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Microbial community characterization of the Gully: a marine protected area

C. William Yeung, Kenneth Lee, Lyle G. Whyte, and Charles W. Greer

Abstract: The Gully is the first Fisheries and Oceans Canada marine protected area off the eastern coast of Canada. To ensure success of conservation efforts in this area, it is essential to develop a better understanding of microbial community composition from the euphotic zone to the deep sea in this previously unsurveyed environment. Denaturing gradient gel electrophoresis (DGGE) and nucleotide sequencing were used to characterize microbial community structure. DGGE results showed a clear difference in the microbial community structure between the euphotic zone and the deep sea water. Cluster analysis showed high similarity (>85%) for all the samples taken from below 500 m, but lower similarity (49%–72%) when comparing samples from above and below 500 m. Changes in microbial community structure with depth corresponded well with changes in oceanographic physical parameters. Furthermore, 16S rRNA gene analysis showed that the bacterioplankton sequences generally clustered into 1 of 9 major lineages commonly found in marine systems. However, not all the major lineages were detected at all the different depths. The *SAR11* and *SAR116* sequences were only present in the surface water, and the *SAR324* and *Actinobacteria* sequences were only present in deep sea water. These findings provide a preliminary characterization of the microbial communities of this unique ecosystem.

Key words: microbial community structure, phylogenetic diversity, DGGE, 16S rRNA.

Résumé : Le Goulet est la première aire marine protégée de Pêches et Océans Canada située au large de la côte Est canadienne. Afin d'assurer le succès des efforts de conservation dans cette aire, il est essentiel de mieux comprendre la composition de la communauté microbienne qui s'échelonne de la zone euphotique vers les eaux profondes dans cet environnement encore non étudié. L'électrophorèse en gradient de gel dénaturant (DGGE) et le séquençage de nucléotides ont été utilisés pour caractériser la structure de la communauté microbienne. Les résultats de la DGGE ont montré une différence marquée entre la structure de la communauté de la zone euphotique et celle trouvée en eau profonde. Une analyse de grappe a montré un haut degré de similarité (>85 %) entre tous les échantillons prélevés à des profondeurs sous les 500 m, mais un degré moins élevé (49 % – 72 %) lorsque les échantillons prélevés au dessus et en dessous de 500 m étaient comparés. Les changements dans la structure de la communauté microbienne en fonction de la profondeur correspondaient bien avec les paramètres physiques océanographiques. De plus, l'analyse du gène de l'ARNr 16S a montré que les séquences des bactérioplanktons se groupaient généralement dans 1 des 9 lignages principaux communément trouvés dans les systèmes marins. Cependant, les lignages n'étaient pas tous détectés à toutes les profondeurs. Les séquences de *SAR11* et de *SAR116* n'étaient présentes que dans les eaux de surfaces alors que les séquences de *SAR324* et d'*Actinobacteria* n'étaient présentes qu'en eaux profondes. Ces résultats fournissent une caractérisation préliminaire des communautés microbiennes de cet écosystème unique.

Mots-clés : structure de la communauté microbienne, diversité phylogénique, DGGE, ARNr 16S.

[Traduit par la Rédaction]

Introduction

The Gully, located at the edge of the continental shelf off Nova Scotia, is the largest submarine canyon in the north-west Atlantic. This deep-sided canyon, with depths of more than 2 km, provides a wide range of environmental conditions and a varied sediment surface that is home to a highly

diverse population of organisms (Gordon and Fenton 2002; Rutherford and Breeze 2002), including cold-water corals and endangered northern bottlenose whales (Whitehead et al. 1996). In 2004, Fisheries and Oceans Canada designated the Gully as a marine protected area (MPA) through regulations under Canada's Oceans Act. With emerging concerns over the impact of commercial fisheries and the proximity

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Fig. 1. Conductivity, temperature, and depth profile. Sample collection depths are indicated by solid lines. Temperature, salinity, oxygen saturation, and chlorophyll fluorescence profiles are presented. (A) From surface to 2000 m. (B) From surface to 500 m.

of the canyon to oil and gas development on the Scotian Shelf (Gordon and Fenton 2002), research and monitoring programs have been initiated to increase our understanding of the Gully and the potential for human impacts on this ecosystem. Fisheries and Oceans Canada recognized that there was a need for baseline studies to provide a reference point for future marine conservation efforts, so that the health and integrity of the Gully ecosystem could be protected. However, to date, there have been no studies of the basic microbial ecology in this area. Since microorganisms are responsible for biogeochemical reactions and are the foundation of marine food webs, it is important to increase our basic knowledge of the microbial community structures within this ecosystem.

The question of “who is there” remains essential to the understanding of microbial community structure and function. The common understanding, however, is that <1% of marine microbes can be cultured (Fuhrman et al. 1992; Rappé and Giovannoni 2003), and the culturing process becomes more difficult with increasing depth (Simonato et al. 2006). Culture-independent surveys of rRNA genes have greatly expanded our knowledge of the phylogenetic diversity of microorganisms. In particular, denaturing gradient gel electrophoresis (DGGE), a method used to separate PCR-amplified DNA fragments based on nucleotide sequence differences (Muyzer et al. 1993), is an extensively used rRNA gene screening method for fast and semiquantitative assessment of the diversity of dominant microbial species, and it allows high sample throughput with DNA-based phylogenetic resolution for an entire community. DGGE can

also be coupled with the sequencing of separated 16S rDNA fragments to rapidly determine taxonomic information about the dominant members of a microbial community. Because large samples can be processed using DGGE, throughput is often higher than that of clone libraries for comparing microbial assemblages across space (Casamayor et al. 2002). It is well documented that many of the new microbial groups that have been discovered via these culture-independent surveys are involved in important processes within natural microbial assemblages in marine systems (Karl 2002).

Using 16S rRNA gene PCR–DGGE, we compared the community composition and the phylogenetic relationships between communities at a variety of depths, from the euphotic zone to the deep sea. This microbial phylogenetic study in the Gully is the first step in the characterization of the microbial community in this previously unsurveyed water column. We hypothesized that the prokaryotic communities experience a high degree of dissimilarity across the vertically stratified water column and are influenced by the strong selective pressure of the Gully’s unique abiotic characteristics. The primary objective of this work was to identify the dominant bacterial and archaeal taxa under these abiotic selective pressures. The findings of this research will provide a preliminary characterization of the microbial communities of this unique ecosystem.

Materials and methods

Site description and sample collection

Four water samples were collected from depths of 1972,

Fig. 2. Cluster analysis of DGGE banding patterns based on band positions using unweighted pair group method using arithmetic average of an S_{AB} matrix. (A) Dendrogram for bacterial DGGE; bands collected for sequencing are marked with letters (a–v) for 10 m sample and with numbers (1–19) for ≥ 500 m samples. (B) Dendrogram for archaeal DGGE; bands collected for sequencing are marked with numbers (1–8). The depths of the different samples are marked below the lanes.

1000, 500, and 10 m at a station located at 43°50'33"N, 58°54'10"W during a CCGS Hudson oceanographic expedition in June 2006. All samples were collected using the Seabird Niskin rosette frame (twenty-four 10 L bottles) containing a Seabird conductivity, temperature, and depth (CTD) detector, a Chelsea AquaTracka Mk3 fluorometer, and a Seabird E43 oxygen detector. The fluorometer was set to 430 nm excitation and 685 nm emission wavelengths for phytoplankton chlorophyll *a* determination. The fluorometer was calibrated with various concentrations of chlorophyll *a* dissolved in pure water, and the zero offset was determined in the laboratory using purified water from a reverse osmosis – ion exchange column. CTD data were plotted with SigmaPlot version 10.0 (Systat Software, Inc. 2006). Acid-washed Nalgene jerrycans were rinsed 3 times with sample water before sample collection. Each individual water sample was immediately filtered through a sterile

0.22 μ m GSWP filter (Millipore, Bedford, Mass.). Five litres of water were filtered for samples from 1000 m and below, but only 4.5 and 1.75 L were filtered for the 500 and 10 m samples, respectively. Following filtration, all filters were transferred to sterile 50 mL Falcon tubes and stored at -20°C until further analyses.

DNA extraction

Total community DNA was prepared by a modified version of the method used by Fortin et al. (1998). Prior to lysis treatment, each 50 mL Falcon tube containing the filter (from the water sample filtering step) was filled with 4.5 mL of sterilized distilled water. A 500 μ L aliquot of 250 mmol/L Tris-HCl (pH 8.0) and 50 mg lysozyme were added, and the samples were incubated for 1 h at 37°C with mixing at low speed. Fifty microlitres of proteinase K (20 mg/mL) was added to the samples, and they were incu-

Fig. 3. Phylogenetic relationship of the 23 bacterial 16S rRNA gene sequences obtained from the 10 m water sample. The bands were labeled with Gully bacteria surface (GBS) and their band letters from Fig. 2A. The tree was inferred by neighbor-joining analysis of sequence from each clone. *Aquifex pyrophilus* was used as the outgroup. Numbers on the nodes are the bootstrap values based on 1000 replicates. The scale bar indicates the estimated number of base changes per nucleotide sequence position.

bated for 1 h at 37 °C with mixing at low speed. The lysis treatment was completed with the addition of 500 mL of 20% SDS solution and 30 min of incubation at 85 °C with gentle mixing. The filter was then removed from the 50 mL Falcon tube. The lysates were treated with 0.5 volume of 7.5 mol/L ammonium acetate, incubated on ice for 15 min to precipitate proteins and humic acids, and centrifuged for 15 min at 4 °C (9400g). The supernatants were transferred to a sterilized Corex tube and treated with 1 volume of cold 2-propanol. The DNA was precipitated overnight at –20 °C, after which samples were centrifuged at 4 °C for 30 min (12 100g). Pellets were washed with 70% cold ethanol and air dried, and the DNA was resuspended in 250 µL of Tris–EDTA (pH 8.0) (10 mmol/L Tris and 1 mmol/L EDTA). DNA concentrations were estimated by agarose gel electrophoresis of 5 µL of purified material against the λ *Hind*III DNA ladder (Amersham Biosciences, Piscataway, N.J.) standard on a 0.7% agarose gel.

PCR amplification of 16S rRNA gene

For PCR amplification of the 16S rRNA gene, the *Bacteria*-specific forward primer U341F (5′-CCTACG-GGAGGCAGCAG-3′) (Muyzer et al. 1993) and the reverse primer U758R (5′-CTACCAGGGTATCTAATCC-3′) (Fortin et al. 2004) were used. These primers, complementary to conserved regions of 16S rRNA gene, were used to amplify a 418 bp fragment corresponding to positions 341–758 in the *Escherichia coli* sequence and covered the variable regions V3 and V4. The *Bacteria*-specific forward primer used for DGGE possessed a GC clamp (5′-GGCGGGGCG-GGGGCACGGGGGGCGCGGGCGGGGCGGGGCGGGG-3′) at the 5′ end. This GC clamp stabilizes the melting behavior of the amplified fragments (Sheffield et al. 1989). The 16S rRNA gene from *Archaea* was PCR amplified using the *Archaea*-specific forward primer ARC344F (5′-ACGGG-GYGCAGCAGGCGCGA-3′) and the reverse primer ARC915R (5′-GTGCTCCCCCGGCAATTCCT-3′). These primers, complementary to conserved regions of the 16S rRNA gene, were used to amplify a 572 bp fragment corresponding to positions 344–915 in the *Escherichia coli* sequence. The *Archaea*-forward primer used for DGGE possessed another GC clamp (5′-CGCCCGCCGCGCCCCG-CGCCCGTCCCGCCGCCCCCGCCCG-3′) at the 5′ end. Each 50 µL PCR mixture contained 1 µL of the template DNA (undiluted, 10^{–1} or 10^{–2}), 25 pmol of each oligonucleotide primer, 200 µmol/L of each dNTP, 1 mmol/L MgCl₂, and 2.5 U of *Taq* polymerase (Amersham Biosciences) in 10× *Taq* polymerase buffer (100 mmol/L Tris–HCl pH 9.0, 500 mmol/L KCl, 15 mmol/L MgCl₂). Briefly, after an initial temperature of 96 °C for 5 min and thermocycling at 94 °C for 1 min, the annealing temperature was set to 65 °C (for bacterial PCR) or 60 °C (for archaeal PCR) for 1 min and decreased by 1 °C every cycle for 10 cycles, followed by a 3 min elongation time at 72 °C. Additional cycles (15–20) were performed with

annealing temperatures of 55 °C for *Bacteria* and 50 °C for *Archaea*. PCR products were loaded onto a 1% agarose gel with SYBR Safe (Molecular Probes, Eugene, Ore.), using a 100 bp ladder (MBI Fermentas, Amherst, N.Y.) to determine the presence, size, and quantity of the PCR products.

Denaturing gradient gel electrophoresis

The 16S rRNA gene products from 8 individual PCRs were combined for each sample and concentrated by ethanol precipitation for DGGE analysis. About 450 ng of the 16S rRNA gene product from each sample was applied to a lane and analyzed in an 8% polyacrylamide gel containing a gradient of 30%–70% denaturant (100% denaturant consisted of a solution with 7 mol/L urea and 40% deionized formamide). DGGE was performed using a DCode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, Calif.). Electrophoresis was run at a constant voltage of 80 V for 16 h at 60 °C in 1× TAE running buffer. The gels were then stained with VistaGreen (Amersham Biosciences) and imaged with the FluoroImager System Model 595 (Molecular Dynamics, Sunnyvale, Calif.). The gel images were analyzed using GelCompar II version 4.6 (Applied Maths, Sint-Martens-Latem, Belgium) to generate dendrogram profiles. The genotypes were visually detected based on the presence or absence of bands in each lane. A band was defined as “present” if its peak intensity was at least 3% of the most intense band in the sample. After conversion and normalization of gels, the degrees of similarity of DNA pattern profiles were computed using the Dice similarity coefficient (Dice 1945), and dendrogram patterns were clustered by the unweighted pair group method using arithmetic average groupings with a similarity coefficient (S_{AB}) matrix.

Sequencing analysis and phylogenetic analyses

Individual bands from the DGGE gels were excised and eluted with 25 µL dH₂O for 48 h at 4 °C before being reamplified with the same set of primers without the GC clamp. One microlitre of DNA was reamplified with the appropriate corresponding eubacterial or archaeal primers as follows: an initial denaturation of 5 min at 96 °C, followed by 30 cycles of 94 °C for 1 min, 60 °C (bacterial) or 55 °C (archaeal) for 30 s, and 72 °C for 1 min. PCR products for sequencing were purified using Illustra GFX PCR DNA and gel band purification kit (GE Healthcare, Piscataway, N.J.). Sequencing was performed at the Université Laval Plate-forme d’analyses biomoléculaires using a model ABI Prism 3130XL (Applied Biosystems, Foster City, Calif.) with their respective primers. Raw sequence data were assembled in BioEdit version 7.0 (Hall 1999). The sequences were manually aligned by comparing forward and reverse sequences. Complete aligned sequences containing the primer regions at both ends were used for further sequencing and phylogenetic analyses. The occurrence of chimeric sequences was

Fig. 4. Phylogenetic relationship of the 14 bacterial 16S rRNA gene sequences obtained from water samples at and below 500 m. The bands were labeled with Gully bacteria deepsea (GBD) and their band numbers from Fig. 2A. The depth from which the band was excised is indicated in parentheses after the accession No. The tree was inferred by neighbor-joining analysis of sequence from each clone. *Aquifex pyrophilus* was used as the outgroup. Numbers on the nodes are the bootstrap values based on 1000 replicates. The scale bar indicates the estimated number of base changes per nucleotide sequence position.

determined manually with the Check Chimera function from the Ribosomal Database Project II (<http://wdecn.nig.ac.jp/RDP/cgis/chimera.cgi?su=SSU>; Cole et al. 2003) and Bellerophon (<http://foo.maths.uq.edu.au/~huber/bellerophon.pl>; Huber et al. 2004). Close relatives of the final selection of different sequences (phylotypes) were tentatively identified by NCBI BLASTN search (<http://ncbi.nlm.nih.gov/blast/>). Sequences were aligned by the MacVector 9.0 software package (Accelrys, Cary, N.C.) with both closely related representatives from NCBI BLASTN as well as novel complete and partial sequences obtained from GenBank. Additional manual alignment was done if necessary. Phylogenetic relationships were constructed with evolutionary distances (Jukes–Cantor distances) and the neighbor-joining method using the MacVector software package. The bootstrap analyses for the phylogenetic trees were calculated by running 1000 replicates for the neighbor-joining data.

Nucleotide sequence accession No.

The 16S rRNA gene sequences obtained in this study have been deposited in the GenBank database under accession No. GQ372917 to GQ372965.

Results

Environmental characteristics of the water column

The depth profiles for temperature, salinity, oxygen content, and chlorophyll *a* content of the water column are shown in Fig. 1. All 4 parameters were very stable below 1000 m but highly variable above 1000 m. The upper water column temperature was highly stratified with 2 major thermoclines, the first showing a temperature maximum of ~16 °C for the 10 m sample and a second temperature maximum of 10 °C at 125 m. The first thermocline was present at depths between 10 and 50 m. The temperature declined rapidly to form the first temperature minimum of 6 °C (Fig. 1B). In addition, a much deeper and smaller thermocline was present at depths between 125 and 500 m, bringing the temperature from 10 °C (the second temperature maximum) to a constant lower water column temperature of around 4 °C. Similarly, there were 2 major haloclines in the upper water column. The first halocline was present at depths between 10 and 100 m. The freshest water had a salinity of 32.5 ppt around 10 m (Fig. 1B), after which salinity increased to ~35 ppt at 100 m. The second major halocline was present between 150 and 250 m, where the salinity decreased to 34.9 ppt and remained uniform to the bottom (Fig. 1A). The chlorophyll *a* concentration was high at depths above 100 m, with a maximum of 0.68 µg/L at 35 m, but declined dramatically below 100 m to near the detection limit (Fig. 1B). Dissolved oxygen exhibited values that matched those of the other parameters. The oxygen maximum of 6 mg/L was present at ~30 m, just above the chlorophyll maximum. The oxygen minimum of 3.5 mg/L was present at ~200 m, just below the second temperature

maximum, after which the oxygen content remained relatively unchanged at around 5.2 mg/L to the bottom (Fig. 1A). The Niskin rosette frame deployed from the ship enabled the collection of physicochemical data from its probes in real time during its downcast and the recovery of discrete water samples at defined depths during its recovery. Therefore, the samples that were collected represent the 2 most extreme physical conditions in the vertical profile. The surface sample from the photic zone of the water column experienced warmer temperatures and lower salinity, as compared to the deep-water samples from the aphotic zone of the water column, which exhibited lower temperature and higher salinity and hydrostatic pressure profiles.

Archaeal and bacterial DGGE analysis

DGGE analysis of bacterial and archaeal 16S rRNA genes was performed to compare the microbial composition of surface-, mid-, and deep-water samples, and dendrograms of the DGGE banding patterns were constructed for statistical analysis. The dendrograms for both *Bacteria* and *Archaea* revealed a division into 2 clusters based on their sampling depths. Cluster analysis of the dendrograms demonstrated lower S_{AB} values between the surface water and 500 m, with 72.7% for *Bacteria* (Fig. 2A) and 49.3% for *Archaea* (Fig. 2B). The S_{AB} values for water samples below 500 m were much higher, with 90%–100% for *Bacteria* (Fig. 2A) and 86.8%–94.1% for *Archaea* (Fig. 2B). Therefore, both bacterial and archaeal DGGE analyses revealed dissimilarities between the community structures of the samples collected from the surface down to 500 m and high similarities between those collected below 500 m.

Bacterioplankton composition in the Gully

Specific phylogenetic information was determined by sequencing of the individual DGGE bands. All bands that migrated to the same position in the gel were first sequenced, then aligned with each other and assigned to an operational taxonomic unit (OTU). If the sequences were identical, they were assigned to the same OTU. The bacterial DGGE gel displayed a much higher number of bands than the archaeal DGGE gel. Both bacterial and archaeal banding patterns, except the archaeal surface sample, showed a high microbial diversity. In the bacterial surface sample, a total of 22 DGGE bands of 26 bands were excised and sequenced. Most of the sequences showed at least a 97% match to the available sequences from GenBank; however, most of them were closely related to uncultured bacteria, but generally clustered well into 1 of the 9 major marine lineages. In the surface sample, most of the sequences were grouped into *Alphaproteobacteria* (45.5% of the sequences) and *Bacteroidetes* (45.5% of the sequences). Only 2 of the 22 sequences were related to *Gammaproteobacteria*, which is the only other group in the phylogenetic tree (Fig. 3).

In the samples below 500 m, 19 DGGE bands of a total of

Fig. 5. Phylogenetic relationship of the 8 archaeal 16S rRNA gene sequences obtained from all depth samples. The bands were labeled with Gully *Archaea* (GA) and their band numbers from Fig. 2B. The depth from which the band was excised is indicated in parentheses after the accession No. The tree was inferred by neighbor-joining analysis of sequence from each clone. *Aquifex pyrophilus* was used as the outgroup. Numbers on the nodes are the bootstrap values based on 1,000 replicates. The scale bar indicates the estimated number of base changes per nucleotide sequence position.

28 bands were excised and sequenced. As in the surface sample, most of the sequences were closely related to uncultured bacteria, as in the surface sample, but again they clustered well into 1 of the 9 major marine lineages. However, the sequences belonged to more diverse groups. *Bacteroidetes* was again represented with the highest number of sequences (47%). Only 1 sequence was identified as *Alphaproteobacteria*. All other sequences belonged to *Gammaproteobacteria*, *Actinobacteria*, *Deltaproteobacteria*, *Gemmatimonadetes*, and an unknown group (Fig. 4).

In the archaeal DGGE gel, a total of 8 bands were excised and sequenced. Many of the faint bands were unique only to the surface water. However, because of the resolution limitation of the gel, we were only able to excise and sequence the dominant band (Fig. 2B). The sequence was related to an uncultured group II *Euryarchaea* from oxic coastal surface water (Fig. 5). The other 7 bands were from below 500 m. Most of them showed a high similarity to available sequences in GenBank from uncultured *Crenarchaea* and *Euryarchaea* (Fig. 5). All sequences clustered well within the 2 common major marine *Archaea* groups: *Archaea* group I and *Archaea* group II.

Discussion

Numerous studies have looked at the 16S rRNA gene to assess microbial diversity in surface-water samples (Giovannoni et al. 1990; DeLong 1992; Fuhrman et al. 1992) and deep-sea samples (DeLong et al. 1997; Kato et al. 1997; Nogi et al. 1998). However, only a limited number of studies have documented a complete depth profile. These full-depth profile studies revealed a shift of microbial community composition over the transition from the photic to aphotic zones using various culture-independent methods (Lee and Fuhrman 1991; Karner et al. 2001; DeLong et al. 2006; Celussi et al. 2009). Similarly, our findings showed a

clear difference in microbial community structure between the photic and aphotic zones (Fig. 2A, 2B).

Since each band on the DGGE gel generally represents a major species that constitutes at least 1% of the total population in a sample (Muyzer et al. 1993), the high number of bands from the DGGE analysis suggests that the Gully water column has a diverse microbial community. Similar to other marine environments, 16S rRNA gene analysis of the Gully samples also showed that the bacterioplankton sequences generally clustered into 1 of the 9 major marine lineages that are commonly found in marine systems: *SAR11*, *Roseobacter/SAR83*, *Gammaproteobacteria/SAR86*, *SAR116*, *Chloroflexi/SAR202*, *Deltaproteobacteria/SAR324*, *Chlorobium/SAR406*, *Cyanobacteria*, and *Bacteroidetes*. However, not all of the major lineages were found at all different depths. Our results showed that *SAR11* and *SAR116* sequences (both *Alphaproteobacteria* clusters) were only present in the surface sample and that *SAR324* (*Deltaproteobacteria*) and *Actinobacteria* sequences were only present below the aphotic zone. Similarly, DeLong et al. (2006) and Sogin et al. (2006) found an increase in the relative abundance of *Deltaproteobacteria* and *Actinobacteria* with increasing depth. A recent study also found that *Actinobacteria* showed a much greater presence in the deep ocean (Jensen and Lauro 2008). Zaballos et al. (2006) have observed that the increase in the percentage of *Deltaproteobacteria* with depth often co-occurs with a decrease in *Alphaproteobacteria*, based on their observations from the Mediterranean and Greenland seas. Morris et al. (2002) and Alonso-Sáez et al. (2007) found that the *SAR11* clade dominated in the ocean surface. Our sequencing results also revealed that 2 of the major clades of *Alphaproteobacteria*, *SAR11* and *SAR116*, were only present in the surface sample. The *Alphaproteobacteria* decreased from 10 representatives in the surface sample to only 1 representative in the deep-water samples,

and this sample is from the *SAR83/Roseobacter* group. Generally, *Alphaproteobacteria* are most competitive at low ambient nutrient concentrations (Pinhassi and Berman 2003) and are better at glucose uptake at low concentration than *Gammaproteobacteria* and *Bacteroidetes* (Alonso and Pernthaler 2006). Glucose is typically the most abundant free neutral aldose in the upper water column in seawater (Skoog et al. 1999). Therefore, the higher diversity of *Alphaproteobacteria*, present mostly in the upper water column, might be related to the higher glucose concentration typical of the upper water column.

We also found an increase in the number of *Gammaproteobacteria* sequences in deep-water samples. Most of the piezophilic microorganisms that have been isolated from many regions of the world belong to the genera *Colwellia* and *Shewanella* within the *Gammaproteobacteria* (DeLong et al. 1997; Kato et al. 1998; Nogi et al. 1998). Zaballo et al. (2006) also found that this increase in the percentage of *Gammaproteobacteria* with increased depth is usually associated with a decrease of *Alphaproteobacteria*, based on their observations from the Mediterranean and Greenland seas.

Interestingly, our phylogenetic tree also revealed a high population of *Bacteroidetes* in both water layers. Usually, *Bacteroidetes* are the most abundant group of bacteria in coastal pelagic habitats (Cottrell and Kirchman 2000; Eilers et al. 2001). Some findings (summary from Kirchman 2002) suggested that members of this phylum could play an important role in the degradation of complex and polymeric organic matter in marine algae in the euphotic zone and of marine snow in the aphotic zone. *Bacteroidetes* are commonly found in high abundance during natural and induced phytoplankton blooms and as primary colonizers of marine phytoplankton, further suggesting a role in algae-derived metabolite consumption (Riemann et al. 2000; Pinhassi and Berman 2003; Grossart et al. 2005). Therefore, high abundances of the *Bacteroidetes* in offshore oligotrophic water suggest a high primary productivity in the Gully from top to bottom. In the deep-water samples, the *Bacteroidetes* was by far the most diverse group, suggesting that the degradation of complex and polymeric organic matter might be occurring throughout the water column.

The archaeal phylogenetic tree showed that the group II euryarchaeal group was the only major band and the only sequence from the surface-water sample. A similar finding to that of Massana et al. (1997) showed that the group II euryarchaeal group dominated in the surface water. The archaeal phylogenetic tree also showed an increase in the number of sequences related to the group I *Crenarchaea* in deeper water samples. Karner et al. (2001) and Massana et al. (1997) found a relative 16S rRNA gene abundance of the crenarchaeal group, which suggests that its members constitute a significant fraction of the prokaryotic biomass in subsurface waters.

Comparison of phylogeny and physical characteristics

The phylogenetic analysis from the different depths corresponded well with the common oceanographic physical parameters in the vertical water column. Differences in physical parameters resulted in an altered microbial community structure. When the abiotic conditions remained con-

stant, the microbial community structures also remained unchanged. Low temperature, absence of solar radiation, and especially high hydrostatic pressure are the characteristics of this environment (Bartlett 1992), and in general, they are physically relatively uniform across space (Fuhrman et al. 1992). The hydrostatic pressure can be more than 3 orders of magnitude higher at the bottom of the Gully than at the surface, and this drastic change in pressure was previously found to be the major factor contributing to the change in microbial community structure (Celussi et al. 2009; Yoshida et al. 2007). However, DGGE results from the deep-water samples showed that the microbial community structure was similar between environments where other abiotic conditions such as temperature, salinity, chlorophyll *a* content, and oxygen content remained nearly constant. This suggests that the hydrostatic pressure does not have as much of an impact on shaping microbial community structure as temperature, salinity, chlorophyll *a* content, and oxygen content. Interestingly, in a recent full-depth profile study, Celussi et al. (2009) found that, in the epipelagic zone, light-affected variables (i.e., oxygen content and fluorescence) had a greater influence than temperature on bacterial community structure. High variations in temperature, salinity, chlorophyll *a* content, and oxygen content were observed in the epipelagic zone in the Gully (Fig. 1B). Further investigation focusing on the epipelagic zone is required to complete the full characterization of this valuable marine protected area.

In general, the aphotic zone is the largest fraction of the ocean, and is populated by a high diversity but low abundance of organisms (Fuhrman et al. 1992; Sogin et al. 2006). It is believed to contain 55% of all the prokaryotes found in all aquatic habitats (Whitman et al. 1998). The findings of this research provide a preliminary characterization of the microbial communities of this valuable marine protected area and contribute to a better understanding of similar unsurveyed ecosystems.

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