Research Article

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Procaryotic Diversity of a Remote Aviation Fuel-Polluted Lentic Ecosystem in Ibeno, Nigeria

Ime R Udotong[1], Mfoniso P Uko[2*] and Justina Ime R Udotong[3]

[1]*Department of Microbiology, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria*

[2]*Department of Biological Sciences, Akwa Ibom State University, Ikot Akpaden, P.M.B. 1167, Uyo, Nigeria*

[3]*Department of Biochemistry, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria*

[*]Corresponding author
Mfoniso P. Uko, Department of Biological Sciences, Akwa Ibom State University, Ikot Akpaden, P.M.B. 1167, Uyo, Nigeria, Tel:+ 08038973046; E-mail: foniso@yahoo.co.uk.

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Abstract

Ibeno in Akwa Ibom State is the operational base of Mobil Producing Nigeria Unlimited (MPNU), a subsidiary of Exxon Mobil in the Niger Delta region, Nigeria and it remains one of the most impacted communities by oil and gas exploration and production (O&G E&P) activities. Natural bodies of water (lotic and lentic systems) in the region receive recent petroleum hydrocarbon inputs almost daily due to oil spills and oily wastes discharges. This research was carried out to determine the prokaryotic diversity in a remote aviation fuel-contaminated lentic ecosystem after 15years of aviation fuel pollution using metagenomic approaches. Water samples from the polluted and a control (unpolluted) sites were collected using sterile 1-litre plastic bottles and transported to the laboratory in ice-packed cooler for analyses. ZR Fungal/Bacterial DNA MidiPrep™ (D6105) Extraction Kit was used to obtain community DNA of all microorganisms present in the water samples. The extracted DNA fragments were amplified by Polymerase chain reaction. The quantity of the amplified product was confirmed by agarose gel electrophoresis and bioinformatic analyses of the extracted fragments were carried out in the NCBI GenBank database using BLAST software. The analysis revealed the dominance of bacterial and archaeal communities in both the polluted and unpolluted water samples. The polluted sample had in composition 93.83% bacteria, followed by 3.43% archaea and 0.36% fungi; the control site sample revealed 58.05% bacteria, 39.69% fungi and 1.05% of archaea. Bacteria are the most dominant organisms in both the polluted and unpolluted ecosystem. These findings suggest that the conditions of the two water bodies are such that allow bacterial growth and proliferation otherwise the archaea would dominate if the conditions were harsh or at extremes.

Introduction

Since the inception of oil and gas exploration and production (O&G E&P) activities in Nigeria and in spite of the increasing revenue from these resources, the communities from which they flow continue to experience deprivation and environmental degradation (Amu, 2006) due to daily inputs of petroleum hydrocarbon spills and oily wastes discharges. Ibeno is one of the thirty one (31) LGAs in Akwa Ibom State, Nigeria. It is the location of massive oil deposits, which have been extracted for decades by Mobil Producing Nigeria Unlimited (MPNU), a subsidiary of ExxonMobil Corporation and some marginal oilfield operators like Frontier Oil Ltd and Network Exploration and Production Nigeria Ltd [1].

The presence of petroleum hydrocarbon is considered one of the major factors that influence microbial diversity and succession in polluted water bodies [2]. Diverse groups of microorganisms naturally are capable of oil hydrocarbon degradation mostly as food due to the ubiquitous distribution of hydrocarbons in the environment from both natural and anthropogenic inputs [3].

This research study was designed to assess, using 16S rRNA gene amplification and sequencing, the prokaryotic diversity of a remote aviation fuel-contaminated lentic ecosystem after 15years of aviation fuel pollution alongside a control which is a lentic ecosystem with no history of aviation fuel pollution.

Materials and Methods

Site description and Sample Collection

Integrated sampling was carried out at an aviation fuel-contaminated lentic ecosystem on longitude 04° 32.647' N, and latitude 007° 59.951' E and on longitude 04° 58.519' N, and latitude 007° 57.908' E as the control. Water samples were collected at different points in one-litre pre-washed plastic containers and taken to the laboratory in ice-packed cooler. Samples from individual site were composited and used for the analyses.

Community DNA Extraction

Community DNA of all the microorganisms was extracted using the ZR Fungal/Bacterial DNA MidiPrep™ (D6105) Extraction Kit according to manufacturer's instructions. The purity of the

extracted DNA was examined by running the extract in 1% agarose gel electrophoresis.

DNA Sequencing

The extracted DNA molecules were amplified by Polymerase Chain Reaction with the aid of 16S rRNA primers in a 50 µl reaction mixture with the following programme: denaturation at 94°C for 3 min, and 30 cycles of 94°C for 20 sec, annealing at 53°C for 30 sec, and extension at 68°C for 5 min, with a final extension at 68°C for 10 min. The PCR products were confirmed by agarose gel electrophoresis. The PCR products were sequenced in Illumina after purification with a Zymo quick Gel Extraction Kit (Zymo Research).

Bioinformatics Analyses

The 16S rRNA sequences were aligned and compared with other 16S rRNA genes in the GenBank by using the NCBI Basic Local alignment search tools BLAST-nucleotide program (https://blast.ncbi.nlm.nih.gov/Blast.cgi#).

Results

Numerous sequences of bacteria and archaea were detected from both water bodies using the 16S rDNA sequencing. Comparatively, the prokaryotic composition of the contaminated water was higher than that of the control water (Table 1). From Table 1, bacteria showed a high occurrence in the two sites with the percentage composition of 93.83% in the contaminated sample and 58.05% in the control sample. The dominance of bacteria in the different ecosystems as revealed above agrees to our previous findings [1].

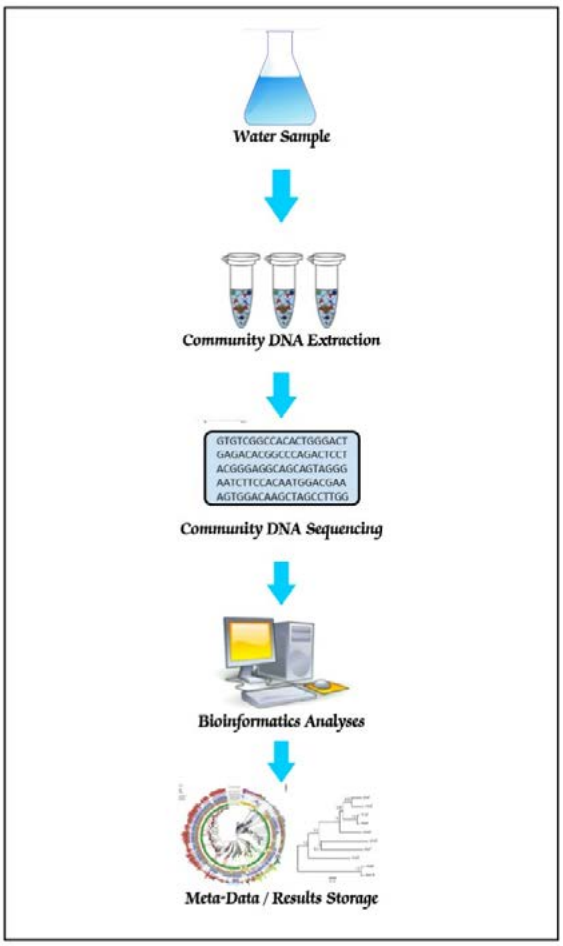

Table 1: Domain Classification of short DNA (reads) sequences detected in the aviation fuel-contaminated and control Water samples

S/N	Domain	Percentage Count / Reads (%)	
		Contaminated site	Control site
1	Bacteria	93.83	58.05
2	Archaea	3.43	1.05
3	Fungi	0.36	39.69
4	Unidentified	2.38	1.21
Total		100	100

Sequences from 26 and 20 phyla were retrieved form the contaminated and control site, respectively. "Other" represents the sum total of all phyla with percentage read of less than one (1) percent. Table 2 presents the dominant phyla in the bacterial community derived from the contaminated water sample and they include *Proteobacteria, Firmicutes, Actinobacteria, Cyanobacteria, Chloroflexi* and *un*known representing 33.86%, 7.31%, 6.19%, 3.65%, 2.84%, and 37.52%, respectively while the dominant phyla in the control water sample were Unknown, *Proteobacteria, Firmicutes*, and *Actinobacteria* and represented 21.50%, 27.87%, 2.89% and 2.61%, respectively. Dominant phyla in the archaeal community retrieved for both waters were *Euryarchaeota* and *Crenarchaeota* representing 1.33% and 0.19% in the contaminated sample and 0.24% and 0.23% in the control sample.

Table 2: Phylum classification of microbial sequences detected in the Aviation fuel-contaminated and control site

S/N	Phylum	Percentage Count (%)	
		Contaminated site	Control site
1	*Proteobacteria*	33.86	27.87
2	*Firmicutes*	7.31	2.89
3	*Actinobacteria*	6.19	2.61
4	*Cyanobacteria*	3.65	0.48
5	*Chloroflexi*	2.84	0.47
6	*Bacteroidetes*	1.88	1.37
7	*Ciliophora*	1.82	0.57
8	*Euryarchaeota*	1.33	0.24
9	*Ascomycota*	0.35	39.69
10	*Unknown*	37.52	21.50
11	*Other**	3.62	4.07

*Other is the sum total of all phylum with percentage read count of <1.

Table 3: Class classification of microbial sequences detected in the Aviation fuel-contaminated and control site

S/N	Class	Percentage Count (%)	
		Contaminated site	Control site
1	*Betaproteobacteria*	20.13	20.75
2	*Alphaproteobacteria*	8.10	1.83
3	*Actinobacteria*	6.17	2.57
4	*Bacill*	3.96	1.49
5	*Cyanophyceae*	3.38	0.44
6	*Clostridia*	3.36	1.40
7	*Gammaproteobacteria*	3.07	4.29
8	*Chloroflexi*	2.84	0.47
9	*Deltaproteobacteria*	1.93	0.64
10	*Gymnostomatea*	1.65	0.24
11	*Bacteroidetes*	1.54	0.37
12	*Methanomicrobia*	1.14	0.09
13	*Other**	4.67	6.11
14	*Not assigned*	0.05	39.55
15	*Unknown*	38.08	21.56
Total			

*Other is the sum total of all Classes with percentage read count of <1

Presented on Table 3 are the sequences belonging to 40 and 34 classes of procaryotes which were obtained from the contaminated and control water samples, respectively. Over thirty eight (38.08) percent of sequences retrieved from the contaminated water sample and 21.56% of sequences from the control water sample were affiliated to the class "Unknown". Also, 39.55% of sequences from the control site had affiliation to the class 'Not assigned' and were the highest in among the classes. While sequences similar to Betaproteobacteria (20.13%), *Alphaproteobacteria* (8.10%) and *Actinobacteria* (6.17%) dominated in the contaminated water, *Betaproteobacteria* (20.75%), *Gammaproteobacteria* (4.29%) and *Actinobacteria* (2.57%) showed highest occurrence in the control sample.

Table 4: Representative Order of procaryotes detected in the Aviation fuel-contaminated and control site

S/N	Order	Percentage Count (%)	
		Contaminated site	Control site
1	*Burkholderiales*	18.82	18.22
2	*Actinomycetales*	5.39	1.93
3	*Bacillales*	3.78	1.40
4	*Rhizobiales*	4.93	0.95
5	*Chroococcales*	3.38	0.44
6	*Clostridiales*	3.34	1.25
7	*Spathidiida*	1.56	0.24
8	*Bacteroidales*	1.54	0.37
9	*Methanosarcinales*	1.14	0.09
10	*Sphingomonadales*	1.04	0.04
11	*Pseudomonadales*	0.77	1.67
12	Neisseriales	0.11	1.13
13	Not assigned	0.03	39.51
14	Unknown	42.20	25.43
15	Other*	10.05	9.32
Total			

*Other is the sum total of all Order with percentage read count of <1.

A lot of the sequences retrieved in the contaminated water sample matched those of bacterial and archaeal diversity belonging to the Order Unknown, *Burkholderiales*, *Actinomycetales*, and *Rhizobiales* while those in the control sample matched those of Not assigned, Unknown, and *Burkholderiales* and were the most top orders.

Majority of the sequences were affiliated to the family Unknown, with the percentage composition of 44.50% followed by *Alcaligenaceae* (16.55%) in the contaminated water and *Coniocybaceae* (39.51%) and *Burkholderiaceae* (9.36%) in the control water (Table 5).

Table 5: Family classification of microbial sequences detected in the Aviation fuel-contaminated and control site

S/N	Family	Percentage Count (%)	
		Contaminated site	Control site
1	Unknown	44.50	25.66
2	*Comamonadaceae*	1.71	8.12
3	*Alcaligenaceae*	16.55	0.72
4	*Bacillaceae*	3.62	0.93
5	*Cyanobacteriaceae*	3.38	0.44
6	*Beijerinckiaceae*	3.06	-
7	*Streptomycetaceae*	2.78	0.77
8	*Coniocybaceae*	0.03	39.51
9	*Burkholderiaceae*	0.38	9.36
10	*Neisseriaceae*	0.11	1.13
11	*Pseudomonadaceae*	0.36	1.11
12	*Clostridiaceae*	1.33	1.04
13	*Eubacteriaceae*	1.64	0.15
14	*Spathidiidae*	1.56	0.24
15	*Chloroflexaceae*	1.16	0.27
16	*Methanosarcinaceae*	1.14	0.04
17	*Sphingomonadaceae*	1.04	0.04
18	Other	23.07	14.07

Other is the sum total of all Family with percentage read count of <1.

Bacterial and archaeal species with gene sequences affiliated to those present at the two study sites together with their accession numbers are represented on Table 6. Both sites share a few common species and are indicated with "+" sign for both sites while most of the organisms don't share species. Majority of the prokaryotic sequences are those of uncultured bacteria as well as uncultured archaea. Some of the species in common include *Bacillus sphaericus* with accession number AY161044.1, *Achromobacter* sp.-AM232721.1, Uncultured *Gloeothece* sp.-AY874086.1, *Pantoea* sp.-AJ534866.1, *Stigonema ocellatum*- AJ544082.1, *Pseudomonas aeruginosa*-AB126582.1, *Simkania* negevensis-SSU68460.2, Uncultured Chloroflexus sp.-AY862018.1. Observably, plenty of the species found in the contaminated sample are not found in the control sample and vice versa.

Table 6: Representative diversity of bacterial and archaeal community detected in aviation fuel-contaminated and control water sample by 16S rDNA analysis

Bacterial and Archaeal Species	Aviation fuel site	Control site	% Identity Match	Accession number
Uncultured bacterium	+	-	93	KF023595.1
Uncultured bacterium	+	-	81	GU632587.1
Mycobacterium sp.	-	+	95	LN876401.1
Uncultured *bacterium*	+	-	83	GQ402641.1
Uncultured *archaeon*	+	-	96	KJ645016.1
Acidovorax sp.	-	+	96	KR088454.1
Uncultured *Streptophyta*	-	+	89	JQ701246.1
Staphylococcus sp.	-	+	83	AJ316320.1
Saprospira sp.	+	+	98	AY929064.1
Uncultured *Methanosarcina* sp.	+	-	95	AY454773.1
Achromobacter sp.	+	+	95	AM232721.1
Uncultured *Gloeothece* sp.	+	+	93	AY874086.1
Uncultured *Verrucomicrobia* bacterium	-	+	73	AY874030.1
Uncultured *Bacteroidetes* bacterium	-	+	95	AY874003.1
Uncultured *Beijerinckia* sp.	+	-	94	AY806011.1
Streptomyces sp.	+	-	86	AB124448.1
Streptomyces sp.	-	+	91	AB124529.1
Simkania negevensis	+	+	98	SSU68460.2
Pseudomonas aeruginosa	+	+	81	AB126582.1
Unidentified bacterium	+	-	86	AJ518513.1
Bacillus anthracis	-	+	83	AE016879.1
Bacillus sp.	+	-	85	AB126768.1
Bacillus sphaericus	+	+	78	AY161044.1
Uncultured *Chloroflexi* bacterium	+	-	95	AY921865.1
Acidovorax delafieldii	+	-	81	AJ518818.1
Uncultured *Chloroflexus* sp.	+	+	95	AY862018.1
Unidentified *eubacterium*	+	-	95	AJ229218.1
Pseudomonas sp.	-	+	75	AJ278108.1
Uncultured *Methanosphaera* sp.	-	+	77	AY454780.1
Uncultured *crenarchaeote*	-	+	87	AY454669.1
Uncultured archaeon	+	-	96	DQ146728.1
Spirulina subsalsa	+	+	95	AF329394.1
Pyramimonas parkeae	-	+	98	AF393608.1
Pseudomonas saccharophila	-	+	77	AF396932.1
Bradyrhizobium sp.	-	+	82	AF363148.1
Uncultured *Methanobacteriaceae*	+	-	91	AM050403.1
Uncultured candidate division OD1	+	-	86	AY921841.1
Uncultured soil bacterium	+	-	96	AY850299.1
Chlorella sorokiniana	-	+	88	X65689.1
Leptolyngbya sp.	+	-	91	X84809.1
Gemmata obscuriglobus	-	+	94	X85248.1
Methylocapsa acidiphila	+	-	93	NR_028923.1

Key: + = detected - = not detected

Evidently, the prokaryotic diversity in the aviation fuel-contaminated water is relatively higher in composition than in the uncontaminated water. This may be attributed to the presence of petroleum hydrocarbons and their effects on the diversity and population of procaryotes especially the bacterial group in the freshwater system, an observation earlier reported by Atlas and Bartha, that the presence of petroleum hydrocarbons affects the diversity, distribution and population of microorganisms in an environment. Regarding fungal diversity, only very few numbers were found in the contaminated water ecosystem (Table 1) [4]. This agrees with Obire and Anyanwu, who reported that crude oil enhances the population of a fewer fungi [5].

Conclusion

The 16S rDNA analysis of the prokaryotic diversity of the remote aviation fuel-contaminated and control lentic ecosystem revealed an enormous composition of bacteria and archaea in both water bodies. The contaminated water had a greater composition of procaryotes than the control water. Bacterial community got a higher diversity of the composition than the archaea in both waters. Fungi were very few in the contaminated water but higher than archaea in the uncontaminated water. Hence, fungi are unable to thrive in hydrocarbon polluted sites. This research study helps to know the trend of microorganism in hydrocarbon polluted and unpolluted freshwater resource [6-12].

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