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Full Length Research Paper

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. Alpha- and 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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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 used to explain the variability observed in bacterioplankton communities in coastal north-west Atlantic Ocean.

Despite the relatively strong influences of several site-specific 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 site-specific 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 areas as previously described by Olapade (2020). At each 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 locus-specific 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

Table 1. Environmental variables measured at the study sites.

Site	Coordinates		Temperature	Conductivity	DO	pH	ORP
	Longitude	Latitude	(°C)	(mS/cm)	(%)		
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

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	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 abundance-based 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 belonging to the *Proteobacteria, Planctomycetes, Acidobacteria, Actinobacteria, Bacteroidetes* and *Chloroflexi* accounted for about 90% of sequencing reads in all the sites (Figure 2). The phylum *Proteobacteria* was

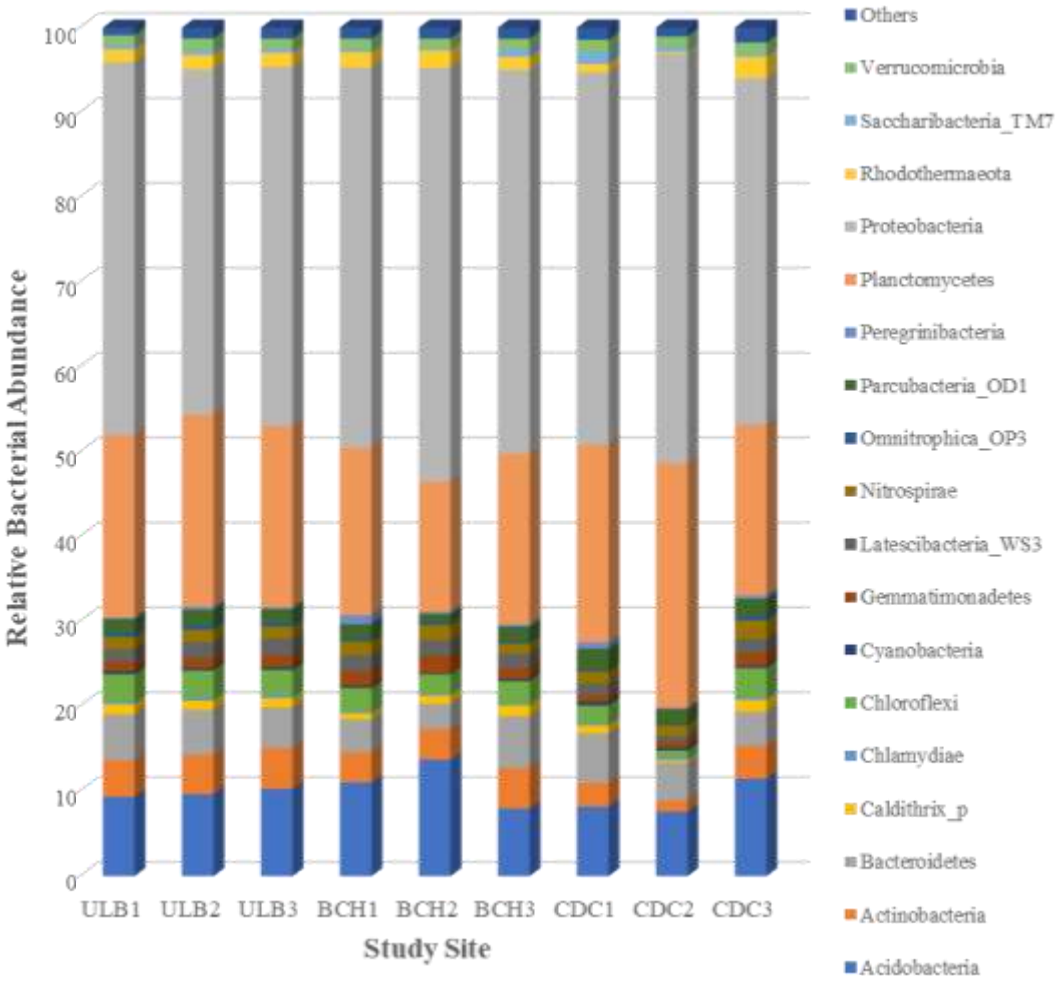

Figure 2. Relative abundances of bacterial phylogenetic taxa at the phylum level.
Source: Author

numerically the most predominant, representing more than 40% of total sequences across all nine sites examined. Within the proteobacterial community, members of the *Gammaproteobacteria* 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 are directly influenced by a freshwater lagoon (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 abundance-based coverage estimators, in particular ACE, CHAO and Jacknife were negatively influenced by some of the

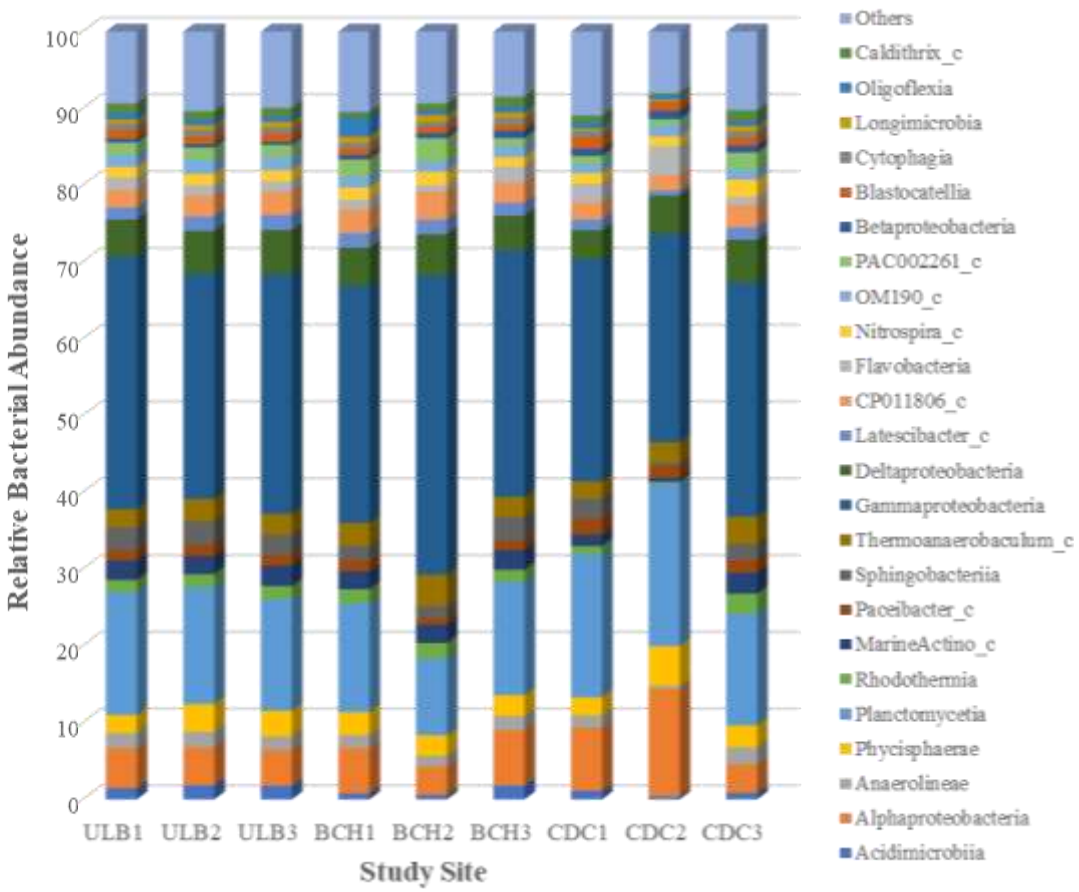

Figure 3. Relative abundances of bacterial phylogenetic taxa at the class level.
Source: Author

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 Town, South Africa. When Lang-Yona et al. (2022) 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

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

Phylum/class	Taxon Name	Site I			Site II			Site III		
		ULB1	ULB2	ULB3	BCH1	BCH2	BCH3	CDC1	CDC2	CDC3
Actinobacteria	Acidimicrobiia	1.5	1.8	1.8	0.8	0.6	1.8	1.1	0.4	0.8
	MarineActino_c	2.5	2.3	2.6	2.3	2.4	2.5	1.5	0.5	2.7
Acidobacteria	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
	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
Proteobacteria	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
	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
Bacteroidetes	Flavobacteria	1.6	1.4	1.3	1.3	0.8	2.1	2.4	3.8	1.0
	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
Planctomycetes	OM190_c	1.6	1.9	1.7	1.4	1.3	1.5	1.2	1.4	1.3
	PAC002607_c	0.9	1.0	0.9	0.7	0.8	0.6	0.5	0.4	0.9
	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

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

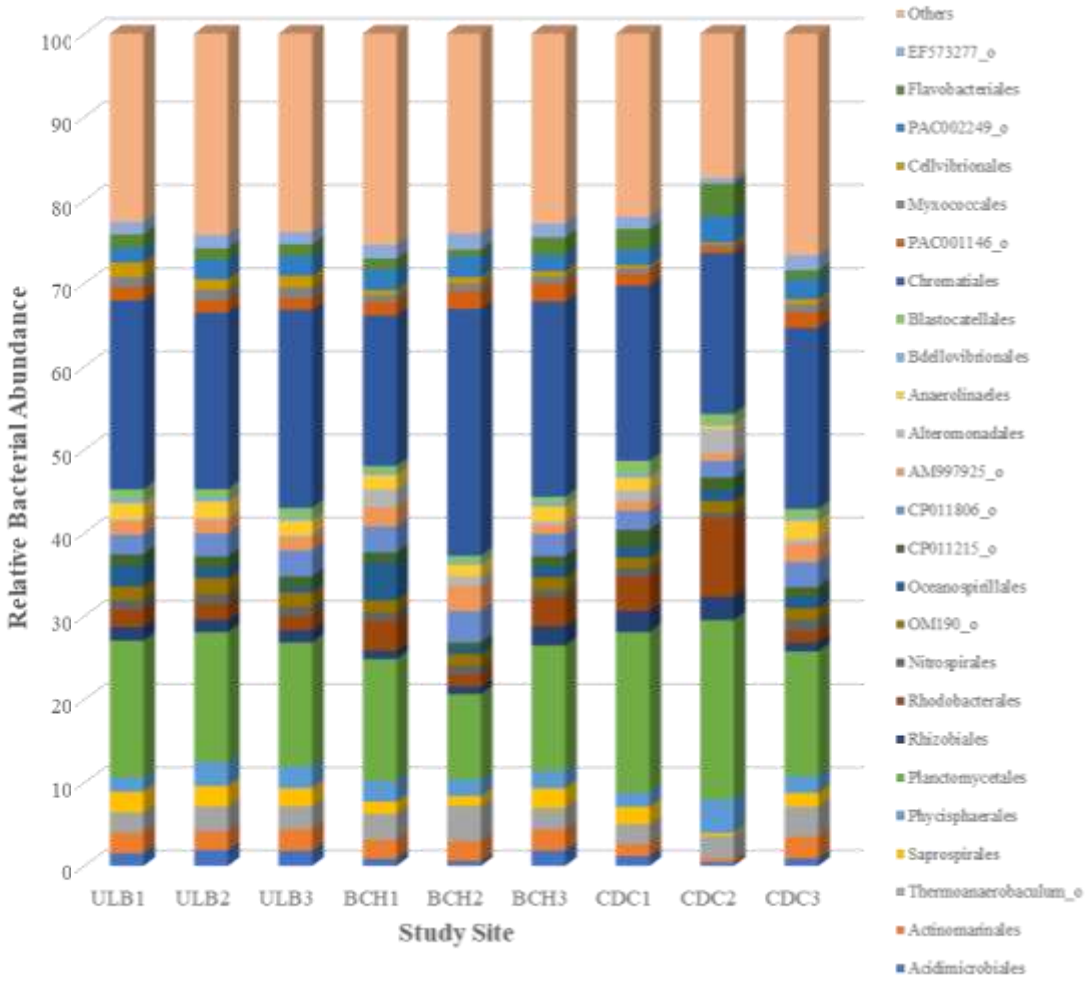

Figure 4. Relative abundances of bacterial phylogenetic taxa at the order level.
Source: Author

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

Correlation	Temp	Cond	DO	pH	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											
pH	-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

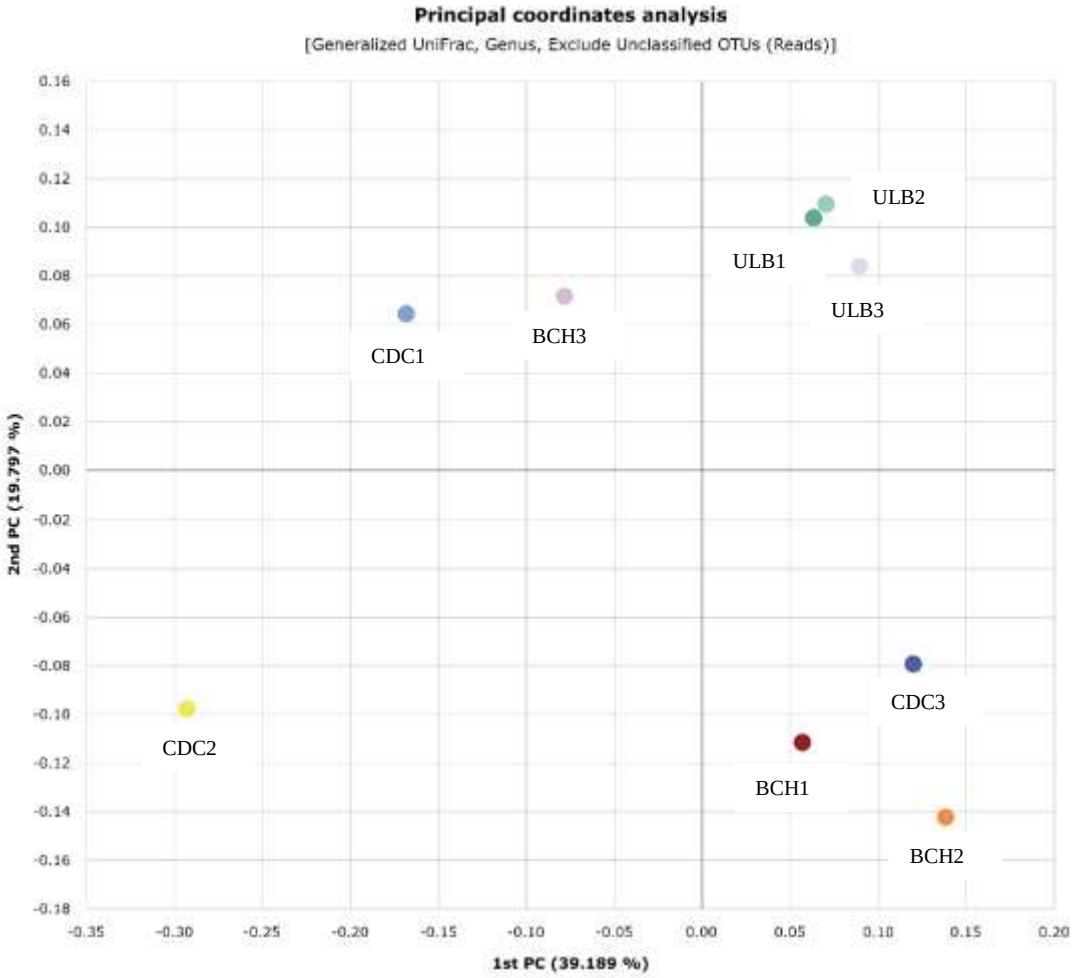

Figure 5. Principal coordinate analysis (PCoA) based on the Unifrac distance matrix of the bacterial assemblages for normalized OTU abundances within the study sites. Results are presented as 2D ordination plots of PCoA. Samples displayed on plots are colored according to the metadata.
Source: Author

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

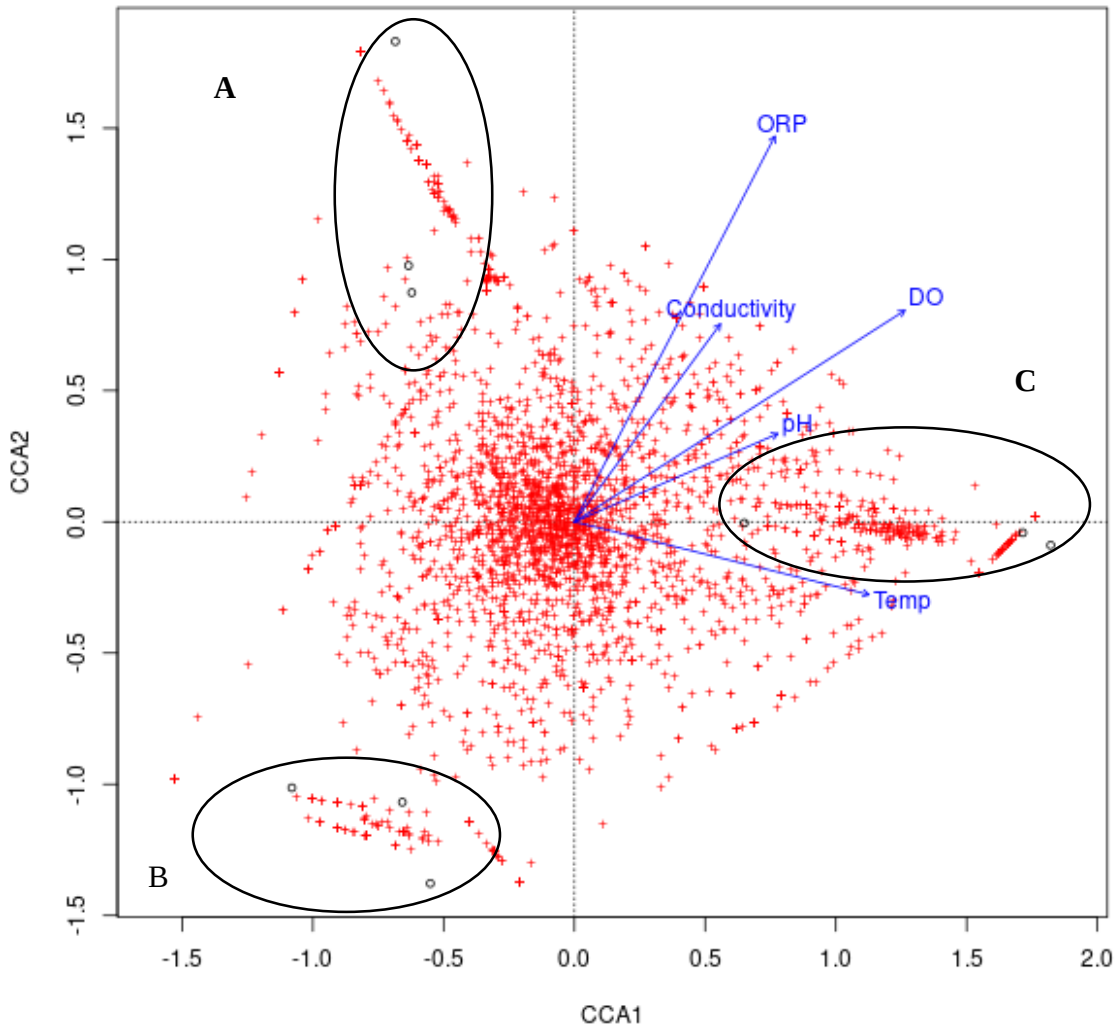

Figure 6. Canonical Correspondent analysis (CCA) of the bacterioplankton assemblages show relationships with environmental variables within the study sites examined.
Source: Author

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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The author thanks Aminata Amiezoe Swaray and many staff members of Bella Casa Hotel for their various assistance during field sampling and visit to Monrovia. The support by Luann Shepherd and Nicole Lee with the processing of paper work associated with the trip to Liberia here at Albion College was appreciated. The support by Shukar Abdullah and colleagues at EZBiome Inc (ChunLab) USA was appreciated for assistance during sequencing and bioinformatics data analysis. The study was generously supported by the awarded A Merton Chickering Endowed Professorship in Biology grant by Albion College.

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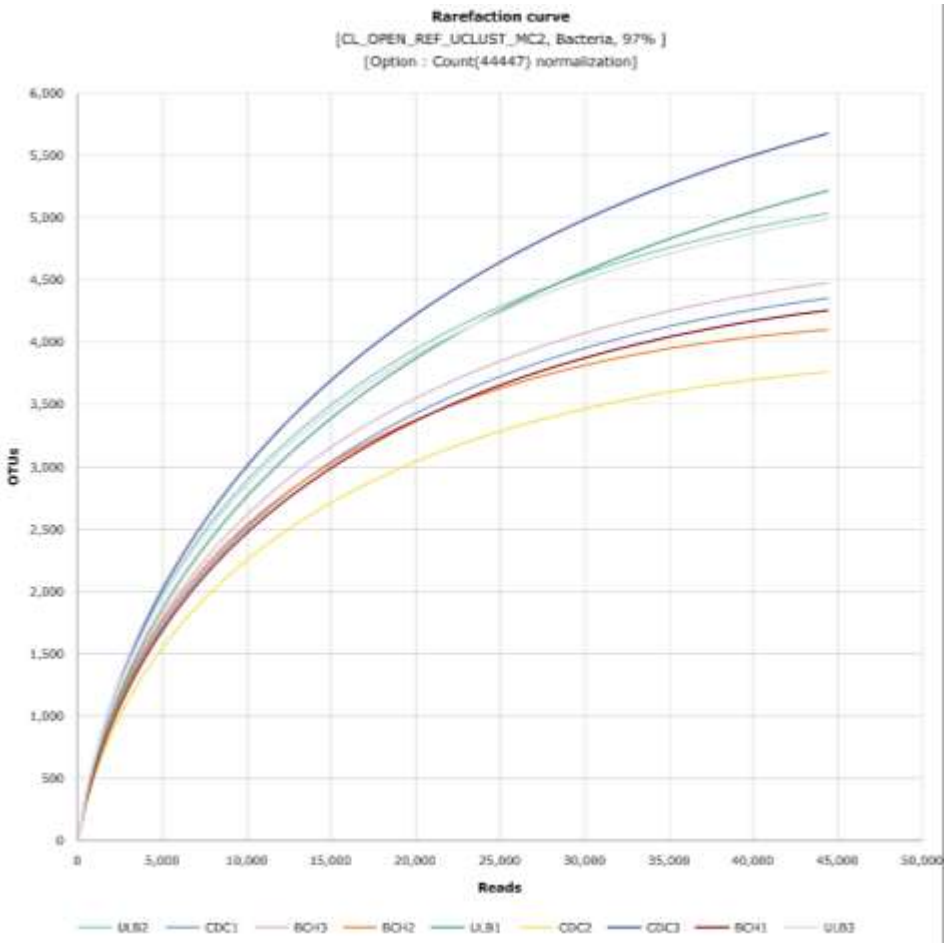
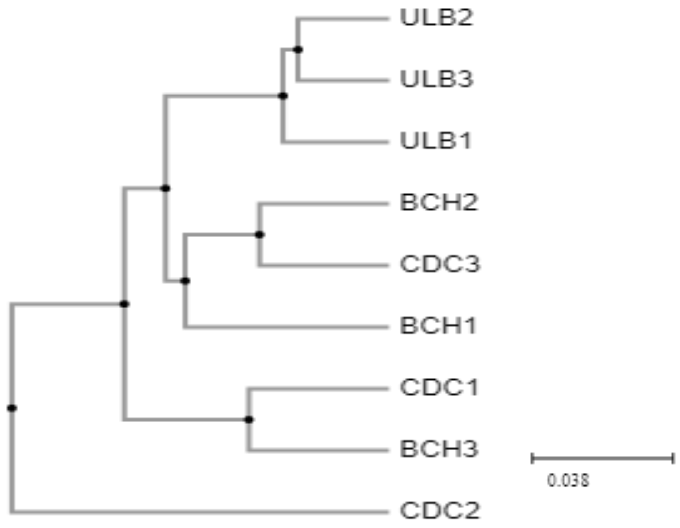
SUPPLEMENTARY MATERIALS



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.

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African Journal of Microbiology Research

Full Length Research Paper

Multiple adaptations of bacteriophages in Lagoon Ebrie: Indicators of permanence microbiological pollution in the environment, Côte d'Ivoire, West Africa

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The Lagoon Ebrié in the city of Abidjan is a typical aquatic ecosystem that can be used to predict the existence and circulation of water pathogens. Phages are viruses that infect bacteria and can be used for the detection of their bacteria host. In this study, bacteria colonies were investigated in their natural habitat within the Lagoon by using phages as indicators. Their impact on pollution was also determined in different sites of the rural Lagoon in the city. Water samples were collected at different sites of the Lagoon for the detection of bacteria and phages. The virulence of phages was tested on bacteria host using diffusion on an agar plate. Our results showed three major bacterial strains; *Pseudomonas aeruginosa, Escherichia coli*, and *Enterobacter cloacae*. Twenty-four phages that infected five bacteria hosts were also isolated. The spectrum revealed that the phages have high virulence against *E. coli*, *P. aeruginosa* and *E. cloacae* by 82.5%, 71 and 70% respectively. The majority (83.3 %) of isolated phages produced lysis on *Enterobacteriaceae.* However, Vibrio phages were not detected in this study. These findings suggested the existence of multiple adaptations of phages and the persistence of diverse bacteria hosts in the Lagoon. The phages that were detected confirmed the bacterial presence in the Lagoon, this portray the ecological system in operation and possible microbiological risks for the riverine populations.

Keys words: Lagoon Ebrie, pollution, indicators, phages, adaptation, Abidjan.

INTRODUCTION

Bacteriophages, viruses that infect bacteria, are the most numerous of all viruses in the biosphere and are estimated to be globally more numerous than bacteria (Weinbauer, 2004; Williamson et al., 2005; Breitbart and Rohwer, 2005; Weitz and Wilhelm, 2012). This abundance plays an important role in the evolution of bacterial communities and may influence global biogeochemical cycles (Abedon, 2008; Harada et al., 2018). Detection of bacteriophage can be used as an indicator of water quality and wastewater treatment processes (Stewart et al., 2008). This is particularly important due to the difficulties that are often encountered with the direct detection of pathogens in water (Schmelcher and Loessner, 2014).

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Table 1. Sampling sites for phage isolation in this study.

Sites coding	Name of sites	GPS position
Ab	Abobodoume	5°18'36'N°4°2'06''W
Ad	Adiopodoume	5°20'05'N°4°7'37''W
Bo	Boribana	5°21'04'N°4°2'28''W
Ca	Carena	5°20'15'N°4°2'06''W
Lo	Locodjoro	5°19'28'N°4°2'06''W

Source: Authors

The potential presence of fecal pollution is typically assessed by studying the presence of Fecal Indicator Bacteria (FIB) which include, fecal coliforms, *Escherichia coli,* and *Enterococci* (Brian et al., 2017; Ashbolt et al., 2001).

Bacteriophage can be used as Microbial Source Tracking (MST) in the environment (Brian et al., 2017; Ezzat and Azzam, 2020). Several based-phage methods are developed to detect pathogenic bacteria using engineered phages that coding fluorescent, luminescent or colorimetric markers. Alternatively, labeled phage protein and phage DNA staining can allow direct detection of phages bound to host bacterial cells (Willford et al., 2011; Schofield et al., 2012; Jain et al., 2012; Vinay et al., 2015).

The poor management of household waste has been reported as one of the factors responsible for the deterioration of the living conditions and the natural environment in Abidjan (Adingra and Kouassi, 2011; Dongo et al., 2013; Tia, 2017).

P. aeruginosa, E. coli, *E. cloacae* and *S. aureus* have been reported as the major opportunistic bacteria responsible for nosocomial infections and diarrheal infections worldwide (Leski et al., 1998; Roman et al., 1997; Turnidge and Bell, 2000; Simor et al., 2001).

In this study, the presence of phages infecting *Enterobacter, E. coli*, *S. aureus, and V. alginolyticus* was investigated in the environmental water of the Lagoon Ebrie of the city of Abidjan.

MATERIALS AND METHODS

Water sampling

Five sampling sites on Lagoon Ebrie were used for this study. At each of the sites, 500 ml of water were collected and transported to the lab for microbiological analysis between September and December 2018. The specific sites were Locodjoro, Abobodoume, Boribana, Carena and Adiopodoume, with GPS locations presented in Table 1. Except for the Adiopodoume site, all the four other sites have the presence of human activities; pictures of these are presented in Figure 1.

Identification of bacteria

Three mL of water samples was inoculated in 10 mL of LB medium. The mixture was then incubated at 37 °C/ 24 h. Thereafter, colonies were identified and isolated. The selected colonies were diluted in distilled water for enrichment. They were then transferred to specific growth media for species identification. The strains were confirmed by biochemical tests, Malditoff and Antibiotic susceptibility tests (Koudou et al., 2021). *E. coli* stains B and C, Phage T4 that were used as the positive controls for virulence tests were provided from Dr Sylvain Moineau, University Laval. Canada (Addablah et al., 2021). Bacteria hosts and viral strains that were isolated in water from the lagoon for this study were stored at 20°C for subsequent analysis.

Phage isolation

A total of sixty-five water samples of 500 mL were collected from different sites and stored at 4 °C as the environmental water. Hundert (100) mL of the water was filtered through 0.45 µm. Tree (3) mL of the filtrate were inoculated in 3 mL LB Medium (DIFCO, Mexico) containing 100 µL of fresh bacteria host. Five major species were selected for bacteria hosts (*E. coli, P. aeruginosa, E. cloacae, S. aureus,* and *V. alginolyticus*). The enrichment solutions were incubated at 37 °C for 48 hours with gentle agitation. The solution was centrifuged at 6000 rpm for 10 min. The supernatant was filtered through a 0.45 µm membrane. The filtrate was inoculated in 3 mL of bacteria media twice times at 37 °C /24 h. After the centrifugation, the supernatant was collected and stored at 4 °C for phage' characterization. The phage T4 was the viral control strain for the lytic assay validation. Bacteria hosts were gifted from the collection of Phagetech project of Pasteur Institute Cote d'Ivoire (Addablah et al., 2021).

Virulence test assay

Twenty µL of filtrate were spotted on LB agar plate with enrichment bacteria to confirm phage presence. Lysates containing phages were tested in five bacteria species to determine host sensitivity to phages. Five (5) mL of fresh culture bacteria (OD = 0.3) were inoculated on agar and removed by sterile pipet. The plates were dried on a laminar flow hood. Twenty microliters of phage solution were spotted on the plate and incubated at 37 °C/ 24 h. Several phages were tested in the same bacteria plate. The method of Kakou-Ngazoa et al., 2020 was used for virulence test of phages.

RESULTS

Distribution of Lagoon phages

A total of twenty-four phages lysates were recovered in the Ebrie Lagoon. This is presented in Table 2. No phage lysate produced lysis on *V. alginolyticus, but* the highest percentage was detected in *E. coli.* A total of 83.3 % of isolated phages that produced lysis are *Enterobacteriaceae*.

Virulence spectrum of phages lysate

The distribution of virulence showed that enriched phages produced lysis on *E. coli*, *P. aeruginosa*, and *S. aureus* bacterial lawn. The study of the virulence spectrum of phages showed that a single enriched phage could lead to cell lysis in 2 or more bacterial species

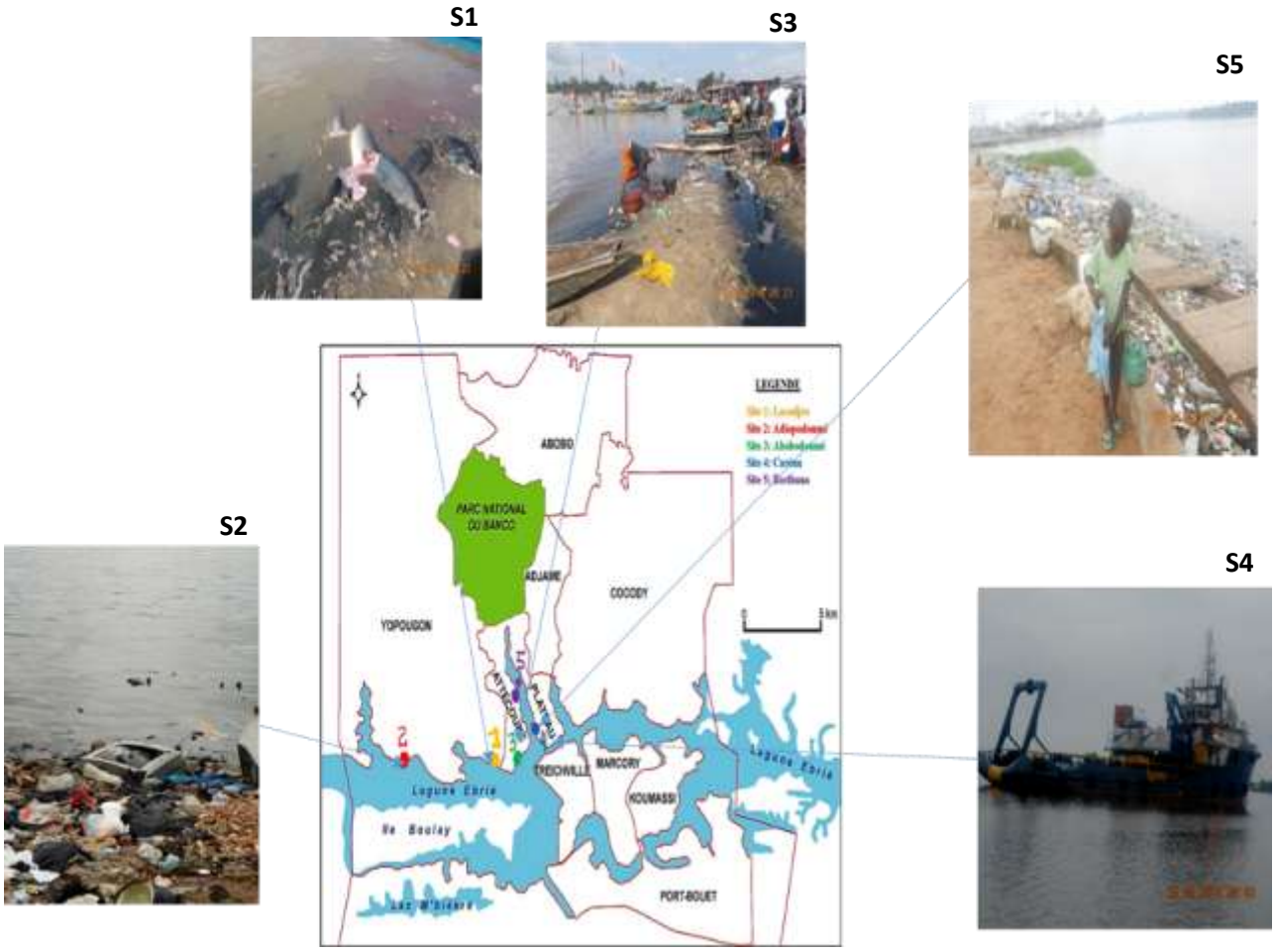

Figure 1. Sampling Sites of Lagoon Ebrie, Abidjan (2018). Sites Locodjo (S1, Site 1), Adiopodoume (S2, Site 2), Site Abobodoume (S3 , Site 3), Site Boribana (S5, Site 5), Site Carena (S4, Site 4).
Source: Pictures Koudou/Ivorycoast/2018 collection

Table 2. Distribution of phage according to their enrichment bacteria.

Bacteria hosts	Phages lysates (n)	Total (%)
Escherichia coli	10	41.67
Enterobacter cloacae	5	20.83
Pseudomonas aeruginosa	5	20.83
Staphylococcus aureus	4	16.67
Vibrio alginolyticus	0	0
Total (%)	24	100%

Source: Authors

(Table 3). Thus lysates such as those obtained after enrichment with the bacteria *P. aeruginosa*, *S. aureus*, and *E. coli* was all able in the vast majority (60 to 100 %) to infect all the bacterial species studied.

The spectrum revealed that the phages have high virulence against *E. coli*, *P. aeruginosa* and *E. cloacae* by 82.5 %, 71 % and 70 % respectively. Vibrio phages were not detected in the Lagoon Ebrie (Table 3).

Pollution of Lagoon Ebrié

By using the indicators of fecal contamination with bacteriophages, the different sites of Lagoon have different level of microbiological pollution. The level of contamination shows that all sites have high or very high pollution, by the presence of major bacteria hosts including *E. coli*, *P. aeruginosa*, *E. cloacae* and *S. aureus* in sites Boribana, Locodjoro and Abobodoume (Table 4). The sites Carena and Adiopoudoume have same pollution level. The lagoon is main contaminated permanent with plural phages and bacteria strains.

DISCUSSION

The surveillance of the ecosystems enhances the

Table 3. Percentage of virulence of enriched phages from Lagoon Ebrié.

Bacteria host/number of phages (n)	Virulence of bacteria strains (%)				
	E. coli B	***E. coli C***	***E. cloacae***	***P. aeruginosa***	***S. aureus***
E. coli /phages (10)	100	80	50	80	90
E. cloacae /phages (5)	60	20	100	0	0
P. aeruginosa /phages (5)	100	100	60	100	80
S. aureus/ phages (4)	100	100	75	100	100
Median virulence (%)	82.5		71	70	67.5

Source: Authors

Table 4. Distribution of bacteria hosts in the lagoon Ebrié.

Lagoon sites	Presence of bacteria	Level of pollution
Boribana	1, 2, 3, 4	Very high
Abobodoume	1, 2, 3	High
Locodjoro	1, 2, 3, 4	Very high
Adiopodoume	1, 2, 3, 4	Very High

1: *E.coli*; 2: *Pseudomonas aeruginosa*; 3: *Enterobacter cloacae*; 4: *Staphylococcus aureus.*
Source: Authors

monitoring of the distribution of bacterial pathogens and therefore, reduces the risk of transmission to humans (European Directive, 2006). These results showed the distribution of phages on the Lagoon Ebrie in Abidjan city. Three major phages were isolated in all sites. Regarding the level of virulence, our study shows that *E. coli, P. aeruginosa,* and *E. cloacae* infecting phages were dominant in the natural lagoon. These findings coincide with earlier results which recorded positive evidence for *E. coli, P. aeruginosa,* and *E. cloacae* infecting phages in all water samples with varying levels. These data have concerned sewage water and Ebrie lagoon which is subjected to sources of microbial pollution. These types of natural waters receiving discharges are known to represent a high public health threat of bacterial infections in various cities in Asia, Africa, and lateen America (Costa et al., 2014; Bonilla et al., 2007; Aka et al., 2017).

But in this study, we have evaluated for each water sample whether there was the possibility of recovering the phages infecting the targeted bacteria. Their presence could be correlated with the presence of their host in the study environment. Indeed, phages are known to be present where their host is located. According to the Kill the winner theory, their abundance is also related to that of their host (Koskella and Meaden, 2013). Since phages adapt to infect the most abundant bacterial population in the ecosystem. In fact, according to these results, it seems that *E. coli* and *P. aeruginosa* are the most abundant of the bacteria studied at the Ebrié lagoon. The presence of bacterial pathogens in tropical lagoons could directly be correlated with the presence of human activities and sewage discharge around/near the lagoon. The natural lagoon of Abidjan is a receptacle for several pollutions in all seasons (Tia, 2017). The studies of Addablah et al. (2021) linked the abundance of the enterophages to the abundance of their host in waste waters that are directly drained into the lagoon without treatment, thus causing pollution.

Regular phage isolation in Lagoon Ebrie has made it possible to monitor the quality of the distribution of phages in the lagoon. But also, this study offers a perspective on using phages as a detection agent to evaluate viable bacteria in the Lagoon Ebrie. The phage-based diagnostics have been successfully conducted for the detection of P. *aeruginosa* in water.

The value of a phage diagnostic assay is mainly dependent on the ability of the phage to specifically target its host species and to infect as many strains as possible. So, the future study will be focused on isolating the phages from these lysates and evaluating their ability to infect a variety of strains belonging to the same bacterial species.

Conclusion

This study shows coli phages and Enterococci phages in Lagoon Ebrie due to human fecal pollution by septic tanks, drain collector of homes in Abidjan city. Several sites of the lagoon have high level pollution. Plural phages were isolated in the lagoon and suggest

permanent pollution and adaptation of bacteriophages in this biotope. Future perspectives will characterize the biocollection of isolated phages, their genomic variation and the application to biocontrol of contaminated biotopes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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