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Cross-site comparison of coastal bacterial occurrences and diversity at selected sites along the North Atlantic Ocean in Monrovia, Liberia

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Coastal marine sites were examined for the occurrences and diversity of indigenous bacterial populations using high resolution 16S rRNA gene pyrosequencing approach to further understand the ecological importance of environmental variables. Samples containing slurries of seawater and sediments were collected from nine different sites along the north Atlantic Ocean in Monrovia, Liberia and were divided into three spatially distinct groups. The bacterial assemblages were found to be quite diverse in their occurrences among the examined sites. The majority of the sequences that dominated among the assemblages were associated with members of the Actinobacteria (1.3 to 4.8%), Bacteriodetes (2.9 to 5.8%), Acidobacteria (8.0 to 13.7%), Planctomycetes (15.3 to 28.8%), and Proteobacteria (40.7 to 48.8%). Gammaproteobacteria was the most abundant bacterial class, representing between 29 and 39% of the operational taxonomic units (OTUs) in all the coastal sites, while members belonging to the Planctomycetia, ranged between 10 and 18.8%, were relatively more abundant in the southern region of Monrovia which is mostly influenced by a freshwater lagoon. Alphaand beta diversity indices as well as rarefaction analysis were used to determine the species richness, evenness and coverage among the sites. Canonical correspondence analysis (CCA) and clustering using UPGMA (Unweighted pair group method with arithmetic mean) revealed the separation of the OTUs into groups probably based on the influence of various site-specific environmental variables at the coastal sites.

Key words: Marine coast, bacterial assemblages, 16S rRNA gene sequencing, diversity.

INTRODUCTION

Bacteria assemblages are ubiquitously present at relatively high occurrences in marine environments where they account for the majority of living biomasses and where they are known to perform several important ecological functions (Falkowski et al., 2008; Whitman et al. 1998; Arrigo, 2005; Pommier 2007; Sunagawa 2015; Salazar and Sungawa 2017; Kirkinci et al., 2021). Generally, ocean microbiomes, specifically those in the

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> temperate, are made up of quite diverse, temporally and spatially dynamic assemblages (Schauer et al., 2010; El-Swais et al., 2015; Seo et al., 2017; Olapade, 2020). While there are several overlapping factors that can potentially be responsible for and used to explain the dynamic changes frequently observed in the occurrences and diversity in microbial communities found in temperate regions, including nutrient availability, spatial distances, stratification, tidal upwelling events, seasonality and anthropogenic influences (Doney et al., 2012; Gao et al., 2021; Sbaoui et al., 2022), however, changing environmental conditions, most especially temperature have been the most plausible explanation (Sunagawa, 2015), especially on the basis of the widely accepted "everything is everywhere, but the environment selects" hypothesis as proposed by Baas-Becking (1934). For instance, Seo et al. (2017) in their study that examined the spatial distribution of bacterial community in the South Sea of Korea found significant influences in the concentrations of phosphate and dissolved oxygen on bacterial community composition. Environmental variables including temperature, pH and conductivity were revealed to be major influencers of bacterial occurrences and diversity along the southern coastlines of the Atlantic Ocean (Olapade, 2020). Similarly, combinations of temperature, nitrate and short-lived algal blooms were explain the variability used to observed in bacterioplankton communities in coastal north-west Atlantic Ocean.

Despite the relatively strong influences of several sitespecific environmental conditions and spatial distances on the structure and compositions of microbial assemblages in marine systems, however, additional studies designed to compare assemblages across larger spatial scales will further positively benefit and enhance current knowledge regarding microbial biogeographical patterns in natural systems. In light of this, this particular study was conducted in the northern coastlines of the Atlantic Ocean in Monrovia, Liberia located at about 435 miles north of the equator with the goals of elucidating and comparing the composition and diversity in the bacterial assemblages to those previously detected in other part of the ocean (Schauer et al., 2010; Olapade, 2020; Sbaoui et al., 2022). On the basis of these past studies on the Atlantic Ocean, there is a need to continue to unravel the occurrences and verse arrays of diversity associated with microbial assemblages in the coastal habitats of the ocean. Therefore, the presence and diversity of various bacterial taxa within microbial assemblages in the surface, water-sediment interphases of coastal locations were studied using the 16S rRNA gene pyrosequencing approach at nine locations in three spatially different beach sites that were selected along the coastline in Monrovia, Liberia. It was hypothesized that the microbial assemblages in these coastal locations will probably be greatly influenced by the various sitespecific environmental conditions, as well as by

differences in terrestrial and anthropogenic impacts among the sampling sites.

MATERIALS AND METHODS

Study location, sample collection and measurement of environmental variables

Slurries of seawater and sediments were sampled between 2nd and 4th of July, 2022 along the coastlines of the Atlantic Ocean in Monrovia, the capital city of Liberia. Liberia is a sub-Saharan, West African country located at 6°N, 9°W that bodies the north of the Atlantic Ocean to the southwest with a relatively long coastline of about 360 miles (580 km). Monrovia is a city that lies along the Cape Mesurado Peninsula, between the Atlantic Ocean to the south and the narrow Mesurado and Saint Paul rivers to the north forming a large natural harbor (Figure 1). Nine locations were selected and slurries of surface water and sediment were collected along the coastal location, various environmental variables including temperature, pH, conductivity, and oxidation-reduction potentials (ORP) were measured in triplicates using the YSI multi parameter probe system, model 556 (YSI Incorporated, USA).

DNA extraction and 16S rRNA gene pyrosequencing

Community DNA was extracted from the preserved filters (~250 ml of slurries filtered through 0.22 um pore-sized membrane filters) containing the water and sediment slurries that were collected using the FastDNA 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). As previously described by Olapade (2020), the Illumina's 16S sequencing library preparation protocol was used in generating amplicon libraries using universal primer pairs that consisted of an Illumina-specific overhang sequence and locusspecific sequence: 92wF_Illum: 5'-TCGTCGGCAGCGTCAGA TGTGTATAAGAGACAGAAACTYAAAKGAATTGRCGG and 1392R Illum: 5'GTCTCGT G GGCTCGGAGATGTGTATAAGAGACAGACGGGCGGTGTGTRC. The pair of primers targets the V6-V8 hypervariable regions of 16S rRNA genes of all microbial groups (Jefferies 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



Figure 1. Map of study sites along the coastlines of the north Atlantic Ocean in Monrovia, Liberia, West Africa. Source: WorldAtlas.com

in the sequences by using the UCHIME version 4.1 program (Edgar et al., 2011) that were later omitted for further analysis.

Bioinformatics, diversity and statistical 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 and beta-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). The Student t-test and ANOVA analyses were used to examine the differences in environmental variables and bacterial diversity between the sites. Linear correlations were used to determine strengths of relations between each environmental variable and the diversity in the assemblages. The unweighted pair group method with arithmetic mean {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).

Sequence availability and accession numbers

Raw sequences were deposited into the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under the BioProject ID: PRJNA882826 and BioSample ID: SAMN30952274.

RESULTS

Environmental variables

Several physicochemical variables including temperature, pH, conductivity, dissolved oxygen and oxidation-reduction potential (ORP) were measured to determine the water chemistry conditions at the coastal sites along the north Atlantic Ocean in Liberia (Figure 1). Specifically, water temperature in the studied sites ranged between 27.75 and 28.94°C (Table 1), with the ULB site showing more significantly warmer temperature than the other two coastal sites (P = 0.028). Water pH was very similar among the sites, ranging from 7.87 in one of the ULB sites to as high as 7.97 in the southern part of CDC sites. ORP measurements were found to differ significantly (P < 0.001) at a high average of 53 in the BCH sites and lowest of 44.9 at the CDC coastal site examined.

Bacterial diversity of the 16S rRNA genes

The use of the 16S rRNA gene high throughput pyrosequencing approach applied in this study to analyze

Site	Coord	linates	Temperature	Conductivity	DO	mU	
	Longitude	Latitude	(°C)	(mS/cm)	(%)	рп	UNF
ULB1	6° 18' 31.50" N	10° 48' 42.3" W	28.24	21.85	0.00	7.87	43.8
ULB2	6° 18' 31.50" N	10° 48' 42.3" W	28.25	28.32	0.00	7.91	44.6
ULB3	6° 18' 31.50" N 10° 48' 42.3" W		28.33	22.75	0.10	7.95	46.1
BCH1	6° 17′ 26.04" N	10°47′10.68" W	27.75	28.51	2.40	7.96	52.6
BCH2	6° 17′ 26.04" N	10°47′10.68" W	28.33	31.77	2.40	7.94	53.9
BCH3	6° 17´ 26.04" N 10°47´10.68" W		28.52	30.75	2.40	7.95	52.4
CDC1	6° 16′ 34.12" N	10°45′48.72" W	28.60	25.46	6.80	7.97	51.4
CDC2	6° 16′ 34.12" N	10°45′48.72" W	28.94	29.68	6.60	7.92	52.5
CDC3	6° 16′ 34.12" N	10°45′48.72" W	28.79	24.94	6.50	7.86	52.8

Table 1. Environmental variables measured at the study sites.

Source: Author

 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.

Site	OTUs	ACE	CHAO	Jackknife	NPShannon	Shannon	Simpson	Phylogenetic diversity	Good's coverage of library (%)	
ULB1	5219	6599.20	6317.33	6794.94	7.27	7.15	0.00	3751	96.49	
ULB2	5037	5702.93	5445.81	6044.00	7.48	7.36	0.00	3808	97.73	
ULB3	4991	5668.58	5410.12	6004.00	7.47	7.35	7.35 0.00 3620		97.72	
BCH1	4258	4709.69	4499.56	4999.00	6.95	6.84	0.01	3695	98.33	
BCH2	4101	4322.39	4193.53	4550.00	7.16	7.05	0.00	3542	98.99	
BCH3	4478	4966.77	4777.38	5286.00	7.24	7.13	0.00	4012	98.18	
CDC1	4355	4843.81	4630.25	5147.00	7.16	7.05	0.00	4020	98.22	
CDC2	3763	4033.58	3888.28	4272.00	6.61	6.50	0.01	3751	98.85	
CDC3	5679	7066.73	6680.06	7306.78	7.54	7.41	0.00	4017	96.36	

Source: Author

samples that were collected from nine samples generated a total of 425, 415 valid reads after quality control filtering and removal of chimeras. The valid reads were then clustered into 41881 OTUs and assigned into the domain Bacteria before analyzed for various alpha-diversity indices. On average, the OTUs from the ULB sites were significantly higher (P = 0.006) than what was obtained from the other coastal sites examined, however the majority of the bacterial sequences were adequately covered in all the sites as reflected by the results from both the Good Library Coverage (Table 2) and rarefaction analyses (Supplementary Figure 1). While abundancebased coverage estimators as ACE, CHAO and Jacknife revealed that bacterial diversity was relatively much higher in the ULB site when compared with the BCH, but not much difference to the CDC site, in contrast, the other indices utilized, that is, Shannon, NPShannon, Simpson

and Phylogenetic diversity showed no differences in diversity among the nine sampling locations.

Cross-site comparison of bacterial community structures and distribution

Phylogenetic analysis was used to compare variations in the occurrences and diversity of bacterial assemblages across the nine coastal locations sampled by determining the relative abundance of bacterial taxa at different taxonomic levels including at the phylum, class and order levels. Among the 18 distinct phyla, bacterial members belonaina the Proteobacteria. Planctomvcetes. to Bacteroidetes Actinobacteria, Acidobacteria. and Chloroflexi accounted for about 90% of sequencing reads in all the sites (Figure 2). The phylum Proteobacteria was



Figure 2. Relative abundances of bacterial phylogenetic taxa at the phylum level. Source: Author

numerically the most predominant, representing more than 40% of total sequences across all nine sites examined. proteobacterial Within the community, members Gammaproteobacteria of the and Alphaproteobacteria were the dominant subclasses (Figure 3) and accounted for greater than 27 and 4%, respectively of total sequences in all nine bacterial communities (Table 3). The order Chromatiales was the most prevalent group occurring at between 17 and 19% within the Gammaproteobacteria among the sampled sites. Also, the order Planctomycetales was well represented within the members of the Planctomycetes occurring at about 10% at one of the BCH sites to as high as 15% in the CDC site (Figure 4).

In order to better understand the distributions of bacterial patterns with respect to spatial and environmental variables, various statistical analyses were performed including clustering using the UPGMA Fast UniFrac, Principal coordinate and the Canonical Correspondent analyses. The clustering analysis showed that the bacterial community within the northern region of the ocean in Monrovia, that is, the three ULB coastal sites, clustered together and were distinctly different from the other six the southern locations in of the ocean that directly influenced by a freshwater lagoon are (Supplementary Figure 2). The clustering patterns observed among the bacterial assemblages that were indigenous to the 9 sampling stations was also confirmed by the PCoA analysis based on the Unifrac distance matrix, which also revealed a similar clustering profile (Figure 5). The CCA plot also showed clustering into 3 separate groups in relations to the various environmental variables measured at the studied sites and also in support of the clustering patterns observed previously (Figure 6). In addition, correlation analyses between the diversity metrics used in examining the bacterial assemblages and the various environmental variables revealed that diversity indices, especially the abundancebased coverage estimators, in particular ACE, CHAO and Jacknife were negatively influenced by some of the



Figure 3. Relative abundances of bacterial phylogenetic taxa at the class level. Source: Author

environmental variables including conductivity (R = -0.73), pH (R = -0.76), and ORP (R = -0.52), while Good's Coverage was strongly corelated with conductivity (R = 0.76); pH (R = 0.77) and ORP (R= 0.51) at the nine locations sampled (Table 4).

DISCUSSION

In this study, 16S rRNA genes obtained from microbial assemblages of 9 different coastal sites, including three sites in the northern part of the ocean in Monrovia, three located mid-distance and the remaining sites on a beach closely influenced by a freshwater lagoon were examined to elucidate and compare their bacterial community compositions as structured in response to various site-specific environmental conditions and their spatial differences. The results obtained from the sampled sites revealed distinct differences between the assemblages from the three northern coastal sites and the sites closer to the freshwater lagoon. The assemblages were dominated by members of the *Proteobacteria*, which accounted on average for 42.3, 46.3 and 44.3% of all

total sequences in the ULB, BCH and CDC sites, respectively. The numerical dominance of this phyla as documented in all the coastal locations examined in this study is not unique and corroborates results from earlier similar studies in marine environments (Seo et al., 2017; Olapade, 2020; Lang-Yona et al., 2022). Seo et al. (2017) found the phylum to have accounted for > 50% of total sequences at the 7 different locations examined in the South Sea of Korea, while the sequences affiliated with the phyla ranged between 52 and 59% in locations along the southern coastlines of the Atlantic Ocean in Cape When Lang-Yona et al. (2022) Town, South Africa. compared their occurrences across oceans, they were found to dominate among other phyla at an average of 58% in the Pacific and 66% the north Atlantic Oceans. The dominance of members of both the Gammaproteobacteria and Alphaproteobacteria classes as observed here appears consistent with previous observations in assemblages found in oceanic waters (Kirchman, 2002; Rappe and Giovanni, 2003; Raes et al., 2017; Wang et al., 2018; Wu et al., 2019).

Members of the *Planctomycetes* were the next prominent phyla occurring at an average of between 10

	T	Site I				Site II			Site III			
Phylum/class	Taxon Name	ULB1	ULB2	ULB3	BCH1	BCH2	BCH3	CDC1	CDC2	CDC3		
Actinghactoria	Acidimicrobiia	1.5	1.8	1.8	0.8	0.6	1.8	1.1	0.4	0.8		
Actinobacteria	MarineActino_c	2.5	2.3	2.6	2.3	2.4	2.5	1.5	0.5	2.7		
	Blastocatellia	1.1	1.0	1.0	0.8	0.8	0.7	1.5	1.1	1.0		
	CP011806 c	2.3	2.7	3.0	3.0	3.6	2.6	2.2	1.9	2.9		
Acidobacteria	 PAC002261_c	1.6	1.5	1.5	2.2	3.0	1.0	1.0	0.8	2.2		
	Thermoanaerobaculum c	2.4	2.8	2.7	3.0	4.1	2.5	2.3	2.6	3.5		
	Alphaproteobacteria	5.2	4.9	4.6	5.8	3.7	7.2	8.3	13.9	3.7		
	Betaproteobacteria	0.5	0.4	0.4	0.6	0.7	1.0	1.0	1.1	0.9		
Proteobacteria	Gammaproteobacteria	32.6	29.1	30.9	30.6	38.5	31.9	29.0	27.1	30.1		
	Deltaproteobacteria	4.9	5.4	5.7	4.9	5.4	4.4	3.8	5.0	5.5		
	Oligoflexia	0.8	0.8	0.7	2.5	0.5	0.8	0.8	0.5	0.7		
Chloroflexi	Anaerolineae	2.0	2.0	1.8	1.7	1.4	1.8	1.6	0.4	2.2		
Caldithrix_p	Caldithrix_c	1.2	1.0	1.1	0.7	1.0	1.2	0.9	0.3	1.4		
	Flavobacteria	1.6	1.4	1.3	1.3	0.8	2.1	2.4	3.8	1.0		
Bacteroidetes	Sphingobacteriia	2.9	3.1	2.7	1.8	1.5	3.1	2.6	0.4	2.1		
Latescibacteria_WS3	Latescibacter_c	1.5	1.9	2.0	1.9	1.9	1.7	1.4	0.7	1.6		
Nitrospirae	Nitrospira_c	1.4	1.5	1.4	1.6	1.8	1.2	1.4	1.2	2.2		
	OM190_c	1.6	1.9	1.7	1.4	1.3	1.5	1.2	1.4	1.3		
Dianatamuaataa	PAC002607_c	0.9	1.0	0.9	0.7	0.8	0.6	0.5	0.4	0.9		
Planctomycetes	Phycisphaerae	2.3	3.6	3.3	3.0	2.6	2.7	2.3	5.1	2.8		
	Planctomycetia	15.9	15.0	14.4	14.0	9.9	14.6	18.7	21.1	14.4		
Parcubacteria_OD1	Paceibacter_c	1.3	1.4	1.3	1.4	0.8	1.2	2.1	1.5	1.6		
Peregrinibacteria	Peribacteria	0.2	0.4	0.2	1.0	0.3	0.3	0.7	0.2	0.4		
Rhodothermaeota	Rhodothermia	1.5	1.7	1.7	1.8	2.0	1.5	1.0	0.2	2.6		
Saccharibacteria_TM7	Saccharimonas_c	0.5	0.5	0.5	0.3	0.2	0.9	1.4	0.6	0.3		

Table 3. Relative abundance of bacterial groups among the coastal sites examined.

Only Phylogenetic groups with more than 1% of total reads in at least one the sites are included. Source: Author

and 15% in all the 9 coastal locations. The high representation by members of this phyla in this study is somewhat surprising, given that while they are usually detected in temperate coastal sites, likely in association with the breakdown of complex organic organic carbons (Glöckner et al., 2003), they are usually not considered major players in coastal surface waters in oceans in terms of their abundance (Rusch et al., 2007). When compared with other coastal sites where they have been documented, their abundance has typically been found to be less than 10% of total bacterial numbers (Glöckner et al., 1999; Pizzetti et al., 2011; Olapade, 2020). Pizzetti et al. (2011) reported their abundance at about 6% at Kabeltonne station in the North Sea, while these members were found in abundance between 2 and 4.5% of total sequences in several locations along the southern coastline of the Atlantic Ocean in Cape Town, South Africa (Olapade, 2020). Also, surprising is the relatively low occurrences of members of the *Cyanobacteria* across all sampled locations (ranging from 0.1 to 0.4%), given that numbers of the *Planctomycetes* have been directly associated with the direct flow of carbon from algae in marine systems (Morris et al., 2006). The numbers recorded in this study are far lower than those reported in earlier similar studies (Seo et al., 2017; Gao et al., 2021). The differences as observed with these other studies could be partly due to differences associated with methodological approaches that were invariably utilized. For instance, some of these other studies examined filtered seawater as compared to slurries of pelagic



Figure 4. Relative abundances of bacterial phylogenetic taxa at the order level. Source: Author

Table 4. Relationship between environmental variables and bacterial diversity measures.

Correlation	Temp	Cond	DO	рН	ORP	OTUs	ACE	CHAO	JK	NPS	Shan	Simp	PD	GC
Temperature	1.00													
Conductivity	0.04	1.00												
DO	0.69	0.18	1.00											
рН	-0.28	0.39	0.02	1.00										
ORP	0.28	0.64	0.70	0.32	1.00									
OTUs	-0.02	-0.68	-0.27	-0.68	-0.53	1.00								
ACE	0.01	-0.73	-0.22	-0.76	-0.51	0.98	1.00							
CHAO	0.01	-0.73	-0.23	-0.76	-0.52	0.98	1.00	1.00						
Jackknife	0.00	-0.73	-0.23	-0.73	-0.53	0.99	1.00	1.00	1.00					
NPShannon	-0.12	-0.46	-0.41	-0.33	-0.46	0.86	0.77	0.77	0.79	1.00				
Shannon	-0.13	-0.45	-0.41	-0.32	-0.46	0.85	0.76	0.76	0.78	1.00	1.00			
Simpson	-0.12	0.32	0.29	0.21	0.36	-0.59	-0.53	-0.54	-0.54	-0.83	-0.83	1.00		
Phylogenetic (PD)	0.47	-0.10	0.52	-0.15	0.17	0.30	0.32	0.31	0.33	0.21	0.21	-0.25	1.00	
Coverage (GC)	-0.03	0.76	0.16	0.77	0.51	-0.94	-0.98	-0.98	-0.98	-0.66	-0.65	0.44	-0.36	1.00

*JK: JackKnife; NPS: NPShannon; Shan: Shannon; Simp: Simpson; PD: Phylogenetic Diversity; GC: Good's Coverage. Source: Author



Principal coordinates analysis

[Generalized UniFrac, Genus, Exclude Unclassified OTUs (Reads)]

Figure 5. Principal coordinate analysis (PCoA) based on the Unifrac distance matrix of the bacterial assemblages for normalized OTU abundances within the study sites. Results are presented as 2D ordination plots of PCoA. Samples displayed on plots are colored according to the metadata. Source: Author

sediments in this study, thereby containing different microbial communities.

The bacterial diversity and richness of 16S rRNA sequences from each of the 9 nine sites sampled were extensively analyzed. Bacteria richness (number of OTUs) was much lower in the BCH as compared to the northern ULB locations (P= 0.0058), but quite similar to those in the CDC sites. However, community diversity based on Phylogenetic and Shannon indices both showed no differences in diversity among the nine sampling locations. A possible explanation for the differences in richness among these spatial locations along the coast may be associated with the direct influence of the freshwater lagoon that perpetually flows directly into the southern CDC site, changing the hydrodynamic conditions and water chemistry around this coastal location. Various environmental variables have been suggested as important regulators of bacterial community composition in marine systems (Guo et al., 2018). In this study, some of the environmental factors at the locations e.g., conductivity, pH and ORP had strong negative correlations with diversity measures at the coastal locations. At a larger spatial scale, comparison between the results from this study conducted along coastal sites in the northern parts of the ocean to those reported previously on several locations along the rocky headland coasts of the same ocean in Cape Town, South Africa (Olapade, 2020), revealed similarities in the major bacterial representations in the assemblages derived from the locations, however there clear differences in environmental factors as well as bacterial species richness and diversity between the two. The clear differences observed between the assemblages located in these extreme sites of the Atlantic Ocean is not at all surprising given the divergent geographical locations, with Cape Town, South Africa located in the southern hemisphere with a distance of about 2000 miles south of the equator and Monrovia, Liberia in northern hemisphere



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

and about 450 miles closer to the equator. The water temperature on average was far higher (28°C) in the northern Liberian more diverse sampling sites as compared to about 15°C in the southern coastal locations, which further corroborates the suggestion that bacterial diversity increases with temperature as the main driver in marine environments (Fuhrman et al., 2008; Sunagawa, 2015).

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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REFERENCES

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25(17):3389-3402.

- Arrigo KR (2005) Marine microorganisms and global nutrient cycles. Nature 437(7057):349-355.
- Baas-Becking LGM (1934). *Geobiologie of Inleiding Tot De Milieukund*. WP Van Stockum and Zoom (in Dutch): The Hague, the Netherlands.
- Chao A (1984). Nonparametric estimation of the number of classes in a population. Scandinavian Journal of Statistics 11:265-270.
- Chao A (1987). Estimating the population size for capture-recapture data with unequal catchability. Biometrics 43:783-791.
- Chao A, Lee SM (1992). Estimating the number of classes via sample coverage. Journal of American Statistical Association 87(417):210-217.
- Doney SC, Ruckelshaus M, Emmett Duffy J, Barry JP, Chan F, English CA, Galindo HM, Grebmeier JM, Hollowed AB, Knowlton N, Polovina J (2012). Climate Change Impacts on Marine Ecosystems. Annual Review of Marine Science 4:11-37.
- Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011). UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194-2200.
- El-Swais H, Dunn KA, Bielawski JP, Li WKW, Walsh DA. (2015). Seasonal assemblages and short-lived blooms in coastal north-west Atlantic Ocean bacterioplankton. Environmental Microbiology.17(10): 3642-3661.
- Falkowski PG, Fenchel T, DeLong EF (2008). The microbial engines that drive Earth's biogeochemical cycles. Science 320: 1034-1039.
- Fuhrman JA, Steele JA, Hewson I, Schwalbach MS, Brown MV, Green JL, Brown JH (2008). A latitudinal diversity gradient in planktonic marine bacteria. Proceedings of the National Academy of Sciences USA 105:7774-7778.
- Gao P, Du G, Zhao D, Wei Q, Zhang X, Qu L, Gong X (2021). Influences of Seasonal Monsoons on the Taxonomic Composition and Diversity of Bacterial Community in the Eastern Tropical Indian Ocean. Frontiers in Microbiology 11:615221.
- Glöckner FO, Fuchs BM, Amann R (1999). Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. Applied and Environmental Microbiology 65:3721–3726.
- Glöckner FO, Kube M, Bauer M, Teeling H, Lombardot T, Ludwig W, Gade D, Beck A, Borzym K, Heitmann K, Rabus R (2003). Complete genome sequence of the marine planctomycete Pirellula sp. strain 1. Proceedings of the National Academy of Sciences 100(14):8298-303.
- Good IJ (1953). The population frequencies of species and the estimation of population parameters. Biometrika 40(3-4):237-264.
- Guo XP, Lu DP, Niu ZS, Feng JN, Chen YR, Tou FY, Liu M, Yang Y (2018). Bacterial community structure in response to environmental impacts in the intertidal sediments along the Yangtze Estuary, China Marine Pollution Bulletin 126:141-149.
- Hamady M, Lozupone C, Knight R (2010). Fast UniFrac: facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. The International Society for Microbial Ecology Journal 4:17-27.
- Jolliffe IT (1989). Principal Component Analysis. Springer-Verlag, New York.
- Kemp PF, Aller JY (2004). Estimating prokaryotic diversity: When are 16S rDNA large enough? Limnology and Oceanography Methods 2:114-125.
- Kirchman DL (2002). The ecology of *Cytophaga-Flavobacteria* in aquatic environments. FEMS Microbiology Ecology 39(2):91-100.
- Kirkinci SF, Edbeib MF, Aksoy HM, Marakli S, Kaya Y (2021). Identification of Dalapon degrading bacterial strain, *Psychrobacter* sp. TaeBurcu001 isolated from Antarctica. Polar Science 28:1-9.
- Lang-Yona N., Flores JM, Haviv R, Alberti A, Poulain J, Belser C, Trainic M, Gat D, Ruscheweyh HJ, Wincker P, Sunagawa S (2022). Terrestrial and marine influence on atmospheric bacterial diversity over the north Atlantic and Pacific Oceans Communications Earth and Environment 3:121.
- Morris RM, Longnecker K, Giovannoni SJ (2006). Pirellula and OM43 are among the dominant lineages identified in an Oregon coast diatom bloom. Environmental Microbiology 8:1361-1370.
- Olapade OA (2013). Molecular Characterization of Bacterial Phylogenetic and Functional Groups at the site of the Deepwater Horizon Oil Spill along the Gulf of Mexico. Journal of Petroleum and Environmental Biotechnology 4:144.

- Olapade OA (2015). Phylogenetic characterization and community diversity of hydrocarbon- degrading bacterial populations in soil microcosms enriched with various aromatic hydrocarbons. Journal of Bioremediation Biodegradation 6(4):1.
- Olapade OA (2020). Bacterial community composition and diversity along the southern coastlines of the Atlantic Ocean in cape Town, South Africa 28:2.
- Pizzetti I, Fuchs BM, Gerdts G, Wichels A, Wiltshire KH, and Amann R. (2011). Temporal Variability of Coastal Planctomycetes Clades at Kabeltonne Station, North Sea. Applied and Environmental Microbiology 77(14):5009-5017.
- Pommier T, Čanback B, Riemann L, Boström KH, Simu K, Lundberg P, Tunlid A, Hagström Å (2007). Global patterns of diversity and community structure in marine bacterioplankton. Molecular Ecology 16(4):867-880.
- Raes EJ, Brodrossy L, Van de Kamp J, Bisset A, Waite AM (2017). Marine bacterial richness increases towards higher latitudes in the eastern Indian Ocean. Limnology and Oceanography Letters 3:10-19.
- Rappe MS, Giovannoni SJ (2003). The uncultured microbial majority. Annual Review of Microbiology 57(1):369-394.
- Rusch DB, Halpern AL, Sutton G, Heidelberg KB, Williamson S, Yooseph S, Wu D, Eisen JA, Hoffman JM, Remington K, Beeson K (2007) The Sorcerer II Global Ocean Sampling Expedition: Northwest Atlantic through Eastern Tropical Pacific. PLoS Biology 5(3):e77.
- Salazar G, Sunagawa S (2017). Marine microbial diversity. Current Biology 27:R489-R494.
- Sbaoui Y, Ezaouine A, Toumi M, Farkas R, Kbaich MA, Habbane M, El Mouttaqui S, Kadiri FZ, El Messal M, Tóth E, Bennis F (2022). Effect of Climate on Bacterial and Archaeal Diversity of Moroccan Marine Microbiota. Microorganisms 10(8):1622.
- Schauer R, Bienhold C, Ramette A and Harder J. (2010). Bacterial diversity and biogeography in deep-sea surface sediments of the South Atlantic Ocean. The International Society for Microbial Ecology 4(2):159-170.
- Schloss PD, Handelsman J (2006). Introducing SONS, a tool for operational taxonomic unit- based comparison of microbial community memberships and structures Applied and Environmental Microbiology 72:6773-6779.
- Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW (2009). Introducing mothur: open-source-, platform-independent, communitysupported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75(23):7537-7541.
- Seo JH, Kang I, Yang SJ, Cho JC (2017). Characterization of spatial distribution of the bacterial community in the south sea of Korea. PLOS ONE 12(3):e0174159.
- Sunagawa S, Coelho LP, Chaffon S, Kultima JR, Labadie K, Salazar G, Djahanschiri B, Zeller G, Mende DR, Alberti A, Cornejo-Castillo FM (2015). Structure and function of the global ocean microbiome. Science 348(6237):1261359.
- Ter Braak CJF, Verdonschot PFM (1995). Canonical correspondence analysis and related multivariate methods in aquatic ecology. Aquatic Sciences 57:255-289.
- Wang S, Yu M, Wei J, Huang M, Shi X, Chen H (2018). Microbial community composition and diversity in the Indian Ocean deep sea REY-rich muds. PLOS ONE.
- Whitman WB, Coleman DC, Wiebe WJ (1998). Prokaryotes: The unseen majority. Proceedings of the Natural Academy of Sciences 95(12):6578-6583.
- Whittaker RH (1972). Evolution and measurement of species diversity. Taxon 21:213-251.
- Wu C, Kan J, Liu H, Pujari L, Guo C, Wang X, Sun J (2019). Heterotrophic bacteria dominate the diazotrophic community in the eastern Indian Ocean (EIO) during pre-southwest monsoon. Microbial Ecology 78:804-819.
- Zhang T, Shao M-F, Ye L (2012). 454 Pyrosequencing reveals bacterial diversity of activated sludge from 14 sewage treatment plants. International Society for Microbial Ecology Journal 6(6):1137-1147.

SUPPLEMENTARY MATERIALS



Supplementary Figure 1. Rarefaction Curve of OTUs based on 16S rRNA clone sequences from the coastal marine assemblages from the nine study sites.



Supplementary Figure 2. UPGMA (Unweighted pair group method with arithmetic mean) dendogram showing the clustering of bacterial assemblages from the study sites.