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Molecular Studies of Bacterial Isolates of Some Benthic Seafood Harvested from Oil Producing Areas in the Niger Delta, Nigeria

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Abstract

The benthic region of the sea consists of a wide variety of organisms including the seafood (molluscs and crustaceans), which serve as a rich source of nutrients for man and other organisms. Oil exploration and exploitation activities in the Niger Delta region of Nigeria has resulted in environmental, socioeconomic and physical damage of the ecosystem over. Bacteria were isolated from selected benthic seafood harvested from oil producing areas in the Niger Delta using standard methods. The molecular identification of the isolates were carried out using the BigDye Terminator kit and the bioinformatics algorithm Trace Edit. Similar sequences from the National Center for Biotechnology Information (NCBI) database were matched using Basic Local Alignment Search Tool Nucleotide (BLASTN). Sequences were aligned using MAFFT and the evolutionary history inferred using the Neighbour-Joining method. The extracted DNA samples all had acceptable range of DNA concentration and DNA purity. All the twelve organisms isolated and identified were associated with contaminated water and seafood. Identified bacteria include *Pseudomonas aeruginosa, Myroides odorantimimus, Bacillus flexus, Bacillus velezensis, Shewanella chilikensis, Enterobacter hormaechei, Enterobacter cloacae, Escherichia fergusonii, Escherichia coli and Klebsiella pneumoniae*. Some of the isolates had been reported to be pathogenic and food spoilage. Environmentally friendly practices in oil production will ensure a healthy aquatic environment in the Niger Delta.

Keywords: Molecular studies; Bacterial isolates; Seafood's.

1. Introduction

The complex community of organisms living on, or in the bottom of a water body is known as "benthos". Benthic community includes a wide range of organisms ranging from bacteria to plants and animals from different levels of the food web [1]. They include polychaetes and oligochaetes; molluscs (bivalves and gastropods); and crustaceans (ampiphods and decapods). The benthic invertebrates are important in transitional ecosystems as they filter phytoplankton and act as food source for larger organisms e.g. fishes linking the primary production with the higher trophic levels. Marine benthic fishes assess the quality of marine environments due to their ability to concentrate large amounts of various substances including microorganisms and heavy metals in their muscles and gills. Gills are sensitive to changes of water components and thus serve a good indicators of water quality because the gill filaments provide a large surface area for contact with contaminants in water [2].

Benthic seafood are common sources of diet in the Niger Delta region of Nigeria. The Niger Delta with diverse ecosystems is the hub of oil production in Nigeria [3] with the associated problems of oil spillage, a global occurrence that is common in Nigeria [4]. Oil spillage affects inhabitants and damages the ecosystems. Unsustainable practices has rendered the Niger Delta region of Nigeria as one of the five most damaged ecosystems in the world resulting in the region characterized by contaminated streams and rivers, forest destruction and the loss of biodiversity.

2. Materials and Methods

Seafood samples were obtained from the markets and water bodies of selected locations using the methods described by Hewitt and Martin [5] and Hoedt, *et al.* [6]. Samples collected include Periwinkle, Prawn, Oysters and Crabs. The samples were preserved in ice and transported to the laboratory for analysis. Samples were collected from five locations within the study area (Ox-bow Lake (Yenagoa) 4.55^{0}N, 6^{0}16'E; Azuzuama 4^{0}43'N, 5^{0}57'E; Nembe 4^{0}32'N, 6^{0}17'; Ogbia 4^{0}39'N, 6^{0}16'E and Brass 4^{0}18'N, 6^{0}14').

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Samples with shells were treated by removing their shells using standard sterile procedures. Ten grams (10g) of flesh from sample was homogenized with 90 ml sterile distilled water for 3 mins in a stomacher blender. Ten fold serial dilution was made using 1% (w/v) sterile distilled water [7]. Homogenate samples were then inoculated on Nutrient Agar and selective media [8] using spread plate technique. These were then incubated at room temperature and observed after 24 – 48 hrs.

Isolated bacteria were subjected to molecular identification using the method described by Lee and Ringdale [9]. Bacteria was detected using PCR based multiplex assay. Target genes that encode for bacterial DNA subunits were used for the identification of closely related strains of bacteria [10-12]. The method of identification include DNA extraction, Quantification, Amplification; Detection and Sequencing; and Phylogenetic tree. Isolates were sub-cultured in Luria Bertami broth media for 24 hrs.' and used for the physical method of DNA extraction. Quantification was done using Nanodrop 1000 Spectrophotometer and Amplification carried out using a Mutiplex PCR machine (Gene Amp* PCR System 9700). Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer. Sequences were edited using the bioinformatics algorithm Trace edit. Similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using Basic Local Alignment Search Tool Nucleotide (BLASTN) aligned using MAFFT. The evolutionary history was inferred using the Neighbour-Joining method in MEGA 6.0 [13]. The bootstrap consensus tree inferred from 500 replicates [14] is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method [15].

3. Results and Discussion

After 12-48 hrs incubation, 12 representative isolates were identified and labeled A-J using their colonial morphology. The results of DNA quantity and purity are presented in Table 1. All extracted DNA samples had values above the recommended lower limit (5.0 - 100 ng/µL). Samples A, D, E, F, H, I and L had concentrations above the recommended limits, the highest being 87 Ng/µL. All the extracted samples had purity values within the recommended range of 1.0 – 2.5 NaN. Table 2 shows the isolated organisms and their corresponding match with the NCBI database using the BLAST and Fig. 1 shows the Phylogenetic relationship of the extracted DNA samples.

Isolate A had a 100% match with *Pseudomonas aeruginosa* strain AU4850. *Pseudomonas* spp are indigenous to aquatic environments occurring in large numbers in polluted aquatic environment [16]. Isolate B has a 100% match with *Myroides odoratimimus* strain G13. *Myroides odoratimimus* is a non-fermenting Gram negative, aerobic and non-motile bacteria that cause urinary tract infection in man but have recently been isolated from fishes [17, 18]. Isolate C has a 100% match with *Bacillus flexus* strain PBCS2. Isolate D and isolate E have a 61.8% match with *Bacillus velezensis* strain KACC_15894. *Bacillus* spp are common in aquatic systems [19], whereas isolate F is a 100% match with *Shewanella chilikenesis* strain BOB2. Isolate G showed a 100% match with *Enterobacter hormaechei* strain N706. Isolates H and L have 57% match with *Escherichia fergusonii* strain CICC 24137 while isolate I has 57% match with *Escherichia coli* strain AR_0067. Isolate J has a 100% match with *Enterobacter cloacae* strain DL01 while K has 97.4% match with *Klebsiella pneumoniae* strain IRQBAS50. *Shewanella chilikensis* is common in chilled seafood while species of *Enterobacter* and *Escherichia* are coliforms that are commonly distributed by humans. The identified organisms were similar to those reported by Akinyemi and Buoro [20].

4. Conclusion

Oil production activities has resultant effects on the aquatic ecosystem with corresponding contamination of the benthic region. The benthic seafood that serve as reservoir of nutrients for human consumption also serves as a reservoir for a variety of bacteria some of which are pathogenic to man. Oil production activities which has devastated the environment of the Niger Delta also contributes to the bacterial load of water bodies within the region [21]. With the aid of molecular identification techniques, bacteria that are transferred down the food chain in the benthic ecosystems can be properly identified and documented.

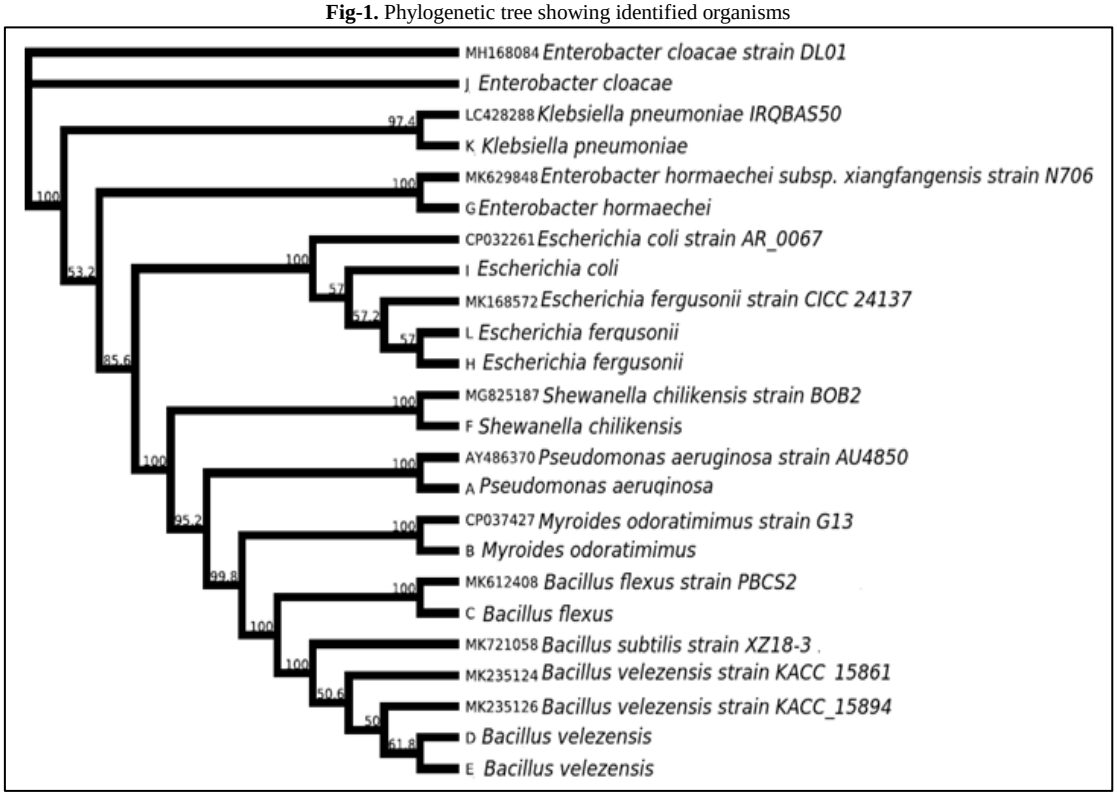
Table-1. Results of DNA Quantification of Isolated Organisms

S/N	Sample	DNA Concentration (ng/µL)	DNA Purity NaN
1.	A	154.1	2.03
2.	B	48.7	2.15
3.	C	35.3	2.16
4.	D	251.4	2.07
5.	E	874.4	2.11
6.	F	144.6	1.92
7.	G	10.1	1.14
8.	H	190.3	2.06
9.	I	200.1	2.04
10.	J	30.4	2.25
11.	K	65.9	1.90
12.	L	236.0	2.10

Table-2. Molecular identification of isolated organisms

S/N	Sample	Sequence	NCBI match	Isolates
1	A	AY486370	*Psuedomonas aeruginosa* strain AU4850	*Psuedomonas aeruginosa*
2	B	CP037427	*Myroides odoratimimus* strain G13	*Myroides odoratimimus*
3	C	MK612408	*Bacillus flexus* strain PBCS2	*Bacillus flexus*
4	D	MK721058	*Bacillus velezensis* strain KACC_15894	*Bacillus velenesis*
5	E	MK 235124	*Bacillus velezensis* strain KACC 15861	*Bacillus velenesis*
		MK235126	*Bacillus velezensis* strain KACC_15894	
6	F	MG825187	*Schewanella chilikensis* strain BOB2	*Schewanella chilikensis*
7	G	MK629848	*Enterobacter hormaechei* sussp. xiangfangensis strain N706	*Enterobacter hormaechei*
8	H	MK168572	*Escherichia fergusonii* strain CICC 24137	*Escherichia fergusonii*
9	I	CPO32261	*Escherichia coli* strain AR_0067	*Escherichia coli*
10	J	MH168084	*Enterobacter cloacae* strain DL 01	*Enterobacter cloacae*
11	K	LC428288	*Klebsiella pneumoniae* IRQBAS50	*Klebsiella pneumoniae*
12	L	MK168572	*Escherichia fergusonii* strain CICC 24137	*Escherichia fergusonii*

Fig-1. Phylogenetic tree showing identified organisms



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