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Isolation and identification of microorganisms from bitumen, and soil around bitumen deposit site

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Abstract

Isolation of microorganisms from hydrocarbon and oil polluted environment has been reported over the years. This study focused on the isolation and identification of microorganisms from bitumen, and soil around bitumen deposit site. Microorganisms were isolated from soil sample collected around bitumen deposit and bitumen itself using pour plate method. The cultural and morphological characteristics of each bacteria isolates such as colony shape, elevation, pigmentation, colour, cell shape, gram reaction, motility, and endospore test were carried out. From the result, the following bacteria were identified; *Acinetobacter* sp (soil and bitumen), *Pseudomonas Aeruginosa* (soil), *Bacillus subtilis* (soil and bitumen), *Staphylococcus aureus* (soil). Mould isolation was carried out by cutting a small portion of mycelium, stained it with cotton blue in lactophenol and the examined carefully under light microscope with x40 0bjective. The result revealed the following mould isolates; *Aspergillus niger* and *Arthrobotrys oligospora* both isolated from the soil. The result showed the isolation of microorganisms from bitumen and soil around bitumen deposit, thus their potential usefulness in remediation of bitumen polluted environment if the exploitation of the resource commenced.

Keywords: Hydrocarbon; Bitumen; Nigeria; Bacteria; Mould

1 Introduction

Bitumen is a viscous liquid, or a solid consisting essentially of hydrocarbons and their derivatives, and is soluble in carbon disulphide, substantially non-volatile and softens gradually when heated. It occurs naturally on its own or as a by-product of vacuum distillation of crude oil. It is blackish or brownish in colour and possesses water proofing and adhesive properties (Olutoye,[1]). Elemental analysis showed that bitumen is composed principally of carbon and hydrogen, with trace of mineral matter such as nitrogen, sulphur and oxygen (Meyer and Witt, [2]; Guma *et al.,* [3]). Road pavement is the principal use of bitumen and it takes about 80% of the total bitumen consumption.

Nigeria has a considerable large deposit of natural bitumen which is found in the Eastern Dahomey (Benin) basin (Oboh *et al.,*[4]; Fagbote and Olanipekun, [5]; Ayoade *et al.,*[6]). Nigeria has a proven reserve of 42.47 billion metric tonnes, the second largest in the world, covering about 120 × 4.3km (Oboh *et al.,* [4]; Ayoade *et al.,* [6]).

Although exploration of the Nigerian tar sand oil deposit has not yet commenced, the environment is already battling with the problem of pollution arising from seepages of the natural resource into the surface water and soil in the communities where the resource is found (Adesanya *et al.,* [7]). During dry season period, the tar sand oil is free flowing contaminating the surrounding environment, water surface and under aquifer (Olabemiwo *et al.,* [8]). The presence of this hydrocarbon in the environment has resulted in the infertility of the land, reduction of water quality, shortage of oxygen and reduction in aquatic population (Abii and Nwosu, [9]; Akpor *et al.,* [10]; Ajayi, [11]). The dominance of

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polyaromatic hydrocarbon in soil and water polluted with tar sand oil has been linked to health issues such as damage to body organs, kidney, liver, carcinogenic and mutagenicity (Fagbote and Olanipekun, [12; Olabemiwo *et al.,* [8]).

Isolation and identification of microorganism isolates from soil and petroleum contaminated environment have been widely reported by many researchers.Species of *Alternaria, Aspergillus, Candida, Cephalosporium, Cladosporium, Fusarium, Geotrichum, Mucor, Penicillium, Trichoderma* has been isolated from the soil and has been proved to degrade petroleum hydrocarbon (Obire and Anyanwu, [13]; Al-Ghamdi, [14]; Al-Jawhari, [15]). Oboh *et al.* [4] studied hydrocarbon degrading potential of bacteria strains, namely *Pseudomonas stutzeri, Pseudomonas mulli* and *Alcaligenes* sp. isolated from bitumen sample and undisturbed soil of Agbabu, Ilubirin and Mile 2, all in Odigbo local government area, to degrade kerosene, diesel and naphthalene. In a study done by Adesanya *et al.* [7], species of bacteria *Pseudomonas, Bacillus, Citrobacter, Flavobacterium, Micrococcus, Acinetobacter,* and *Staphylococcus* isolated from the bitumen contaminated water and sediments in Ilubirin, Temidire camp, and Agbabu communities were found to utilized diesel as sole carbon source. The present study was designed to isolate and identify microorganisms from bitumen, and soil around bitumen deposit site.

2 Material and method

The site for collection was Agbabu village in Odigbo Local Government Area of Ondo State, Nigeria, within the geographical grids of latitude 6^0 35’ 16.3’’N and 6^0 37’ 13.9’’N and longitude 4^0 29’ 29.0E and 4^0 50’ 20.7’’E (Amigun *et al.,* [16]).

Soil samples were collected around the bitumen deposit site at two different locations. The distance was about 2m for the first sample point and 5m for the second sample point, to the bitumen borehole well. The soil was at collected at 0-5cm depth and the other at 10-15cm depth from the surface.

The bitumen sample was collected from the bitumen well at mile 2 where the resources seep to the surface of the land. Sterile metallic spoon was used to fetch the bitumen sample and collected in a sterile reagent bottle.

2.1 Isolation and Identification of Microorganisms

Isolations were carried out from the soil and bitumen sample using pour plate method under aseptic conditions. Nutrient agar and acidified Malt extract agar were employed for bacteria and mould isolations respectively. Sub-culturing was carried out until pure cultures of each organism were obtained.

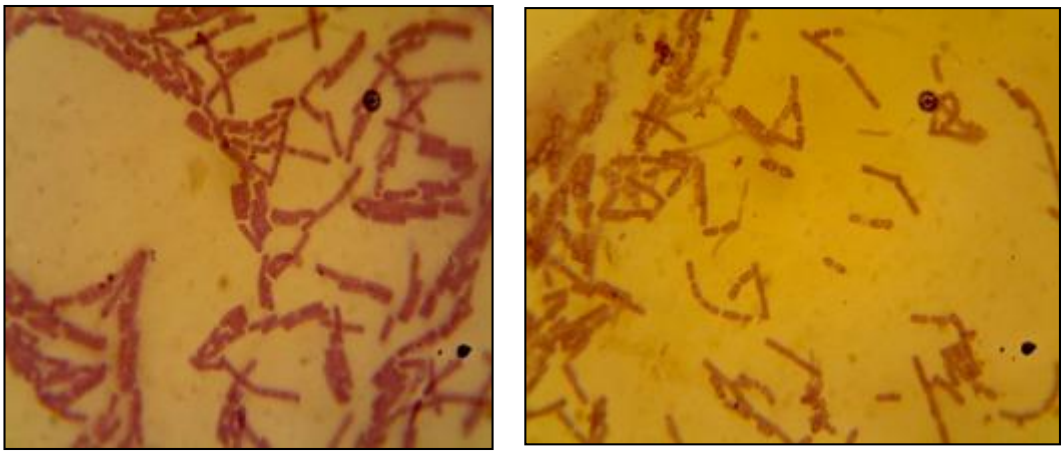
Bacteria isolates were identified by cultural. Morphological, and biochemical characteristics using the taxonomical scheme of Bergey’s manual of Determinative Bacteriology (Bergey *et al.,* [17]).The macroscopic and microscopic characteristics of colour on the plate, hyphal diameter, spore shape and size among other criteria were used for mould identification as reported by Barnatte and Hunter [18] and Watanabe [19].

3 Results and discussion

3.1 Microbial identification

The cultural and morphological characteristics of each bacteria isolates such as colony shape, elevation, pigmentation, colour, cell shape, Gram reaction, motility and endospore test are indicated on table 1. The table also has the results of biochemical test for the bacteria isolates such as catalase test, oxidative fermentative test, sulphate reduction test and starch hydrolysis test, and also the sugar fermentation test.

The isolate A had regular shape, raised elevation, short rod, negative to Gram staining, non-motile, positive for both oxidative and fermentative test, negative for starch hydrolysis and sulphate reduction test, catalase positive, glucose fermentative positive without production of gas and negative for fructose fermentation. The isolate A was isolated from both soil and bitumen sample. On the basis of the cultural, morphological, biochemical and sugar fermentation, isolate A was identified as *Acinetobacter* sp.



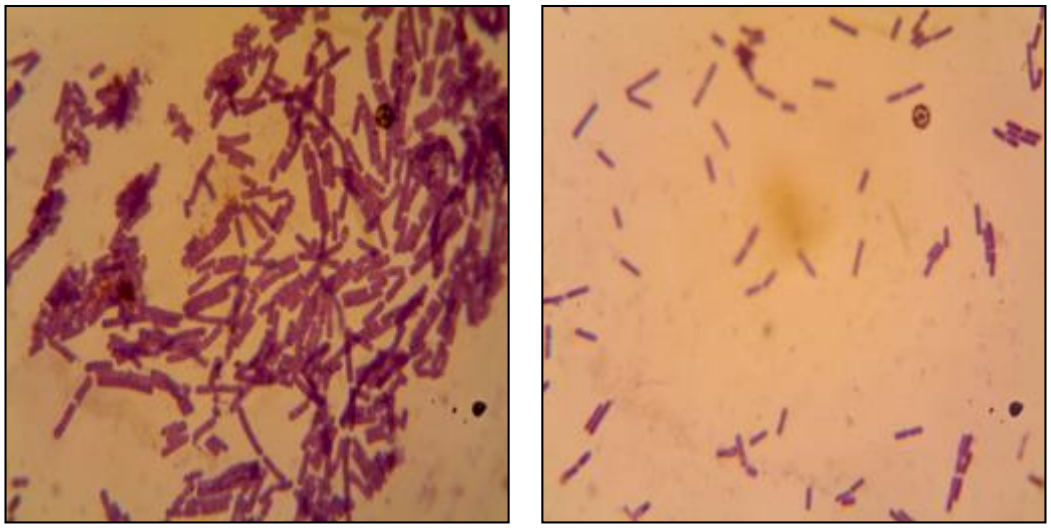
A B

Figure 1 Photomicrograph of *Acinetobacter* sp from soil (A) from bitumen (B) (X 1000)

Isolate B had regular shape, raised elevation, greenish pigmentation, short rod, Gram negative and motile organism, positive for both oxidative and fermentative test, hydrolyses starch, sulphate reduction negative, catalase positive ferment glucose and fructose. The isolate was from the soil sample. The above cultural, morphological, biochemical and sugar fermentation revealed isolate B to be *Pseudomonas Aeruginosa.*

Figure 2 Photomicrograph of *Pseudomonas aeruginosa* (X1000)

Isolate C had an irregular shape, raised elevation, long rod, Gram positive and motile bacterium, positive for both oxidative and fermentative test, starch hydrolysis and sulphate reduction negative, positive for catalase test, ferment glucose, fructose, sucrose and maltose. From the cultural, morphological, biochemical and sugar fermentation, isolate C was deduced to be *Bacillus subtilis*. The *Bacillus subtilis* was isolated from both the soil and bitumen sample



A B

Figure 3 Photomicrograph of *Bacillus subtilis* from soil sample (A) and bitumen sample (B) (X1000)

Isolate D had regular shape, raised elevation, cocci in cluster, Gram positive organism, non-motile, positive for both oxidative and fermentative test, hydrolyses starch, negative to sulphate reduction test, catalase positive, ferment glucose, fructose, sucrose, maltose and lactose. Isolate D was isolated from the soil sample. The cultural, morphological, biochemical and sugar fermentation test revealed isolate D to be *Staphylococcus aureus*.

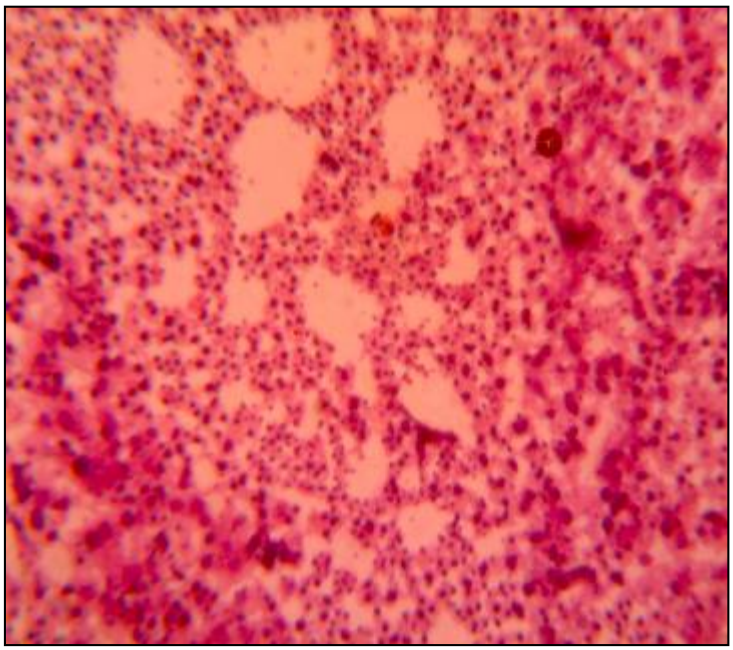


Figure 4 Photomicrograph of *Staphylococcus aureus* from soil (X1000)

Isolate E was black mould on malt extract agar. Microscopic observation revealed that isolate E hyphae was black, an aseptate hyphae, long erected conidiophore and spherical spore. The cultural and microscopic observation of isolated E showed that it was *Aspergillus niger*. *Aspergillus niger* was isolated from the soil.

Isolate F was brown mould on malt extract agar and microscopic examination revealed that isolate F had brown hyphae, septate hyphae, long erected conidiophore and an oval spore. On the basis of the cultural and microscopic observation, isolate F was identified to be *Arthrobotrys oligospora*. This isolate was from the bitumen sample.

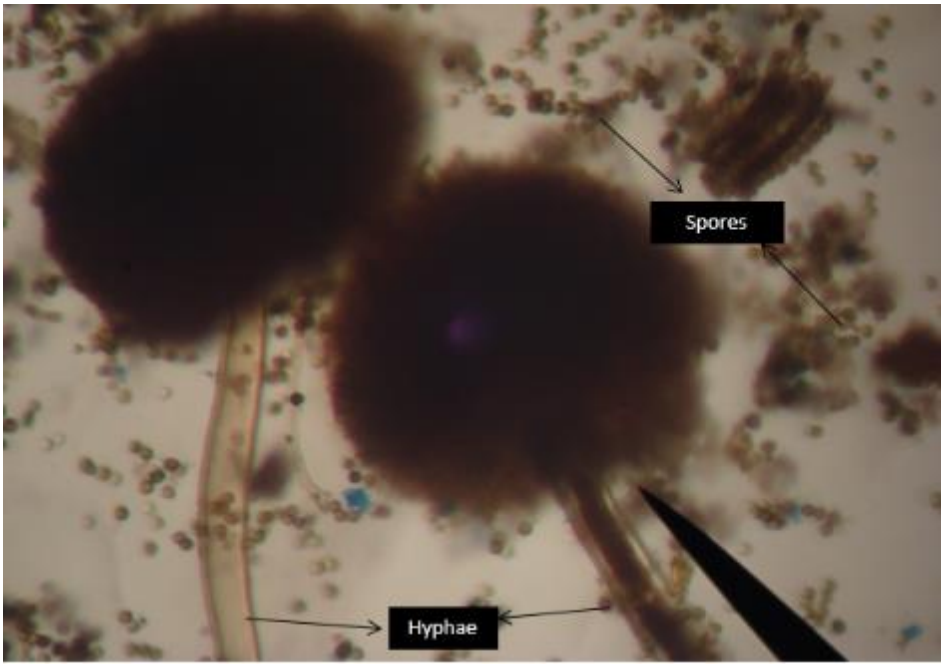


Figure 5 Photomicrograph of *Aspergillus niger* (X400)

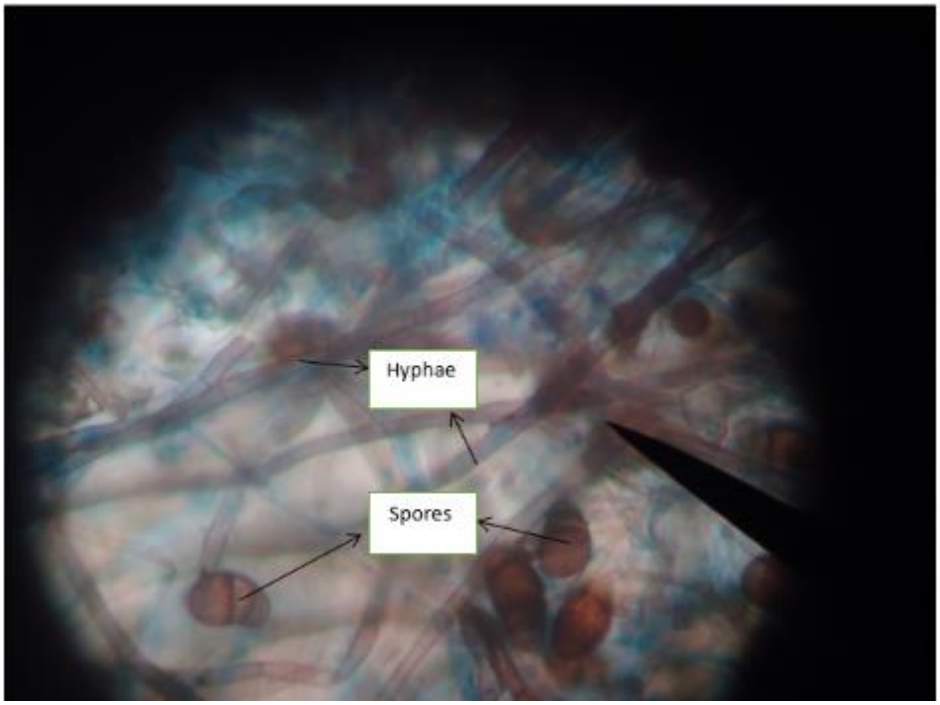


Figure 6 Photomicrograph of *Arthrobotrys oligospora* (X400)

Table 1 Cultural, Morphological and Biochemical characteristics of bacteria isolates from soil around Agbabu bitumen site

Cultural Characteristics					**Morphological Characteristics**			**Biochemical Characteristics**				**Sugar Fermentation**						
Colony Shape	**Elevation**	**Pigmentation**	**Colour**	**Cell Shape**	**Gram Reaction**	**Motility**	**Endospore Test**	**Oxidative Fermentation Test**	**Starch Hydrolysis**	**Sulphate Reduction**	**Catalase Test**	**Glucose**	**Fructose**	**Sucrose**	**Maltose**	**Lactose**	**Raffinose**	**Probable Organism**
Regular	Raised	_	White	Short rod	_	_	_	O/F	_	_	+	+	_	_	_	+	_	*Acinetobacter* sp
Regular	Raised	Greenish	White	Short rod	_	+	_	O/F	+	_	+	+	+	_	_	_	_	*P.aeruginosa*
Irregular	Raised	_	Cream	Short rod	+	+	+	O/F	_	_	+	+	+	+	+	_	_	*B. subtilis*
Regular	Raised	_	White	Cocci	+	_	_	O/F	+	_	+	+	+	+	+	+	_	*S. aureus*

Key: - = Negative Test, + = Positive Test, O/F = both oxidative and fermentative positive

The features revealed the following probable bacteria: *Acinetobacter* sp., *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus.* The probable mould isolates were *Aspergillus niger* and *Arthrobotrys oligospora*. Most of these microorganisms have been isolated from soil and hydrocarbon polluted environment by other researchers (Ogunjobi and Fagade, [20]; Oboh *et al*., [4];Boboye *et al*., [21]; Olabemiwo *et al*., [22]; Omotayo *et al*., [23]; Al-Jawhari [15]. The study revealed for the first time the isolation of *Arthrobotrys oligospora* from natural tar sand bitumen.

4 Conclusion

The isolation of microorganism from Bitumen and soil collected around Bitumen deposit was carried out in this study. The ability to isolate high numbers of petroleum hydrocarbon degrading microorganisms from an environment indicates that these microorganisms are the active degraders in that environment, thus these microorganisms should be tested to ascertain their role in bioremediation of bitumen polluted environment.

Compliance with ethical standards

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Disclosure of conflict of interest

There was no conflict of interest among the authors. Prof Mrs Aborisade Abiola Titilola played supervisory role during the research work and I duly notified her of my intension of sending the research work for publication. She gave in her full support for the ongoing publication.

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