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International Journal of Environment and Waste Management

ISSN online: 1478-9868 - ISSN print: 1478-9876
https://www.inderscience.com/ijewm

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DOI: 10.1504/IJEWM.2023.10055256

Article History:

Received:	17 September 2020
Last revised:	27 June 2021
Accepted:	06 July 2022
Published online:	30 December 2024

In-sight into microbial structure and function of an Eastern Obolo estuarine water using metagenomics

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Abstract: Estuary microbes play several crucial roles that are not fully understood. We utilised metagenomics, a culture-independent technique to evaluate microbial composition and functions in an estuary. Sampling and physicochemical analyses were carried out using standard techniques. Extracted metagenomic DNA was sequenced using next-generation sequencing on the Illumina Miseq platform. In addition to taxonomic analysis, functional genes assessment was carried out using several pipelines for a robust analysis. The result of the physicochemical analysis showed anthropogenic influence on the estuarine water. Structural composition revealed two kingdoms (bacteria and archaea), and the dominant phyla to be firmicutes and proteobacteria. All three pipelines combined showed genes associated with energy metabolisms, post-translational modifications, and environmental information processing. Interestingly, these functions were linked to either proteobacteria (alpha and gamma proteobacteria) or firmicutes. The wide array of energy metabolisms and environmental information processing pathways confirm microbial versatility in an estuary. Evaluation of microbial structure and function as done in this study can infer the status of an ecosystem.

Keywords: metagenomics; bioinformatics; Niger-Delta; estuary; microbial function.

Reference to this paper should be made as follows: Edet, U.O., Bassey, I.U., Andy, I.E., Ebana, R.U.B. and Rufus, U.I. (2025) 'In-sight into microbial structure and function of an Eastern Obolo estuarine water using metagenomics', *Int. J. Environment and Waste Management*, Vol. 36, No. 1, pp.111–126.

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1 Introduction

The Niger Delta region of Nigeria is well known for its crude oil deposits and concomitant pollution (UNEP, 2011). It harbours the third largest mangrove forest in the world and Africa's biggest Delta (Ajibade and Awomuti, 2009). Estuary resources such as its water and sediment are very important for the estuarine communities as they support activities such as farming, transportation, waste disposal and fishing among other activities (Edet et al., 2018a, 2018b). Estuary habitats have characteristics high inflow of anthropogenic nutrients from activities of their estuarine settlements. These include organic matter such as sewage as the estuarine communities use it as a source of waste disposal (Edet et al., 2018b). There are reports of crude oil contamination in the Niger Delta region of Nigeria due to incessant oil spills (UNEP, 2011; Udotong et al., 2015, 2017). Furthermore, there may be variations in salt concentration due to the influx of salt from ocean water (Chen et al., 2016). These changes can alter the microbial community of such estuaries, as well as their functions (Edet et al., 2017a; Xu et al., 2018). In addition, hydrocarbons from crude oil and other pollutants have been shown to alter microbial composition in various habitats around the world (UNEP, 2011; Udofia et al., 2018). These pollutants are utilised by some microbes as carbon sources and in the process, they select the microbes while killing off those that cannot utilise them (Udofia et al., 2018).

Microbial communities play important roles such as recycling and decomposition of organic matter, and demineralisation of inorganic pollutants (Sun et al., 2014; Edet et al., 2017a). Furthermore, these communities are intricately involved in the biogeochemical cycles of nitrogen, phosphate, and other elements (Edet et al., 2017a; Sun et al., 2014). Several studies have shown the presence and abundance of various groups of microbes in estuaries using cultural and non-cultural techniques (Crump et al., 2004; Smith et al., 2017; Dang et al., 2019; Xu et al., 2018). Those studies based on non-culturable techniques such as metagenomics allowed for a better description of the non-culturable fraction in estuaries and other ecosystems (Edet et al., 2017a; Xu et al., 2018).

Metagenomics is a versatile technique that allows the description of microbial diversity whether it is bacteria or fungi in any ecosystem without the need to culture the organisms. This property has revealed a lot of diversities and microbial functions including putative biodegradation pathways and genes previously not known in various habitats or processes (Salam et al., 2017; Edet et al., 2017a, 2018b; Li et al., 2019). However, few studies exist that have evaluated the structural compositions of estuaries in the Niger Delta. In previous studies, microbial diversities but not functions of various ecosystems in the Niger Delta were carried out using metagenomics (Udotong et al., 2015; Edet et al., 2018a, 2018b). Furthermore, studies exist that have shown seasonal

variations in microbial loads using culturable techniques and physicochemical parameters of estuaries (Unimke et al., 2014). In this study, we utilised metagenomics on the Illumina Miseq platform to evaluate the microbial diversity of the estuarine habitat and the functions of the microbial communities therein in addition to the physicochemical parameters of the estuary.

2 Materials and methods

2.1 Sampling

The study site was Eastern Obolo Estuary in the Niger Delta region of Nigeria (see map for further details). Three sampling points were identified 10m apart from each other. Collection of the samples was done as previously reported (Bassey et al., 2015, 2016, 2018) and in triplicates from each location. Using a dual pH and temperature metre (Jenway pH meter model 3020), pH and temperature were determined *in-situ* (Brook et al., 2006). Samples were transported to the laboratory in ice immediately for further analysis.

2.2 Physicochemical analysis

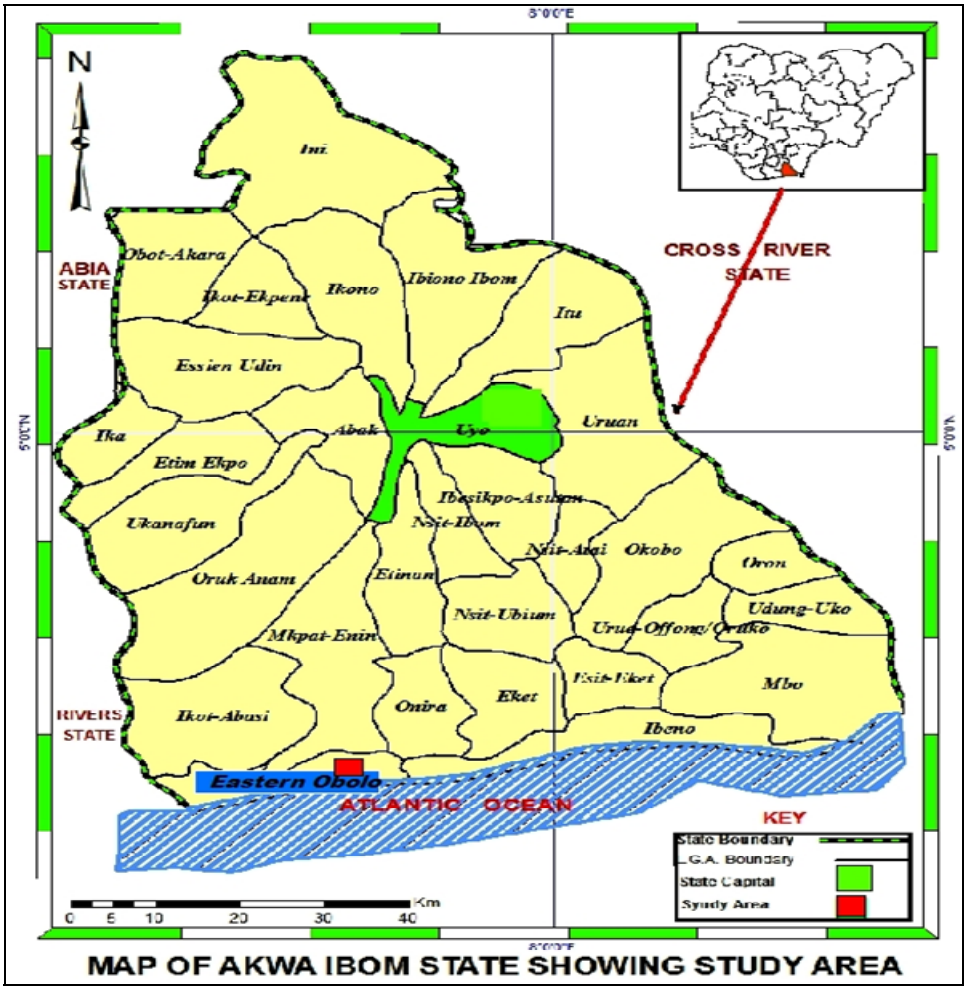
In addition to pH and temperature, the following parameters were also evaluated and these were electrical conductivity, total organic carbon, colour, chloride, nitrite and nitrate, sulphate, turbidity, dissolved oxygen, and biological oxygen demand (BOD_5), and heavy metals. Determination of heavy metals (zinc, cobalt, nickel and lead) was carried out as previously reported by Ediae et al. (2020) and Vincent-Akpu et al. (2015). Electrical conductivity, turbidity, total organic carbon, total hydrocarbon content, colour, chloride, nitrite, nitrate, and BOD were carried out as previously reported (Ekpo et al., 2000; APHA, 2008).

2.3 Metagenomics and bioinformatics analysis

Metagenomic DNA was extracted from 10 ml of the composite sample. This was done using the Zymo DNA extraction kit with strict compliance with the protocols outlined by the manufacturer. The DNA was then subjected to Illumina next-generation sequencing (NGS) using the universal primer pair – 16S: 27F: 5'-GAGTTTGATCCTGGCTCAG-3' and 518R: 5'-ATTACCGCGGCTGCTGG-3' carrying its specific adapter on the 5' ends of the forward and reverse primers. Cycling conditions were the same as those reported earlier (Salam et al., 2017; Edet et al., 2018a, 2017b; Anika et al., 2020) while NGS was carried on Illumina Miseq platform at Inqaba biotechnology, South Africa. Initial sequence alignment was carried out using Vecton NTI suite 9 (Infor Max, Inc.) bioinformatics tool and analysis was performed with sequences with q-scores of 20 and above using NCBI BLAST-2.2.24 and CLC BioGenomics workbench v7.5.1. For functional gene analysis, the Illumina pair-end sequence reads obtained were first assembled using the make-contig tool in MOTHUR v 1.33 (Schloss et al., 2009), and gene calling was then performed using MetaGene to predict open reading frames (ORFs). These ORF (length $\geq$ 400) were then functionally annotated onto the Kyoto Encyclopedia of Genes and Genomics (KEGG), GhostKOALA, the Clusters of Orthologous Groups

(COG) of proteins (Tatusov et al., 2000), and NCBI PRK functional analysis tools for a robust analysis. In GhostKOALA, each query sequence was given a taxonomic category as previously described (Kanehisa et al., 2016).

Figure 1 Map of the study area (see online version for colours)



2.4 Statistical analysis

Replicate readings obtained for the physicochemical analyses were analysed using a one-way analysis of variance and significance set at 95% (0.05). Values were then presented as mean plus or minus standard deviation (mean ± standard deviation).

3 Results

The results of the physicochemical analysis from all sampling points are presented in Table 1 together with World Health Organization (WHO, 2006) portable water standard. All the parameters apart from Zn were less than their respective standards. Across all three sampling points, pH was highest at sampling point EW3 with a value of 6.94. The temperature range was 27.56 ± 1.43–29.35 ± 1.51°C. Turbidity values ranged from 14.23 ± 0.77 at EW1 to 16.41 ± 0.81 (NTU) at EW3. The BODs in EW1 was the highest with a value of 12.35 ± 0.67 mg/l followed by EW2 and EW3. The values of nitrite and nitrates were lower than those of their respective controls. Sulphate values were 2.10 ± 0.04, 2.45 ± 0.03 and 3.41 ± 0.03 mg/l, respectively. Comparatively, dissolved oxygen values were lower than BOD and these were 3.21 ± 0.27, 2.80 ± 0.14 and 2.91 ± 0.16 mg/l, respectively for EW1, EW2 and EW3. Cobalt, nickel, iron and chromium were all within range or lower than the standard. Chromium and nickel values were fairly similar across all three sampling points and their values ranged from 0.06 ± 0.01 to 0.07 ± 0.01 mg/l and 0.04 ± 0.01 to 0.06 ± 0.01 mg/l, respectively.

Table 1 Physicochemical analysis of the estuary samples

Parameters	*EW1*	*EW2*	*EW3*	*WHO (2006, 2011)*
pH	6.81 ± 0.31	6.75 ± 0.42	6.94 ± 0.52	6.5–8.0
Temperature (°C)	28.53 ± 1.21	29.35 ± 1.51	27.56 ± 1.43	-
Colour (TCU)	78.45 ± 3.31	68.74 ± 2.53	57.25 ± 2.27	-
Turbidity (NTU)	14.23 ± 0.77	15.36 ± 0.97	16.41 ± 0.81	< 0.10
BOD_5 (mg/l)	12.35 ± 0.67	8.94 ± 0.57	7.45 ± 0.61	-
Nitrite (mg/l)	2.40 ± 0.03	2.03 ± 0.02	1.98 ± 0.02	3
Nitrate (mg/l)	5.43 ± 0.05	4.32 ± 0.03	3.53 ± 0.04	50
Sulphate(mg/l)	2.10 ± 0.04	2.45 ± 0.03	3.41 ± 0.03	500
Dissolved oxygen (mg/l)	3.21 ± 0.27	2.80 ± 0.14	2.91 ± 0.16	-
Cobalt (mg/l)	0.25 ± 0.02	0.26 ± 0.02	0.31 ± 0.01	ND
Nickel (mg/l)	0.05 ± 0.01	0.04 ± 0.01	0.06 ± 0.00	0.07
Zinc (mg/l)	1.12 ± 0.04	0.98 ± 0.03	0.78 ± 0.03	0.01–0.05
Iron (mg/l)	1.02 ± 0.05	0.78 ± 0.04	0.86 ± 0.02	> 1.0
Chromium (mg/l)	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.07

Note: ND = not detected; - = no value available and EW1, 2 and 3 = estuary water locations 1, 2 and 3.

Figure 2 shows the microbial structural composition of our estuarine sample. Kingdom classification revealed two kingdoms and these were archaea and bacteria with an abundance of 39% and 61%, respectively. Phyla classification revealed a total of eight kingdoms namely firmicutes, proteobacteria, diapherotrites, bacteroidetes, actinobacteria, euryarchaeota, cyanobacteria, and verrucomicrobia in decreasing order of abundance. Classes representing the top phyla were well represented and were dominated by unknown, beta proteobacteria, sphingobacteria, bacilli, bacteroidia, alphaproteobacteria, gammaproteobacteria, clostridia, actinobacteria, flavobacteria, and methanomicrobia in decreasing order of abundance. The order classification revealed a total of eight orders and this was dominated by unknown, clostridiales, and bacilllales as

the top three orders in decreasing order of abundance. The top three families were unknown, clostridiceae and burkholderiaceae while the top three genera were *Prevotella, Clostridum* and *Prevotella*. The species classification was dominated by unknown with an abundance of 69.48%.

Figure 2 Taxonomic classification from kingdom to species, (a) kingdom classification (b) phyla classification (c) classes classification (d) orders classification (e) family classification (f) genus classification (g) species classification (see online version for colours)

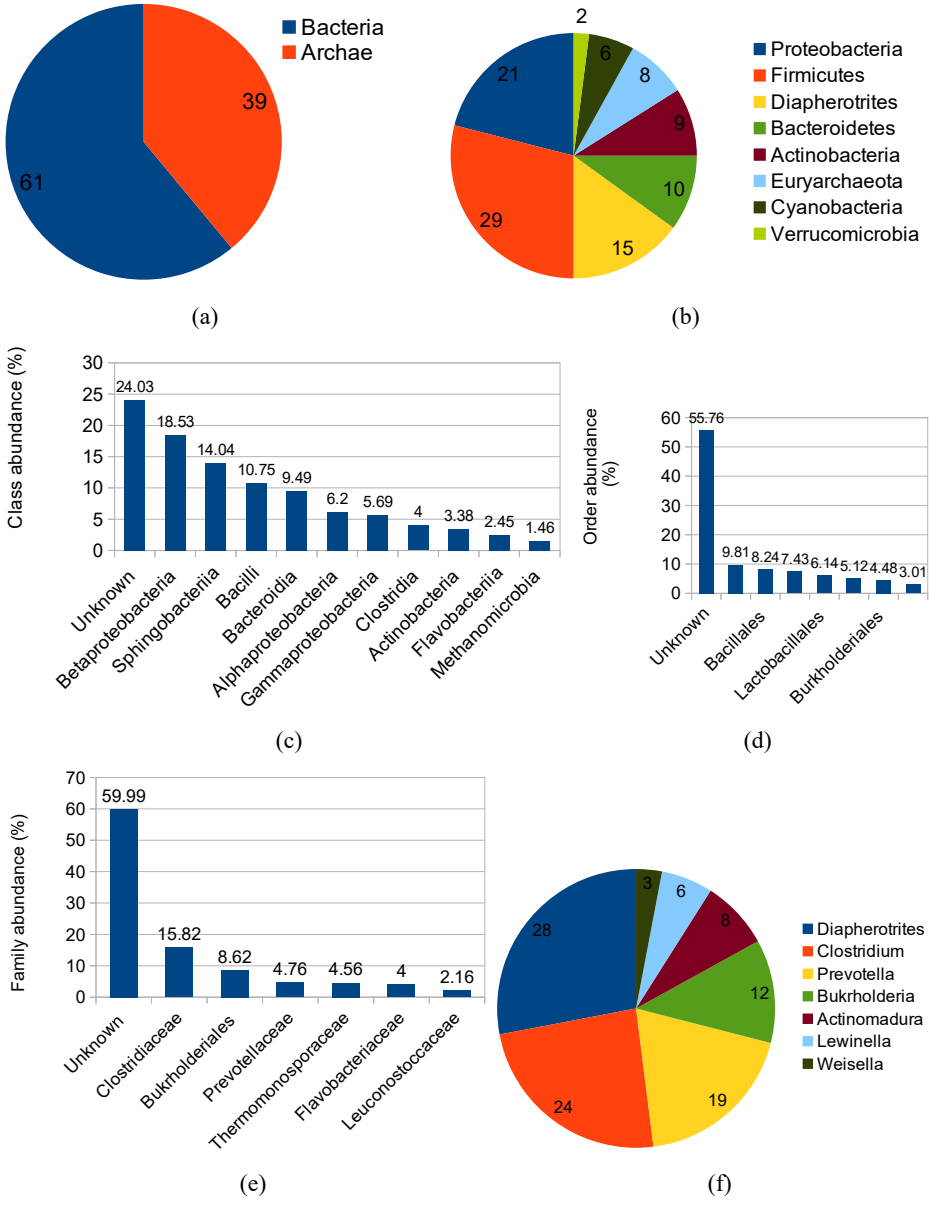
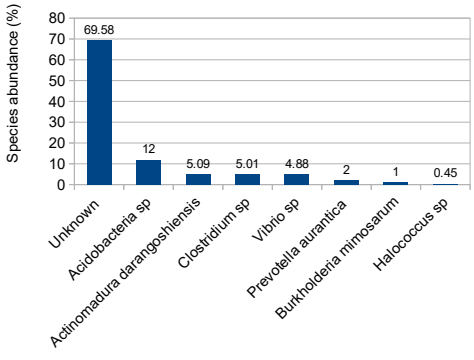


Figure 2 Taxonomic classification from kingdom to species, (a) kingdom classification (b) phyla classification (c) classes classification (d) orders classification (e) family classification (f) genus classification (g) species classification (continued) (see online version for colours)



(g)

Table 2 COG classes of annotated proteins

COG class	*Counts*	*Description*
C	1	Energy production and conversion
G	2	Carbohydrate transport and metabolism
O	8	Posttranslational modification, protein turnover and chaperones
T	2	Signal transduction mechanisms

Table 3 COG family functional annotations

COG families	*Count*	*Description*	*Class*	*Class description*
COG0297	1	Glycogen synthase	G	Carbohydrate transport and metabolism
COG4281	1	Acyl-CoA-binding protein	I	Lipid transport and metabolism
COG5116	11	26S proteasome regulatory complex component	O	Posttranslational modification, protein turnover and chaperones
COG4758	1	Predicted membrane protein	S	Function unknown

Table 2 shows the COG classes of proteins annotated in our sample while Table 3 shows their various families. A total of four COG classes were annotated and these were C, G, O and T with respective counts of 1, 2, 8 and 2, respectively. The descriptions of the classes were C (energy production and conversion), G (carbohydrate transport and metabolism), O (posttranslational modification, protein turnover, chaperones) and T (signal transduction mechanisms). From Table 3, various COG family annotations were COG0297, COG4281, COG5116 and COG7458 with counts of 1, 1, 11 and 1, respectively. The descriptions of the various genes were glycogen synthase, Acyl-CoA-binding protein, 26S proteasome regulatory complex component and predicted membrane S protein, and their respective classes were G, I, O and S. The class descriptions for G and O were similar to those of their respective family classifications while those for I and S were for lipid transport and metabolism, and function unknown respectively.

Table 4 PRK class functional annotation

PRK class	*Count*	*Description*
E	1	Amino acid transport and metabolism
J	1	Translation, ribosomal structure and biogenesis

Table 5 PRK family functional annotation

PRK family	*Count*	*Description*	*Class*	*Class description*
PRK11858	1	Trans-homoaconitate synthase	E	Amino acid transport and metabolism
PRK14552	1	C/D box methylation guide ribonucleoprotein complex aNOP56 subunit	J	Translation, ribosomal structure and biogenesis

Tables 4 and 5 show the respective classes and families of PRK protein annotated in our sample. Two classes of proteins were revealed using PRK and these were E and J with equal counts of one each. The descriptions of the protein classes were for amino acid transport and metabolism, and translation, ribosomal structure and biogenesis. The families were equally 2 and these were PRK11858 and PRK14552, respectively. These families belonged to the same classes and their proteins were trans-homoaconitate synthase and C/D box methylation guide ribonucleoprotein complex aNOP56 subunit.

Table 6 KEGG BRITE energy metabolisms

Metabolism	*Class (organisms)*	*Enzymes*	*Pathway/systems*
Nitrogen metabolism	A (*Hirschiabaltica*)	Assimilatory nitrate reductase catalytic subunit	Assimilatory nitrate reduction, nitrate => ammonia
	A (*Rhodoplanes species*)	Nitrate reductase gamma subunit	Dissimilatory nitrate reduction, nitrate => ammonia
	A (*Rhodoplanes species*)	Nitrate reductase gamma subunit	Denitrification, nitrate => nitrogen
Central carbohydrate metabolism	A (*Pelagibaca abyssi*)	Phosphogluconate dehydratase	Entner-Doudoroff pathway, glucose-6P => glyceraldehyde-3P + pyruvate
Pyrimidine metabolism	A (*Aureimonas species*)	Dihydroorotase	Uridine monophosphate biosynthesis, glutamine (+ PRPP) => UMP
Serine and threonine metabolism	F (*Thermacetogenium phaeum*)	D-3-phosphoglycerate dehydrogenase/ 2-oxoglutarate reductase	Serine biosynthesis, glycerate-3P => serine
Arginine and proline metabolism	A (*Rhizobiales bacterium NRL2*)	Acetyl ornithinedeacetylase	Ornithine biosynthesis, glutamate => ornithine
Bacterial secretion system	G (*Tolumonas auensis*)	General secretion pathway protein A	Type II general secretion system

Note: A = Alphaproteobacteria, G = Gammaproteobacteria and F = Firmicutes.

Table 6 shows the different energy metabolisms annotated in the estuary water sample and implicated species. These metabolisms and the various organisms were nitrogen metabolism (*Hirschi abaltica* and *Rhodoplanes species*), central carbohydrate metabolism (*Pelagibaca abyssi*), pyrimidine metabolism (*Aureimonas species*), serine and threonine metabolism (*Thermacetogenium phaeum*), arginine and proline metabolism (*Rhizobiales bacterium NRL2*), and bacterial secretion system (*Tolumonas auensis*). The various enzymes and pathways/systems were assimilatory nitrate reductase catalytic subunit in assimilatory nitrate reduction (nitrate => ammonia), nitrate reductase gamma subunit in dissimilatory nitrate reduction, (nitrate => ammonia), nitrate reductase gamma subunit in denitrification (nitrate => nitrogen), phosphogluconate dehydratase in Entner-Doudoroff pathway (glucose-6P => glyceraldehyde-3P + pyruvate), dihydroorotase in uridine monophosphate biosynthesis [glutamine (+ PRPP) => UMP], D-3-phosphoglycerate dehydrogenase/2-oxoglutarate reductase in serine biosynthesis (glycerate-3P => serine), acetylornithinede acetylase in ornithine biosynthesis (glutamate => ornithine), and general secretion pathway protein A in type II general secretion system.

Table 7 KEGG BRITE environmental information processing

Environmental information processing	*Class (organisms)*	*Enzymes*	*Pathways/systems*
Mineral and organic ion transport system	A (*Martelella endophytica*)	NitT/TauT family transport system ATP-binding protein	NitT/TauT family transport system
	A (*Octadecabacter antarcticus*)	Thiamine transport system ATP-binding protein	Thiamine transport system
Peptide and nickel transport system	A (*Bosea species*)	Oligopeptide transport system ATP-binding protein	Oligopeptide transport system
ABC-2 type and other transport systems	G (*Rhodanobacter denitrificans*)	Putative ABC transport system/permease protein	Putative ABC transport system

Note: A= Alphaproteobacteria and G = Gammaproteobacteria.

Table 7 shows the various environmental information processing pathways in the estuarine water sample and their respective microorganisms. These were mineral and organic ion transport systems (*Martelella endophytica* and *Octadecabacter antarcticus*), peptide and nickel transport system (*Bosea species*), and ABC-2 type and other transport systems (*Rhodanobacter denitrificans*). The respective enzymes and their pathways/systems were NitT/TauT family transport system ATP-binding protein in NitT/TauT family transport system, thiamine transport system ATP-binding protein in the thiamine transport system, oligopeptide transport system ATP-binding protein in oligopeptide transport system and putative ABC transport system/permease protein in putative ABC transport system.

4 Discussion

Mangrove sediment and estuaries act as a buffer zone between marine water and land. As is common in other habitats, estuaries around the world are under significant pressure from anthropogenic activities. They are known to harbour microbial communities that play crucial roles in biogeochemical cycles (Imchen et al., 2018; Edet et al., 2017a). Some of these crucial roles are relatively unknown (Li et al., 2019). In our study site, the physicochemical assessment showed anthropogenic influence on the estuary and it conforms with our previous findings for the same study site (Edet et al., 2018a, 2018b). Changes in microbial communities have been linked to environmental influence or factors that are capable of shaping them (Udofia et al., 2018). In aquatic and terrestrial habitats, there are diverse factors that can affect the diversities and functions of microbes. These include physicochemical, nutrients and chemicals (inorganic and organic chemicals) (Dang et al., 2019). The distribution of these environmental inputs is important in understanding the ecology and biogeochemistry of the aquatic and terrestrial ecosystems. From the result of the physicochemical analysis, the levels of both nutrients (nitrogen and phosphorus) were lower than the WHO standard used in the comparison. Phosphorus as well nitrogen sources is important nutrients that form a component of biomolecules such as nucleic acids, proteins and carbohydrates metabolisms (El-Sharkawi, 2012; Smith and Prairie, 2004). Similarly, since not all genera of microbes can fix nitrogen, there is also an increased demand for nitrogen in every habitat. The role of these environmental inputs (its gradient) as it alters the diversity and metabolism of these organisms is important in maintaining the stability of and function of estuarine and other ecosystems but at concentrations that are not harmful to the microbes (Dang and Jiao, 2014). In another study, chromium in trivalent form and nickel although needed by microorganisms for daily functioning can be toxic in certain concentrations (Asitok et al., 2019a, 2019b).

In our study, two kingdoms were classified and these were bacteria and archaea. Phyla classification revealed a total of eight phyla. In another study, they obtained some different phyla and classes of bacteria. Their annotated phyla were firmicutes, chloroflexi, chlorobi, actinobacteria, tenericutes, synergistetes, thermotogae, and bacteroidetes, among others in order of decreasing abundance (Priya et al., 2018). In addition to bacteria, Smith et al. (2017) in their study revealed key microbial players involved in a fast-flowing river-dominated estuary and impacted by phytoplankton bloom to include diatoms. The phyla in both studies were also similar to what we obtained earlier from the same study site (Edet et al., 2018a, 2018b).

In our study, the energy metabolisms showed that the microbes were very versatile and utilised an array of compounds for the production of energy and these compounds include nitrogen, carbohydrate, pyrimidine, and various amino acids such as serine, threonine, arginine and proline. Interestingly, all the annotated phylotypes belonged to the proteobacteria class and were among the top five classes. This was followed by the firmicutes and gamma proteobacteria which were linked to amino acid metabolisms for energy utilisation. Nitrogen metabolisms showed that nitrogen cycle is actively going on in the estuary as all three enzymes and pathways annotated were those intricately involved in nitrogen cycling. On the hand, carbohydrate metabolism showed the utilisation of the Entner Duodoroff reaction step that converts glucose-6-phosphate to glyceraldehyde-3-phosphate and pyruvate. The amino acid metabolisms were completely those for the biosynthesis of serine and ornithine.

In an earlier study, the dynamics of microbial composition and function in the Yangtze estuary during the spring and summer season was evaluated. Their findings showed that certain metabolisms such as carbohydrate and transport, energy production and conversion, translation/ribosomal biogenesis, cell wall/membrane/envelope biogenesis for the exit site and cell cycle control and division at the internal sites were significant. Their dominant classes were actinobacteria, alpha, beta and gamma proteobacteria, cyanobacteria, bacteroidetes, chlorobi, verrumicrobia and acidobacteria. In addition, unknown functions accounted for a large chunk of the abundance of the genes and *Synechococcus* was a significant genus. Their COG gene abundance showed the presence of 30 proteins categorised as sulphatase, ABC transporters, and DNA polymerase among others (Xu et al., 2018). Compared to our findings, the ABC transport system was also annotated and this was linked to proteobacteria. Xu et al. (2018) showed that total phosphate correlated with increased metabolisms of carbohydrate and energy, translation/ribosomal and cell wall metabolisms. Li et al. (2019) reported that enhanced sulphate levels favour methanogens and sulphate-reducing bacteria. Metals contamination is a common phenomenon in mangroves and in their study, genes associated with efflux pumps of cobalt, zinc and cadmium were detected indicating that microbes can handle metal pollution even though it could be toxic at certain concentrations. Similarly, in our study, we showed the presence of transport systems, especially for nickel and minerals. Since microorganisms often find themselves in oligotrophic environments, they constantly need to take up nutrients from the environment. Our functional metagenomics revealed the presence of various transport systems and these were the mineral and organic ion, peptide and nickel, and even putative ABC transport systems. These varied transport systems indicate the presence of various anthropogenic inorganic and organic pollutants in the estuary samples.

Li et al. (2019) evaluated the metabolic profile of contaminated and pristine mangrove sediments at subtropical and coastal sites. Their control site showed a reduced abundance of diazotrophs and nitrogen metabolism but an increased abundance of greenhouse gases and methanogenesis and sulphate reduction metabolisms. The presence of nitrogen metabolism abundance confirms the presence of anthropogenic influence in our study estuarine. Furthermore, in their polluted site, there was a high concentration of heavy metals, especially zinc, cadmium and lead, and heavy metal/antibiotics encoding genes. Even though our heavy metal concentrations were not higher than those of the standard, these findings suggest that the presence of contaminants can significantly alter microbial composition and metabolisms. Furthermore, contaminants may weaken microbial metabolisms, especially at higher concentrations. Their microbial phyla were acidobacteria, actinobacteria, firmicutes, spirochates, synergistetes, tenericutes, thermotogae, verrumicrobia, bacteroidetes, proteobacteria (alpha, beta, gamma and epsilon and delta) and planctomycetes, and these were also annotated in our study sample and was dominated by the proteobacteria and firmicutes.

The presence of certain phyla correlates with the biological activity ongoing in a certain habitat (Priya et al., 2018). In their study, Priya et al. (2018) showed certain phyla with an abundance of genes related to carbohydrate metabolism, especially those utilised in the breakdown of plant-based cell wall carbohydrates such as cellulose, pectin and hemicellulose. This also collaborated with an earlier study that evaluated mangrove sediment from India in an attempt to elucidate their microbial composition and function and compared the same to those of Brazil and Saudi Arabia. In their study, the most abundant phyla were proteobacteria, followed by firmicutes and bacteroidetes.

Furthermore, they also obtained a total of 1,942 genes that overlapped. These genes were those of antibiotics (methicillin and fluoroquinolones) and heavy metal (Co, Zn and Cd) resistance across all three ecosystems (Imchen et al., 2018). In their study, Ghai et al. (2011), posited that microbes in an aquatic environment such as a river play essential roles, especially in the breakdown of organic matter of terrestrial origin and their microbial structure was dominated by members of proteobacteria (alpha, gamma and beta proteobacteria), acidobacteria and actinobacteria in the upper course of the Amazon River. Interestingly, genes associated with heavy metals and antibiotics were also obtained in our study.

The findings of Andreote et al. (2012) in their structural and functional metagenomic assessment of mangrove sediment showed the prevalence of carbon (such as methane, formaldehyde and carbon dioxide), methane and sulphur, and nitrogen (dissimilatory reduction of nitrate, nitrogen immobilisation and denitrification) metabolisms. The linked microbial groups were eukaryota, aquificae, tenericutes, synergistetes, verrumicrobia, nitrospira, spirochaetes, unclassified, cyanobacteria, chloroflexi, bacteroidetes, actinobacteria, firmicutes and proteobacteria. Furthermore, their functional analysis showed the various metabolisms to include those of carbohydrate, lipid, nucleotide, biosynthesis of secondary metabolites, energy and amino acid, metabolisms. Others were signal transduction, replication and repairs, and membrane transport among others. Interestingly, all of these functional categories and some of the microbial groups were also captured in our study, further confirming the versatility of microbes in an estuary.

5 Conclusions

Microbial roles are always linked with their structures which in turn are influenced by anthropogenic inputs. To fully elucidate the crucial roles that microbes play in an estuary, we utilised metagenomics and sequenced extracted DNA on the Illumina Miseq platform. Physicochemistry revealed anthropogenic influence on the estuarine water. Bacteria followed by archaea dominated the kingdom taxa while phyla taxa were dominated by firmicutes and proteobacteria. Dominant genes were those of energy metabolisms from carbohydrates, amino acids and nucleic acids, post-translational modifications and environmental information processing metabolisms. The energy and environmental information processing metabolism were diverse further supporting the versatility of microbes in an estuary. Furthermore, there was an abundance of metals and mineral transportation systems/enzymes as well as putative ABC transport system/permease proteins suggesting active transport of nutrients into microbial cells.

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