in an Inner Sea (The Sea of Marmara) Between the Mediterranean and the Black Sea, Turkey. Turkish Journal of Biology. 37, 184-190.
[24] Cardak, M., Altug, G. and Ciftci Turetken, P.S. (2015) Variations of Culturable and Metabolically Active Bacteria in a Stratified Water Column: The Example of Istanbul and Canakkale Straits. International Journal of Environmental Research. 10, 15-21.
[25] Cardak, M., Altug, G., May, M. and Erol, Ö. (2016) Investigation of The Distribution of Antibiotic Resistance and The Presence of Vancomycin-Resistance Genes (vanA and vanB) in Enterobacteriaceae Isolated from The Sea of Marmara, Canakkale Strait and Istanbul Strait, Turkey. Oceanological and Hydrobiological Studies. 1, 1-5.
[26] Dang, H., Zhu, H., Wang, J. (2009) Extracellular hydrolytic enzyme screening of culturable heterotrophic bacteria from deep-sea sediments of the Southern Okinawa Trough World J Microbiol Biotechnol. 25, 71.
[27] Altug, G., Cardak, M., Ciftci, P.S., Gürün, S. (2013) First records and micro-geographical variations of culturable heterotrophic bacteria in an inner sea (the Sea of Marmara) between the Mediterranean and the Black Sea, Turkey. Turkish Journal of Biology. 37, 184-190.
[28] Gurun, S. (2014) Investigation of Diversity, Composition and Abundance of Culturable Heterotrophic Bacteria of Güllük Bay (Aegean Sea). Dissertation. Istanbul: Graduate School of Science and Engineering, Istanbul University. 203p.
[29] Altug, G., Cardak, M., Gurun, S., Ciftci, P.S. (2010) Biodiversity of Culturable Aerobic Heterotrophic Bacteria in the Coastal Areas of Syria, Lebanon and the offshore Area in the Northern Aegean Sea. The International Conference on "Biodiversity of the Aquatic Environment" Towards a Diverse and Sustainable World. Syria.
[30] Ciftci, P.S. (2014) Investigation of the Level and Diversity of Culturable Bacteria around Gökçeada Island (Northern Aegean Sea. Dissertation. Istanbul: Graduate School of Science and Engineering, Istanbul University. 166p.

Received: **01.06.2017**
Accepted: **01.05.2019**

CORRESPONDING AUTHOR

Gulsen Altug
Istanbul University,
Faculty of Aquatic Sciences,
Department of Marine Biology,
Istanbul – Turkey

e-mail: galtug@istanbul.edu.tr