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Bacterial community composition and diversity along the southern coastlines of the Atlantic Ocean in Cape Town, South Africa

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The spatial distribution and diversity within bacterioplankton assemblages in four coastal sites along the southern points of the Atlantic Ocean were examined using the Illumina high-throughput that targets 16S rRNA genes to examine indigenous bacterial assemblages in the littoral zones along the coast of the ocean. Results of the study showed very similar bacterial representation between the coastal sites with majority of the sequences affiliated with members of the *Proteobacteria* (52 to 59%), *Bacteriodetes* (21 to 31%) followed by *Actinobacteria* (3 to 9.5%) and *Planctomycetes* (2.1 to 4.5%). The bacterioplankton assemblages at each site examined were quite diverse, with members of the Gammaproteobacteria found as the most abundant bacterial class among the four sites. However, clear differences were observed among the sites at the order level, with the *Chromatiales* the more dominant in the eastern coastal (CPTI) sites, while clades belonging to the *Flavobacteriales* and *Rhodobacterales* were more prevalent in the two western (CPTA) coastal sites. While the results of unweighted pair group method with arithmethic (UPGMA) clustering and principle coordinate (PCoA) revealed two spatially separate clusters among sites, canonical correspondence (CCA) analysis indicated that environmental variables such as temperature, pH and conductivity were probably the major influencers of bacterial occurrences at the coastal sites.

Key words: Bacterioplankton assemblages, ocean, 16S rRNA gene sequencing.

INTRODUCTION

The bacterioplankton assemblages in oceans have been described as comprising one of the largest and active microbial assemblages in the biosphere (Whitman et al.,1998; Salazar and Sunagawa, 2017), where they actively partake in the biogeochemical influxes and cycling of various nutrients and organic compounds (Azam and Malfatti, 2007; Falkowski et al., 2008, Zehr and Kudela, 2011). Contrasting findings have been previously reported regarding bacterial diversity and biogeographic distributions in marine systems, especially in tropical oceans (Pommier et al., 2007; Fuhrman et al., 2008; Milici et al., 2016). Milici et al. (2016) reported a

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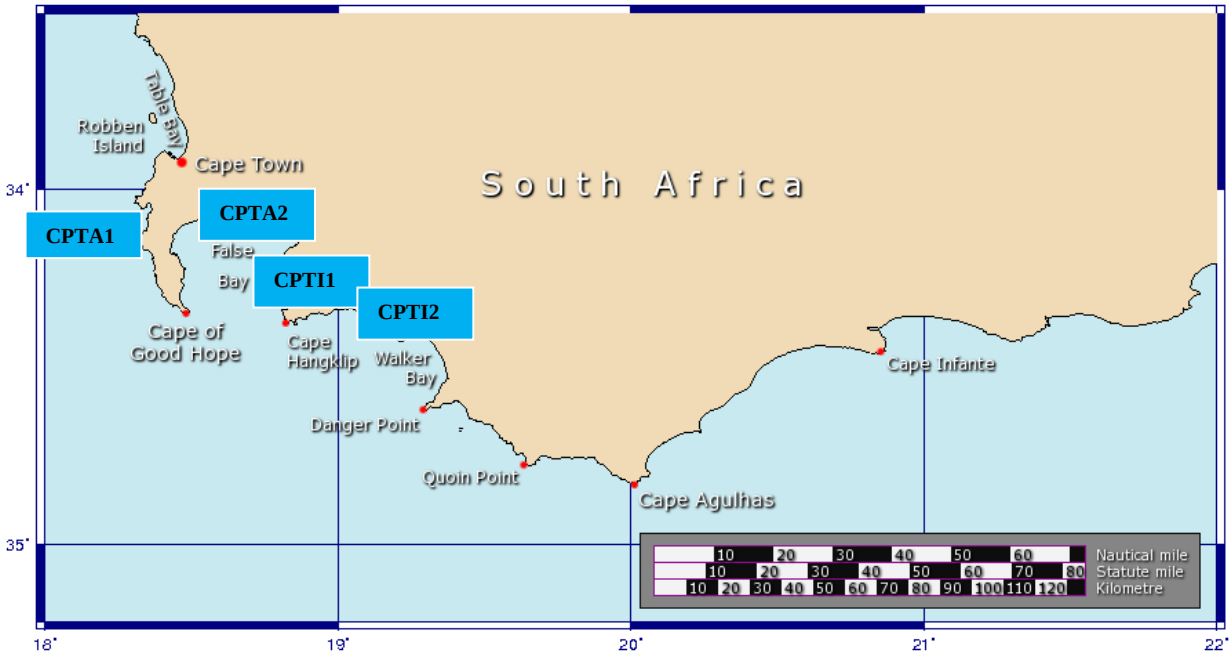


Figure 1. Map of study sites along the coastlines of the Atlantic and Indian Oceans in Cape Town, South Africa.

clear biogeographic pattern with a double inverted latitudinal gradient, with higher diversity in planktonic bacteria population in mid-latitudinal regions, and decreasing towards the equator in the Atlantic Ocean in their study. In contrast, Fuhrman et al. (2008) found a negative correlation between species richness and latitude both in the Northern and Southern hemisphere. Additionally, interesting observations regarding microbial provincialism and their discrete distributions in various marine habitats have been previously documented (Brown et al., 2009; Jeffries et al., 2015).

In order to better understand the structural compositions and diversity within bacterial assemblages in marine systems, this study was conducted on several coastal sites along the southern points of the Atlantic Ocean in Cape Town, South Africa by examining indigenous populations in the bacterioplankton communities using Illumina high-throughput sequencing approach that targets the 16S ribosomal RNA genes. The four sites that were selected for the study are located along the coastline within the metropolitan city of Cape Town, between the Cape of Good Hope and the Cape of Agulhas two touristy landmarks that are prone to various anthropogenic influences. The Atlantic Ocean is considered the second largest of the world's five oceans, second only to the Pacific Ocean, with a body of water located between Africa, Europe, the Arctic, America and the Southern Ocean. The southern parts of the Atlantic Ocean where this study was conducted is located around the metropolitan area of the city of Cape Town in South Africa, between the Cape of Good Hope (~34° 21´24.63" S, 18° 28´ 26.36" E) and Cape Agulhas (~34° 49´ 59.6" S, 20° 00´ 0" E), with mostly rocky headlands of coastlines in between these two landmarks that are about 90 miles apart (International Hydrographic Organization, 2002). The main aim of the study was to examine the taxonomic profiles of the microbial assemblages indigenous to these coastal marine sites as well as determine the influences of various environmental factors, such as temperature, pH and dissolved oxygen concentrations on the structural composition and diversity within the assemblages at the four sites.

MATERIALS AND METHODS

Sample collection and measurement of environmental variables

Samples were collected from the surface waters along the rocky headland coasts of the Atlantic Ocean in Cape Town, South Africa in September 2019. Specifically, water samples were collected from approximately 1 to 5 m depth into sterile falcon tubes from four separate sites along the ocean front close to the metropolitan city of Cape Town in South Africa (Figure 1). Collected samples were filtered through 0.2 µm pore-size polyethersulfone membrane filters and then stored frozen until nucleic acid extraction was performed. While on site, the water chemistry properties were also measured (Table 1) with probes for temperature, conductivity, pH, dissolved oxygen and oxidation-reduction potential using the YSI model 556 MPS multi-probe system (YSI Incorporated, USA).

DNA extraction and 16S rRNA gene pyrosequencing

Community DNA was extracted from the filters using FastDNA

Table 1. Environmental variables measured at the study sites.

Site	Latitude	Longitude	Temperature (°C)	Conductivity mS/cm	DO %	pH	ORP
CPTA1	34° 21' 25." S	18° 28' 26" E	14.80	48.45	127.60	8.92	-26.50
CPTA2	34° 21' 24." S	18° 28' 36" E	14.76	48.50	111	8.80	-16.50
CPTI1	34° 13' 36. " S	18° 28' 7." E	14.93	48.93	389.20	9.14	-23.60
CPTI2	34° 49' 59. " S	18° 28' 7." E	14.89	48.45	263.30	9.07	-20.50

SPIN Extraction kit (MP Biomedicals, Solon, OH, USA) and eluted in 50 µl of sterile deionized water according to the vendor's instructions. Determination of DNA quantity was then carried out with a NanoDrop Spectrophotometer (2% accuracy/range of purity, NanoDrop 2000, Thermo Scientific, Delaware, USA). The quality of extracted DNA was further assessed by amplifying with the 16S rRDA universal primer sets, 27F (5' AGA GTT GTA TCM TGG CTC AG 3') and 1492R (5'GGT TAC CTT GTT ACG ACT T3') as previously described in Olapade (2013, 2015).

The Illumina's 16S metagenomic sequencing library preparation protocol was used in generating amplicon libraries using universal primer pairs that consisted of an Illumina-specific overhang sequence and locus-specific sequence: 926wF_Illum: 5'-TCGTCGGCAGCGTCAGA TGTGTATAAGAGACAGAAACTYAAAKGAATTGRCGG and 1392R_Illum: 5'GTCTCGT GGGCTCGGAGATGTGTATAAGAGACAGACGGGCGGTGTGTRC . The pair of primers targets the V6-V8 hypervariable regions of 16S rRNA genes of all microbial groups (Jeffries et al., 2015).

Quality trimming and filtering of low-quality sequences

The raw pyrosequencing data was processes and analyzed using the open-source software program, Mothur (Mothur v. 1.36.1; http://www.mothur.org) as previously described (Schloss et al. 2009). Barcode and the fusion primers are trimmed before any of the bioinformatics commences. Sequences reads without a barcode or a primer region are dropped and not considered for further analysis. Low quality sequences that is, those less than 300 base pairs as well as those with less than average quality score (value of 25 or less) are filtered out and deleted (Zhang et al., 2012). Operational taxonomic units (OTUs) were constructed by comparing them to close relatives via global pairwise alignment (Altschul et al., 1997) to determine their close relatives using the BLASTN (blast.ncbi.nlm.nih.gov) system. Chimeras were detected in the sequences that were later omitted for further analysis by using the UCHIME version 4.1 program (Edgar et al., 2011).

Statistical and diversity analysis

The sequences were clustered into OTUs after setting 97% distance limit or cutoff similarity value (Tindall et al., 2009; Edgar et al., 2011) and then analyzed for species richness, Shannon Index, Simpson's (Reciprocal) Index of diversity, species evenness, ACE richness estimate and Chao-1 richness indicator (Chao, 1984, 1987; Chao and Lee, 1992; Schloss and Handelsman, 2006). In order to determine whether total diversity was covered by the numbers of sequences screened, Good's Library Coverage values were calculated as previously described (Good, 1953; Kemp and Aller, 2004). Alpha, beta and gamma diversity calculations were also carried out according to Whittaker (1972); in addition to rarefaction analysis that was performed to also determine the diversity of the clone libraries using the freeware program by CHUNLAB Bioinformatics Made Easy (CLcommunity version 3.30). Taxon exclusive (XOR) analysis was carried out based on the taxonomic assignment of sequencing read to reveal the sequences present in one library but absent in the others as described by Li and Godzik (2006). The UPGMA Fast UniFrac analysis was used to cluster the sequenced microbial communities based on phylogenetic relationship and abundance in order to generate a dendogram (Hamady et al., 2010), while the multi-dimensional UniFrac distance matrixes were then converted into vectors using the Principal coordinate analysis (PCoA) as described by Jolliffe (1989). Additionally, canonical correspondent analysis (CCA) was also used to analyze and examine which of the bacterial assemblages corresponds to the independent environmental variables that were measured at the study sites according to Ter Braak and Verdonschot (1995).

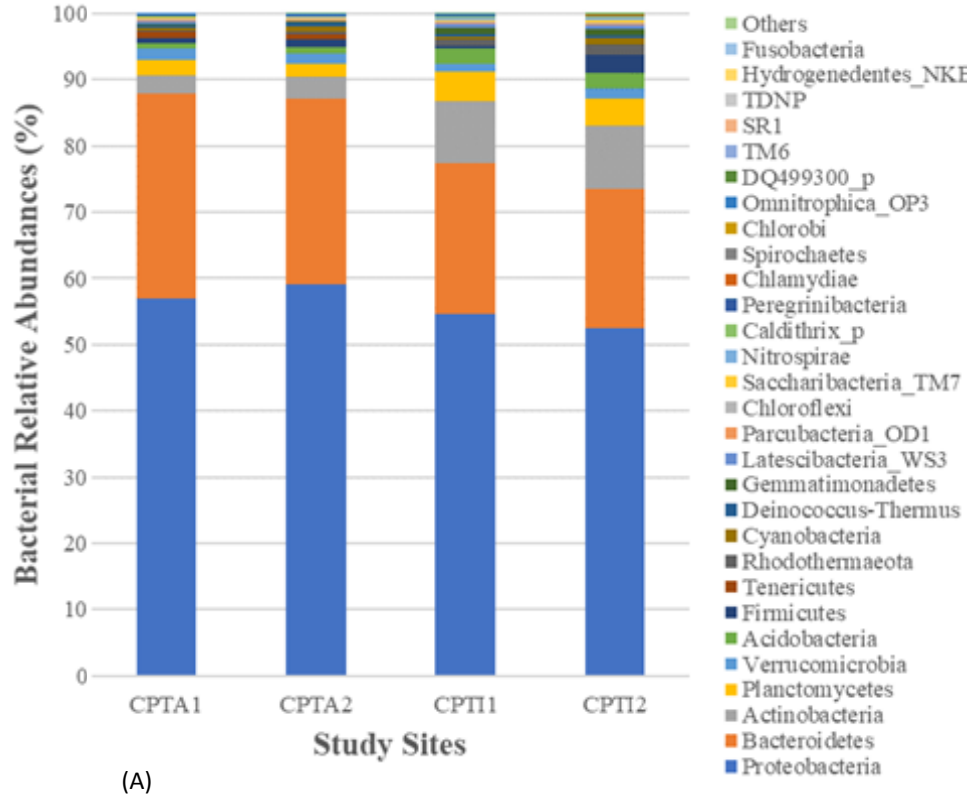
RESULTS

Environmental variables

The environmental variables measured at the four coastal sites examined along the ocean front included temperature, pH, dissolved oxygen (DO), conductivity and oxidation-reduction potential (ORP). Most of these variables were quite similar among the studied sites, with the exception of DO that was relatively higher in the two eastern sites (CPTI1 and CPTI2) closest to the Indian Ocean. Specifically, water temperature among the four coastal sites ranged from 14.76 to 14.89°C and slightly higher in the two easterly located sites, while pH was also in the range of 8.80 to 9.14 between the western and the eastern sites of the ocean, respectively (Table 1).

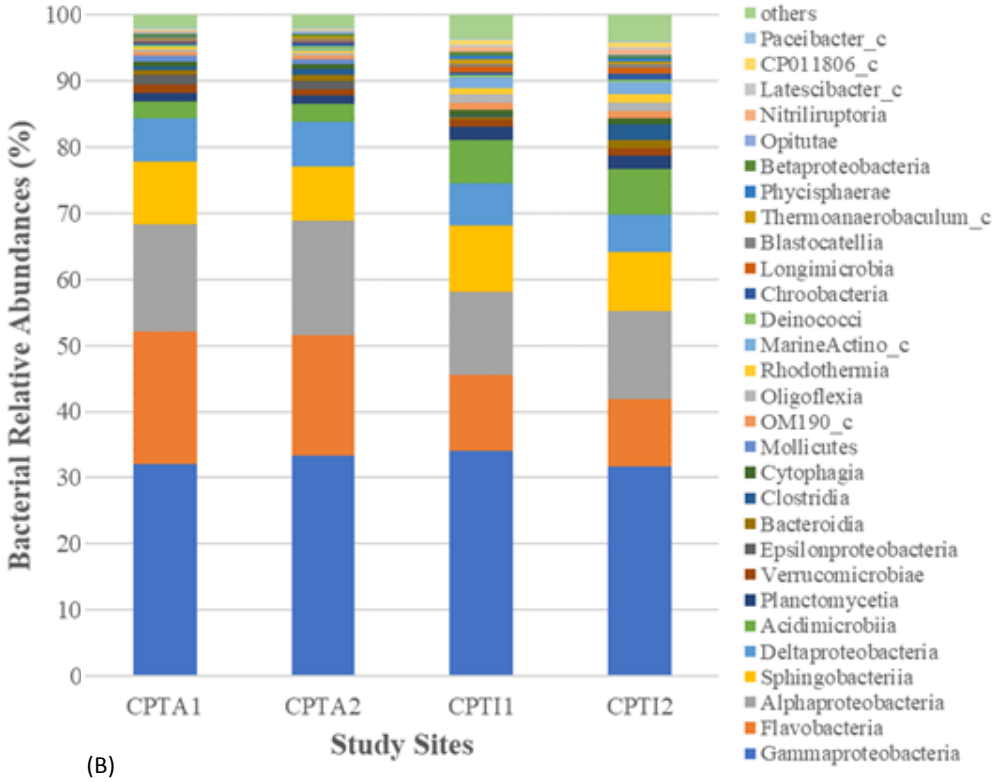
Community composition and diversity analysis

Based on the 16S ribosomal RNA gene sequencing, the relative abundance of bacterial taxa was determined at different taxonomic levels, and majority of the bacterial sequences (~99%) were ascribed to 29 different known bacterial phyla. Out of these 29 different phyla, bacterial members belonging to the *Proteobacteria, Bacteroidetes, Actinobacteria, Planctomycetes* and *Verrucomicrobia* were observed to have accounted for more than 90% of total community compositions among the sequences obtained from the four coastal sites (Figure 2A). Specifically, members of the *Proteobacteria* were the most numerically dominant phyla in the four sites,



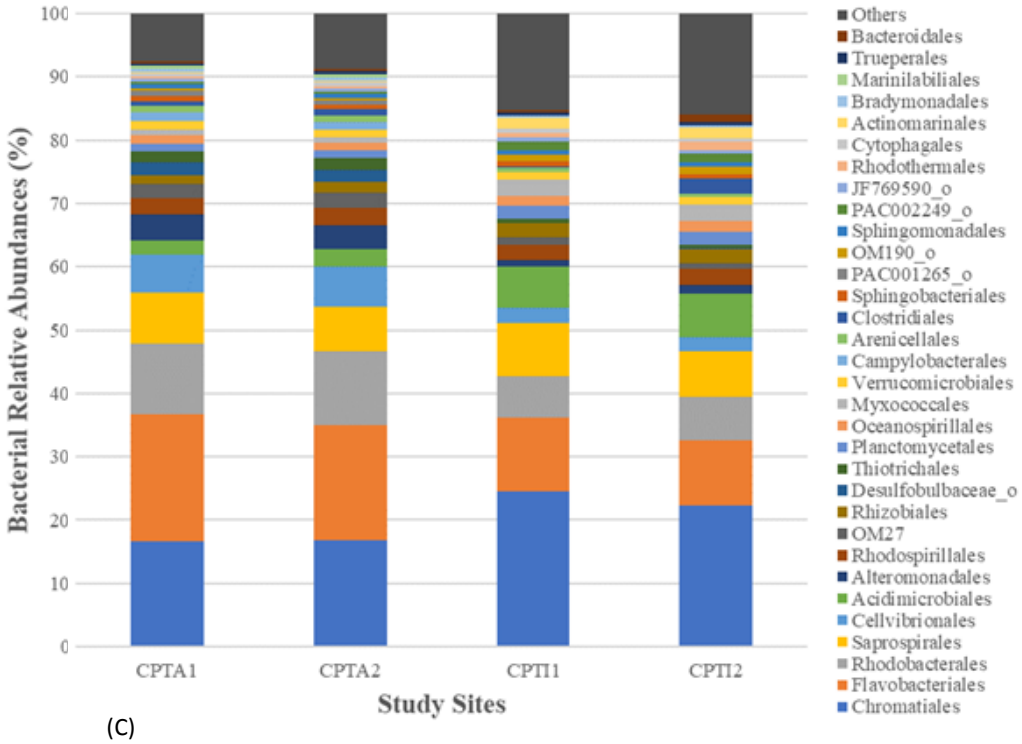
(A)

Figure 2A. Relative abundances of bacterial phylogenetic taxa at the phylum level.



(B)

Figure 2B. Relative abundances of bacterial phylogenetic taxa at the class level.



(C)

Figure 2C. Relative abundances of bacterial phylogenetic taxa at the order level.

Table 2. Community diversity analysis of the 16S ribosomal RNA gene sequences from the Bacterioplankton of the Atlantic and Indian Oceans in Cape Town, South Africa.

Sample	Valid reads	OTUs	Ace	Chao1	JackKnife	NPShannon	Shannon	Simpson	Good library coverage
CPTA1	225897	4712	4846.97	4761.60	5027	6.28	6.26	0.01	99.86
CPTA2	30087	1838	1948.57	1901.30	2055	6.18	6.11	0.01	99.28
CPTI1	44435	2942	3083.15	3010.00	3243	6.70	6.63	0.00	99.32
CPTI2	38470	2910	3087.23	2990.90	3254	6.82	6.73	0.00	99.10

accounting for between 52 and 59% of total bacterial sequences. The *Bacteroidetes* were in close second with between 21 to 31% sequence representations, followed by members of the *Actinobacteria* and *Planctomycetes* (between 3 to 9.50% and 2.10 to 4.50%, respectively). The next 25 bacterial phyla (including the members of the *Acidobacteria, Firmicutes, Tenericutes, Rhodothermaeota, Cyanobacteria, Deinoccocus-Thermus, Gemmatimonadetes*, among others) represented less than 10% of the total bacterial sequence abundance. Among the *Proteobacteria,* members of the *Gammaproteobacteria* were the predominant class, representing between 32 to 34%, followed closely by the *Alphaproteobacteria* with 13 to 14% representation among the four studied sites (Figure 2B). The *Chromatiales* were the dominant groups among the members of the *Gammaproteobacteria* at the order level, followed closely by the *Flavobacteriales* and the *Rhodobacterales*, both members of the *Flavobacteria* and *Alphaproteobacteria* classes, respectively (Figure 2B).

The Good Library Coverage analysis revealed that majority of the bacterial sequences was covered among the sites (Table 2). This result is also somewhat corroborated by the rarefaction curves that showed sufficient coverage in the numbers of the different bacterial phyla contained within three of the four assemblages examined at the coastal sites (Figure 3). The rarefaction curves revealed that three of the four microbial assemblages examined were tending towards saturation, with the exception of the CPTA1 site, that also had the highest Good Library Coverage of 99.86%. Diversity measures such as the Shannon diversity index

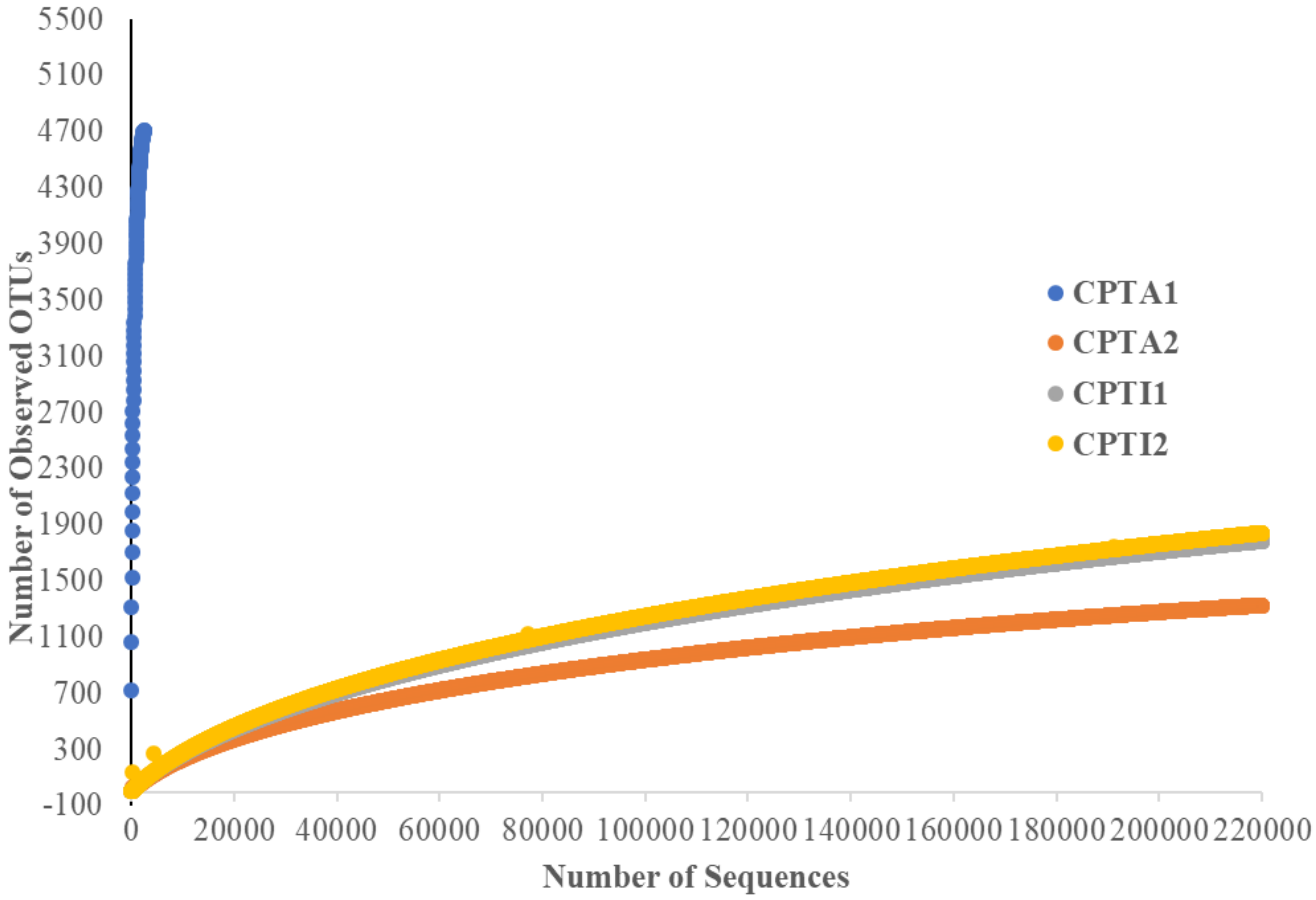


Figure 3. Rarefaction curves of OTUs based on 16S rRNA sequences from the bacterial assemblages from the study sites

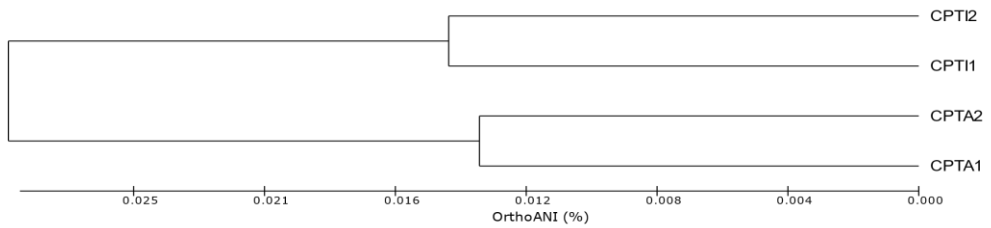
showed that bacterial diversity was comparatively higher in the two eastern coastal sites (CPTI1 and CPTI2) than in the two western Atlantic Ocean sites that were examined. Results of bacterial richness and species diversity based on ACE and Chao1 while not showing a distinct delineation among the four coastal sites, however revealed that the CPTA1 site had the highest diversity compared to the three other sites. This result is further validated by the results of the taxon exclusive analysis of the bacterial assemblages at the phylum level that showed disparate differences in bacterial diversity among the sites (Table 3). The bacterial assemblages found within the CPTA1 coastal site comprised of several bacterial phyla that were totally absent in the three other sites including the *Armatimonadetes, TM6, Lentisphaerae, BRC1, TDNP, SR1*, *Elusimicrobia, Fibrobacteres and WS6*. While the CPTA2 site comprised of *Deferribacteres* that were absent in the other sites, and phyla belonging to *Aminicenantes*, *GN04* and *Synergistetes* were exclusively found in CPTI1 and CPTI2, respectively.

Hierarchical clustering based on the Fast UniFrac distance matrix revealed that the bacterial sequences obtained from the two bacterioplankton assemblages in the western Atlantic Ocean sites were more similar, but were a bit distant from those in the easterly located assemblages (Figure 4). The PCoA that was also carried out to further explain the variations in bacterial community compositions between the four coastal sites also corroborated the results of the UPGMA clustering. Three axes were extracted that together explained 90.1% of the observed variance and showed that the bacterial assemblages within the two western sites (CPTA1 and CPTA2) clustered along the PC1 axis, while those from the eastern sites of the ocean (CPTI1 and CPTI2) clustered around the PC2 axis (Figure 5).

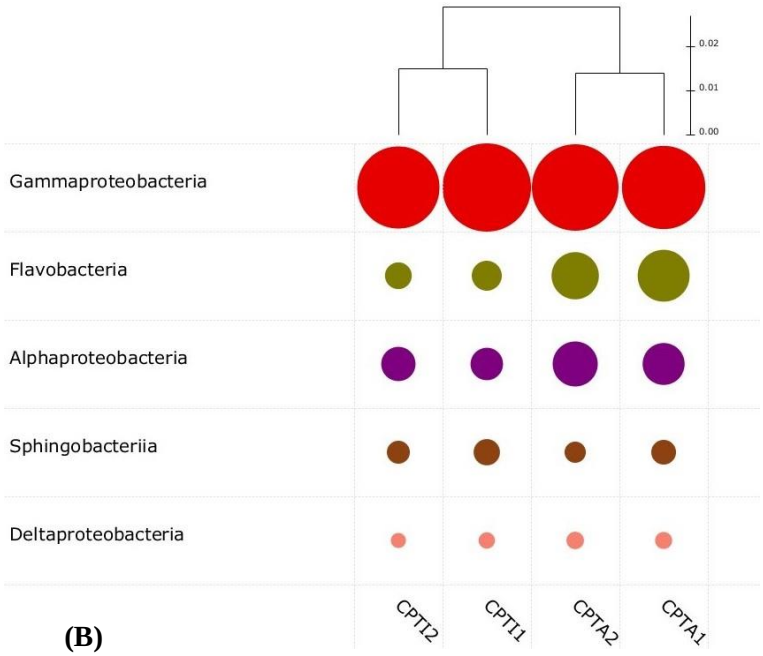
CCA was carried out to better understand bacterial distribution patterns along the coastal sites, especially regarding the spatial occurrences of the various environmental factors that were measured. Therefore, temperature, pH, conductivity, DO and ORP were included in the CCA analysis. The environmental variables in the two CCA axes (that is, CCA1 and CCA2) together explained more than 98.46% of total variations in the bacterial abundance distribution (Figure 6). Temperature, pH, conductivity and DO ($p < 0.01$) all contributed significantly to the total variance and were

Table 3. Result of taxon exclusive analysis at the phylum level to detect taxa that are present in one bacterioplankton assemblage but absent in the others based on 16S rRNA gene sequences/clones

Taxonomic group by phylum	Number of sequences/clones			
	CPTA1	CPTA2	CPTI1	CPTI2
Armatimonadetes	3	-	-	-
TM6	56	-	2	3
Lentisphaerae	6	-	17	7
BRC1	15	-	4	2
TDNP	40	-	-	-
SR1	47	-	-	-
Elusimicrobia	10	-	4	3
Fibrobacteres	13	-	-	-
WS6	5	-	-	-
Deferribacteres	-	3	-	-
GN04	-	-	4	-
Aminicenantes_OP8	-	-	1	-
Synergistetes	-	-	-	2
Bacteria_uc	2	-	-	-



(A)



(B)

Figure 4. UPGMA (Unweighted pair group method with arithmethic mean) dendogram (**A**) and heat map (**B**) showing the clustering of bacterial assemblages from the study sites.

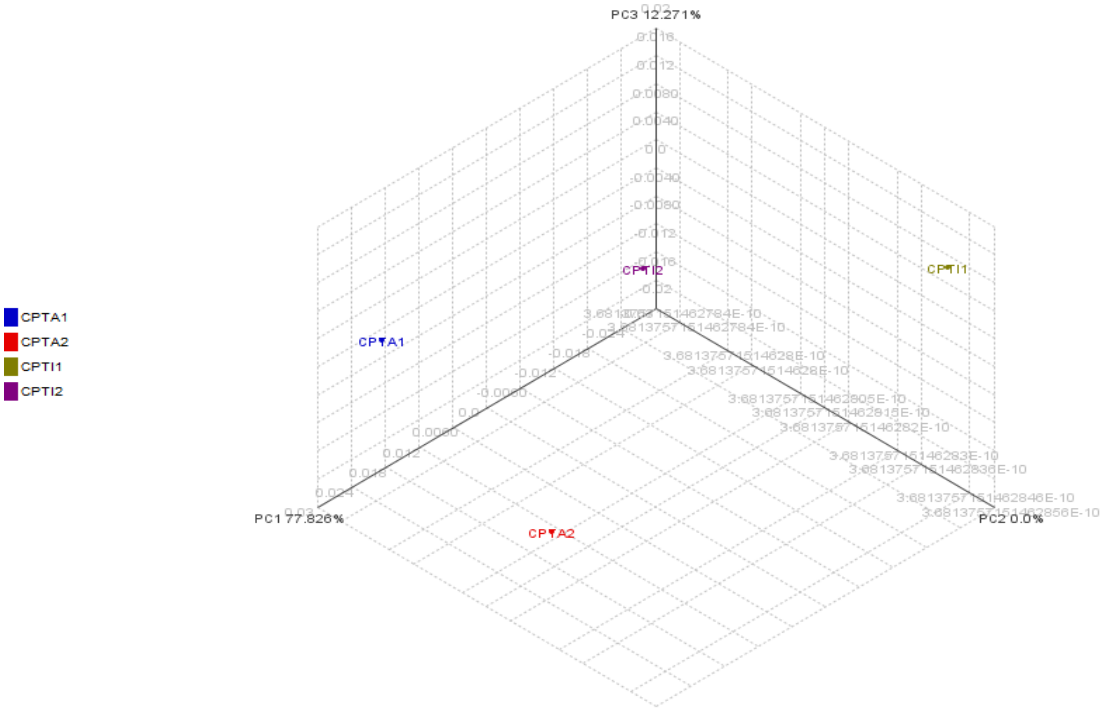


Figure 5. Three-dimensional principal coordinate analysis (PCoA) based on the Unifrac distance matrix of the bacterial assemblages for normalized OUT abundances within the study sites.

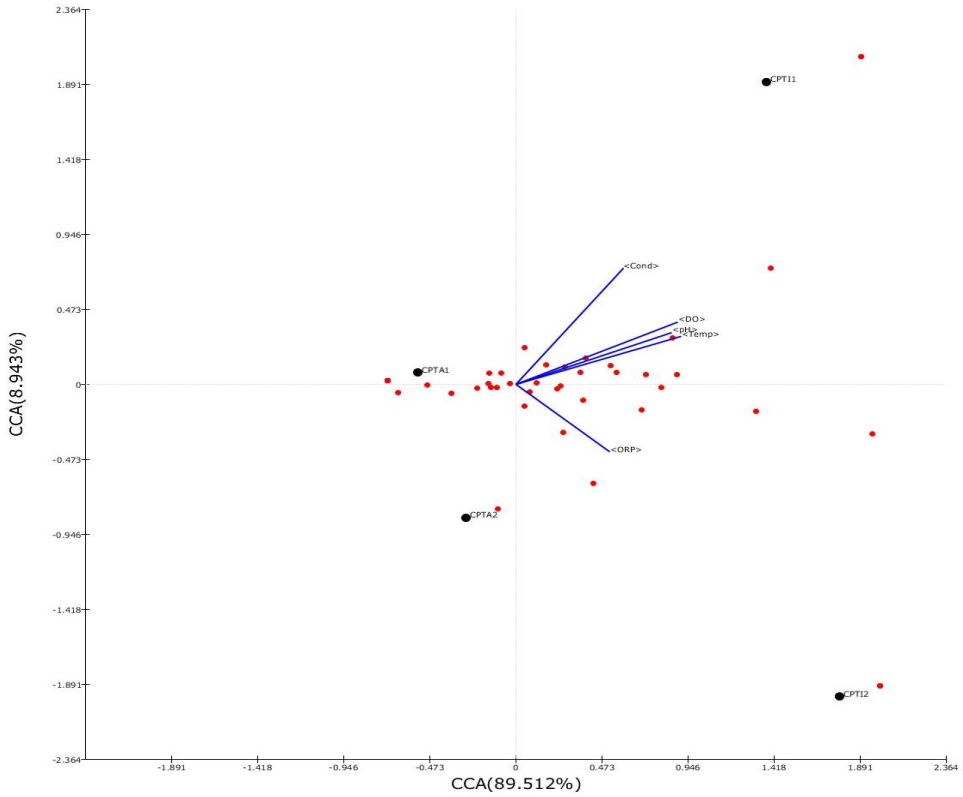


Figure 6. Canonical Correspondent analysis (CCA) of the bacterioplankton assemblages shown relationships with environmental variables within the study sites examined.

closely associated with the first and second CCA axes.

DISCUSSION

In this study, the 16S rRNA gene sequences obtained from 4 different coastal locations along the southernmost parts of the Atlantic Ocean in Cape Town, South Africa were analyzed in order to characterize the bacterial community structures in response to potential changes in environmental variables between these spatially different coastal marine sites. The 16S rRNA occurrences diverse phylogenetic groups within the assemblages revealed close similarity in the dominant taxa among the four coastal sites examined. Bacterial members at each of the sites were mostly dominated by the *Gammaproteobacteria* class followed by the *Flavobacteria.* The numerical dominance of members of the *Proteobacteria* and *Bacteroidetes* as observed in this study is consistent with previous studies that have also reported the high occurrences of these two bacterial phyla in various marine systems (Brown et al., 2009; Seo et al., 2017; Wang et al., 2018; Wu et al., 2019).

The relatively high occurrences of members of the *Gammaproteobacteria, Alphaproteobacteria* and *Flavobacteria* among the sequences in the four coastal sites examined in this study are fairly consistent with those reported for oceanic waters by previous studies (Kirchman, 2002; Rappe and Giovannoni, 2003; Schmidt et al., 1991; Raes et al., 2017; Wang et al., 2018; Wu et al., 2019). These bacterial groups are known to be major constituents of microbial assemblages in various marine systems (Kirchman, 2002; Rappe and Giovannoni, 2003), especially in coastal environments because of their propensity for the high availability of enhanced dissolved organic matter that are copiously produced by photosynthetically active autotrophs in this euphotic area of the ocean. More specifically, previous studies have revealed strong correlations between significant occurrences of these particular bacterial phyla and dissolved organic matters associated with phytoplankton productivity (Calson et al., 2009) and other environmental variables, including phosphate concentration (Morris et al., 2010; Seo et al., 2017), salinity and temperature (Milici et al., 2016; Wu et al., 2019) in coastal marine waters. For instance, Milici et al. (2016) particularly found bacterial diversity to change drastically with changing water temperature in the Atlantic Ocean, with the highest species diversity documented at between 15 and 20°C, but with significant reduction when water temperature was above 20°C. The results documented in this study seems to validate these particular previous observations, given that the water temperature of the four coastal sites examined here were on average around 15°C and that most of the diversity measures, especially as indicated by both ACE and Chao1 showed species richness among the microbial assemblages to be relatively high in all the coastal sites examined.

The CCA results of this study showed that combinations of the major environmental variables that were measured at the sites sufficiently explained the distribution of the different bacterial phyla elucidated among the coastal sites examined. Although other variables such as inorganic nutrients, including phosphate and nitrate concentrations were not included in the analysis conducted in this study, however, other studies have previously shown strong correlations between their concentrations and the spatial distributions and diversity of bacterial assemblages in marine waters (Raes et al., 2017; Seo et al., 2017). Raes et al. (2017) found strong correlations between total dissolved inorganic nitrogen, chlorophyll a, phytoplankton community structure and primary productivity with bacterial richness in their study on the surface waters of the eastern Indian Ocean. While, Seo et al. (2017) in their study on the coastal waters of the South Sea of Korea similarly reported significant influences of both phosphate and dissolved oxygen concentrations on the bacterial community compositions found in different stations along the coast of the sea.

The results obtained from this study that was designed to examine the occurrences, distribution and diversity of bacterial populations within the coastal bacterioplankton assemblages in the Atlantic Ocean around Cape Town, South Africa further validate and strongly corroborate several previous studies where various members of the heterotrophic bacterial populations, especially the *Gammaproteobacteria and Alphaproteobacteria* classes have also been observed dominant, especially within the coastal euphotic zones of marine environments (Wu et al., 2019). Conclusively, the multivariate analysis of the bacterial assemblages from the coastal sites in this study, revealed two spatially separate clusters among the sites, whereas environmental variables such as temperature, pH and conductivity were probably the major influencers of bacterial occurrences at the four coastal sites. Therefore, the results from this study further validate the interconnected of environmental variables and microbial assemblages as well as the potential influences of such factors on the ecology of bacterial populations in coastal aquatic environments.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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