

Original Research Article

ISOLATION AND IDENTIFICATION OF SOME HYDROCARBON UTILIZING

BACTERIA ISOLATED FROM CONTAMINATED SOIL IN ZUMA, BWARI AREA

COUNCIL, FCT, ABUJA, Nigeria.

Abstract

The wide spread use of petroleum products leads to contamination of soil and aquatic environments, thereby posing a serious threat to all life forms including humans. Therefore, isolation of oil-degrading microorganisms and optimizing conditions for biodegradation process are important. Thus, this study was aimed at isolating and characterizing hydrocarbon utilizing bacteria from hydrocarbon contaminated soil in Bwari area council, Federal Capital Territory, Abuja, Nigeria, using three different petroleum products (petrol, kerosene and diesel) thereby checking their catabolic capacities on the products. Enrichment culture technique (using mineral salt medium; MSA) was employed to obtain fourteen (14) bacterial isolates capable of utilizing the petroleum products. Biochemical characterization showed the isolates identified and their prevalence to be *Pseudomonas* spp. 21.43%, *Bacillus* spp. 21.43%, *Acinetobacter* spp. 7.14%, *Corynebacterium* spp. 7.14%, *Staphylococci* spp. 7.14%, *Citobacter* spp. 14.29%, *Aeromonas* spp. 14.29%, and *Flavobacterium* spp. 7.14%. The hydrocarbon utilizing bacteria count ranged from 1.8×10^4 to 1.5×10^8 cfu/ml showing a high range of different bacteria found in polluted soil due to formation of consortia to degrade the substrates. The highest growth was found in petrol sample (PS) followed by kerosene sample (KS) and then diesel sample (DS), implying the ability of these organisms to rapidly utilize lower molecular and low density hydrocarbon than higher ones. Applications of this adaptation of microorganisms have been seen in bioremediation, oil recovery, indicators of environmental pollution of petroleum hydrocarbons, etc which are all environmental friendly and cost effective.

Key words: Bacteria, Soil, Hydrocarbon, Nigeria.

1.0 Introduction

Oil spillage and oil pollution in water environment have been a major threat to the ecosystem and human being through the transfer of toxic materials including polycyclic aromatic hydrocarbons (PAHs) into the food chain (Sei and Fathepure, 2009). Presence of polycyclic aromatic hydrocarbons in the soil and water is a major problem as environmental contaminants and most of these PAHs are recalcitrant in nature. Physical and chemical methods like volatilization, photooxidation, chemical oxidation, and bioaccumulation (Zhao *et al.*, 2008) are rarely successful in rapid removal and cleaning up PAHs (Dasgupta *et al.*, 2013), and also these methods are not safe and cost effective when compared to microbial biodegradation.

In many ecosystems there is already an adequate indigenous microbial community capable of extensive oil biodegradation, provided that environmental conditions are favourable for oil-degrading metabolic capacity (Capelli *et al.*, 2004, Kim *et al.*, 2004). There are several advantages relying on indigenous microorganisms rather than adding microorganisms to degrade hydrocarbons. First, natural populations have developed through many years. These microorganisms are adapted for survival and proliferation in that environment. Secondly, the ability to utilize hydrocarbons is distributed among a diverse microbial population. This population occurs in natural ecosystems and either independently or in combination metabolizes various hydrocarbons. A number of hydrocarbon-degrading microorganisms produce emulsifying agents (Rahman *et al.*, 2002, Sood and Banwari, 2009, Venvolden and Copper, 2003 and Jain, 2011). Some of these bioemulsifiers have been considered for use in cleaning oil storage tanks, such as supertankers (Zhang *et al.*, 2012). In some cases, the emulsifying agents appear to be fatty acids or derivatives of fatty acids; in other cases, more complex polymers are active emulsifying agents. Although the production of emulsifying should increase the susceptibility of hydrocarbons in oil to microbial degradation, microbial strains which effectively emulsify oil often do not extensively degrade the hydrocarbons in the oil.

2.0 Materials and Methods

Sampling area

The sampling area was Zuma in Bwari area council of Federal Capital Territory, Abuja, lying between latitude 8.25 and 9.20 north of the equator and longitude 6.45 and 7.39 east of the Greenwich meridian, Abuja is geographically located in the center of the country.

Sample collection

Soil sample was obtained from the generator house in Liberty Hotel from Zuma in Bwari area council of Federal Capital Territory, Abuja, Nigeria. The samples were aseptically collected using sterile spatula in different portions and depth of 15-20cm. The samples were stored in sterile aluminum foils and transported to the laboratory within 24 hours of collection.

Isolation procedure

The modified method of Francy et al., 2000 was employed. About 20.0 grams of the contaminated soil sample was inoculated into 180ml of mineral salt broth (MSB) in a 250ml conical flask. The mixture was then shaken thoroughly to produce a well dispersed suspension and this was incubated at 30°C for 72 hours. This aid at resuscitating the stressed microorganisms present in the sample. The mixture was serially diluted (10^{-1} to 10^{-6}) from the stock sample using sterile distilled water and plated in triplicates on mineral salt agar (MSA) incorporated with nystatin to inhibit the growth of fungi. A filter paper (Whatman No. 1)saturated with the different hydrocarbon/petroleum products (kerosene, diesel, and petrol) was aseptically placed on the plates over the lid of the same petri dish and these were incubated while inverted (vapour phase transfer method) for 14 days at 30°C.Pure cultures of each isolate were prepared on agar slants for further analysis.

3.0 Results and Discussion

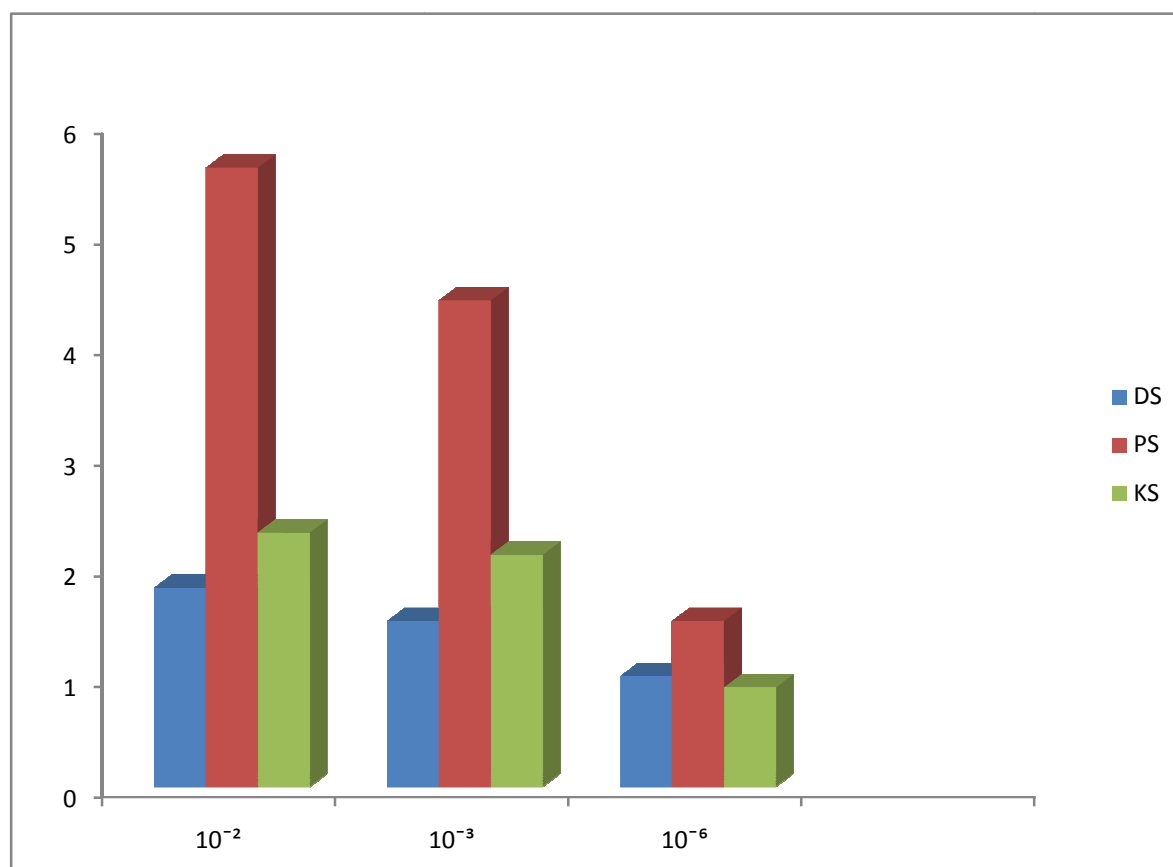
Table 2.1 below shows total heterotrophic count which ranges from 1.8×10^4 to 1.5×10^8 cfu ml⁻¹, isolate code number PS had the highest number of hydrocarbon utilizing bacteria count followed by KS and DS, these varies from the average colony count.

Table 2.1: Total Heterotrophic Count (Colony forming unit per milliliter)

Isolates	Colony count			Average colony count	Dilution factor	Total count (CFU/ml)
DS 1	18	25	11	18	10^{-2}	1.8×10^4
PS 1	55	51	62	56	10^{-2}	5.6×10^4
KS 1	20	23	23	23	10^{-2}	2.3×10^4
DS 2	18	17	10	15	10^{-3}	1.5×10^5
PS 2	38	54	41	44	10^{-3}	4.4×10^5
KS 3	21	19	23	21	10^{-3}	2.1×10^5
DS 3	12	8	10	10	10^{-6}	1.0×10^8
PS 3	13	21	11	15	10^{-6}	1.5×10^8
KS 3	6	11	10	9	10^{-6}	9.0×10^7

Fig 1 below shows the comparison of the number of hydrocarbon utilizing bacteria on mineral salt medium with the different carbon sources. It can be deduced that for all the dilutions, the

80 highest number of hydrocarbon utilizing bacteria was observed in the plate having petrol as the
 81 carbon source(PS) followed by that with kerosene (KS) and then lowest in that with diesel (DS).
 82 This consistency shows that for all the petroleum products used, petrol was the most preferred
 83 followed by kerosene and then diesel.



84 **FIG I:** Graph showing the cfu/ml of the hydrocarbon utilizing bacteria on mineral salt medium
 85 having the different carbon sources.

86 **Table 2.2** below shows the colonial morphology of the test isolates. Out of the 14 isolates, 8
 87 were convex, and 6 were flat in terms of elevation. All the isolates displayed circular shapes, 8
 88 isolates were smooth with regular shaped edges like ring and the remaining 6 isolates showed
 89 irregular shaped edges. In terms of their characteristic colouration, 6 isolates showed cream
 90 colouration, 1 was milky white in colour while 3 showed brownish white colouration, 2 had a
 91 greenish white colouration, 1 was creamy yellow and 1 was brownish yellow and the diameter of
 92 the isolates ranges from 0.1 to 3.7 cm in sizes. 11 isolates were opaque and 3 transparent.

S/N	Isolate code number	Shape	Elevation	Transparency	Colour	Edges	Size (cm)
1	DSa	Circular	Flat	Opaque	Cream	Rough	3.7
2	DSb	Circular	Convex	Transparent	Cream	Smooth	2.3
3	KSc	Circular	Convex	Opaque	Cream	Smooth	2.7
4	DSd	Circular	Flat	Opaque	Greenish white	Rough	0.6
5	KSf	Circular	Flat	Opaque	Cream	Rough	3.1
6	PSf	Circular	Convex	Opaque	Brownish white	Smooth	1.3
7	PSg	Circular	Convex	Transparent	Cream	Smooth	0.1
8	KSh	Circular	Convex	Opaque	Brownish white	Smooth	0.4
9	Psi	Circular	Flat	Opaque	Milky white	Rough	2.5
10	PSj	Circular	Flat	Opaque	Cream	Rough	0.8
11	KSk	Circular	Flat	Opaque	Greenish white	Rough	0.5
12	PSl	Circular	Convex	Transparent	Creamy yellow	Smooth	1.2
13	KSm	Circular	Convex	Opaque	Brownish White	Smooth	0.3
14	PSn	Circular	Convex	Opaque	Brownish yellow	Smooth	0.3

93

94 **Key:**

95 PS1 – Petrol Sample plate 1 PS2 –Petrol Sample plate 2 PS3 – Petrol sample plate 3

96 KS1 –Kerosene Sample plate 1 KS2 –Kerosene Sample plate 3 KS3 –Kerosene Sample plate 3

97 DS1 –Diesel Sample plate 1 DS2 –Diesel Sample plate 2 DS3 –Diesel Sample plate 3

98 Table 2.3 below shows the cellular morphology, biochemical characterization and Gram reaction
99 of the isolates from this study with reference to Bergey’s manual of determinative bacteriology
100 9th edition (1995). It was observed that *Bacillus* and *Pseudomonas* species were the predominant
101 isolates in the whole of the hydrocarbon contaminated soil samples screened followed by

102 *Citrobacter* and *Aeromonas* species, then *Acinetobacter*, *Corynebacterium*, *Staphylococcus* and
103 *Flavobacterium* species.

104

105

106

107

108

109

110

111

112

113

114

115

116

117

118

119

120

121

122

123 Table 2.3: Gram stain, Morphology and Biochemical characteristics of isolates

S/N	Isolates	Cell morphology	Grams	Reaction	Catalase	Indole	MR	VP	H ₂ S	Spore test	Motility	Citrate	Glucose	Sucrose	Lactose	Mannitol	Urea	Coagulase	Heamolysis	Probable genera
1	D S a	R o d s	+	+	-	+	+	+	-	+	-	+	A/G	-	A	+	-		β	<i>B a c i l l u s</i> s p p .
2	D S b	R o d s	-	+	+	+	+	+	+	-	-	-	A/G	-	-	+	-		α	<i>A e r o m o n a s</i> s p p .
3	K S c	R o d s	-	+	+	+	+	+	+	-	-	-	A	-		+	-		α	<i>A e r o m o n a s</i> s p p .
4	D S d	R o d s	-	+	-	-	-	-	-	-	+	+	A	-	-	-	-		α	<i>P s e u d o m o n a s</i> s p p .
5	K S e	R o d s	-	+	-	+	+	+	-	+	+	+	A/G	-	-	+	-		β	<i>B a c i l l u s</i> s p p .
6	PSf	Coccobacilli	-	+	-	-	-	-	-	-	-	+	A/G	-	A	+	-		γ	<i>Acinetobacter</i> spp.
7	P S g	Rods	+	+	-	+	-	-	-	-	-	+	A/G	-	A	-	-		γ	<i>Corynebacterium</i> spp.
8	K S h	R o d s	-	+	-	+	-	+	-	-	+	+	A/G	A	A	+	-		γ	<i>Citrobacter</i> spp.
9	P s i	R o d s	+	+	-	+	+	+	-	+	+	+	A/G	-	A	+	-		β	<i>B a c i l l u s</i> s p p .
1 0	P S j	R o d s	-	+	-	-	-	-	-	-	+	+	A	-	-	+	-		α	<i>Pseudomonas</i> spp.
1 1	K S k	R o d s	-	+	-	-	-	-	-	-	+	+	A	-	-	+	-		α	<i>Pseudomonas</i> spp.
1 2	P S l	C o c c i	+	+	-	+	+	+	-			+	A	A	A	+	-	+	β	<i>S t a p l y l o c o c c u s</i> <i>aureus</i>
1 3	K S m	R o d s	-	+	-	+	-	+	-	-	-	+	A/G	A	A	+	-		γ	<i>Citobacter</i> spp.
1 4	P S n	R o d s	-	+	-	-	-	+	-	-	-	-	A	-	A	+	-		γ	<i>Flavobacterium</i> spp.

124

125

Discussion

Soil had been known to be a favourable habitat for the proliferation of microorganisms, with micro colonies developing around soil particles, but the addition of refractory humic substances slow down the activities of these organisms, thus giving room to organisms that have the ability of metabolizing such products and limiting the growth of non-metabolizers of the products (Obire and Nwaaubeta, 2002). Biodegradation has been considered as efficient, economic, versatile and environmentally sustainable treatment. Degradation of petroleum hydrocarbons by environmental micro flora involves microorganisms having specialized metabolic capacities.

Contaminated soil was analyzed for hydrocarbon utilizing bacteria. The hydrocarbon utilizing bacteria ranged from 1.5×10^3 to 2.8×10^7 cfu/ml. Thus the counts of hydrocarbon- utilizing bacteria obtained from the contaminated soil were very high. This may be attributed to the fact that contaminated soils often harbour a vast array of microbial flora that is capable of utilizing the hydrocarbon as energy and carbon source and also due to the presence of residual hydrocarbon in the polluted soil which boosts the carbon supply in the soil, hence favours the growth of the hydrocarbon utilizing bacteria (HUB) which is in line with the report of Ijah and Abioye (2003). It has been observed that hydrocarbon discharge to the ecosystem may result in the high microbial population in the soil (Okpokwasili, 2000). Fourteen bacteria isolates which include organisms from the genera *Corynebacterium*, *Acinetobacter*, *Pseudomonas*, *Citobacter*, *Staphylococci*, *Bacillus*, *Flavobacterium* and *Aeromonas* isolated from the contaminated soil were similar to the reports of Obire and Nwaauebeta. The genera *Pseudomonas* and *Bacillus* were the most predominant as expected because they are commonly ubiquitous especially the ones in oil polluted areas (Makkar and Cameotra, 2002). Previous reports have proved extensive microbial diversity with population estimated between approximately 4×10^3 to 10^4 species per g of uncontaminated soil (Adesemoye *et al.*, 2006). Some microorganisms are more abundant in areas of high concentration of hydrocarbons as they are very resistant such as *Pseudomonas* spp. able to form adaptive structures such as spores as seen in *Bacillus* spp. and their ability to use nitrate as an electron acceptor and in the process producing or giving off hydrogen sulphide gas as seen in *Citobacter* spp. and *Aeromonas* spp. The microorganisms isolated have been implicated in hydrocarbon degradation and are among such organisms. The hydrocarbon-utilizing bacteria isolated were dominated by Gram negative bacteria belonging to a wide range of taxa which is in

accordance with the report of Adesemoye *et al.*, (2006). On the basis of the growth data, we could infer that petrol was highly preferred by the isolates in relation to the reports of Amanchukwu *et al.*, (2000) and Okpokwasili (2000). This accounts for the ability to rapidly degrade low density and low molecular weight hydrocarbon as compared to those of kerosene and diesel which were utilized but at a much slower rate.

References

- Adesoye, E., Adedamo, F., and Okere, S. (2006). Utilization of hydrocarbon utilizing bacteria by *Micrococcus sp.* From hydrocarbon contaminated soil. *Environmental International Microbiology*, 3, 211-234.
- Amanchukwu, U. I., Okoro, E. S. and Lawson, E. (2013). Isolation and identification of hydrocarbon utilizing bacteria from crude-oil contaminated site. *Applied Environmental Microbiology*, 3(8), 78-90.
- Atlas, R. and Bragg, J. (2009). Bioremediation of marine oil spills: when and when not-The Exxon Valdez experience. *Microbiology of Biotechnology*, 2, 213-221.
- Capelli, S. M., Busalmen, P. J. and De Sánchez R. S. (2001). Hydrocarbon bioremediation of a mineral-base contaminated waste from crude oil extraction by indigenous bacteria. *International Bio deterioration and Biodegradation*; 47, 233-238.
- Dasgupta, D., Ghosh, R. and Sengupta, T. K. (2013). Biofilm mediated enhanced crude oil degradation by newly isolated *Pseudomonas* species. *Improvement Science Research Network Biotechnology*, 2013, 1-13.
- Ijah, J. and Abioje, R. (2003). Degradation and mineralization of crude oil by bacteria. *Letter Microbiology*, 12 (4), 72-76
- Makkar, O and Cameotra, F. O. (2002). Isolation of hydrocarbon of hydrocarbon utilizing bacteria from gasoline contaminated soil. *Journal of Biotechnology*, 4(2), 68-75.

- 171 Nwachukwu, S. U. (2001). Bioremediation of sterile agricultural soils polluted with crude petroleum by application of the soil
172 bacterium *Pseudomonas putida*, with organic supplements. *Current Microbiology*, 42(2), 231-236.
- 173 Obire and Nwaaubeta, T. (2002). Effects of refined petroleum hydrocarbon on soil physic-chemical and bacteriological
174 characteristics. *Journal of Applied Science and Environmental Management*, 6(1), 39-44.
- 175 Okpokawsili, O. (2000). Isolation and characterization of hydrocarbon degraders from contaminated soil. *African Journal of*
176 *Biotechnology*, 5, 233-333.
- 177 Sei, A. and Fathepure, B. Z. (2009). Biodegradation of hydrocarbons in high salinity by an enrichment culture from hypersaline
178 sediments of Rozel point of great Sal lake. *Journal of Applied Microbiology*, 107(6), 2001-2008.
- 179 Sood, N. and Banwari, L. (2009), Isolation of a novel yeast strain *Candida digboiensis* TERI ASN6 capable of degrading petroleum
180 hydrocarbons in acidic conditions. *Journal of Environment Management*, 90, 1728-1736.
- 181 Venvolden, K. A. and Copper, C. K. (2003). Natural seepage of crude oil into the marine environment. *Geo-Marine Letters*, 23(3-
182 4), 140-146.
- 183 Zhang, D. C., Mortelmaier, C. and Margesin, R. (2012). Characterization of the bacterial archaeal diversity in hydrocarbon-
184 contaminated soil. *Science Total Environment*, 421, 184-196.
- 185 Zhao, H. P., Wang, L., Ren, J. R., Li, M. and Gao, H. W. (2008). Isolation and characterization of phenanthrene-degrading strains
186 *Sphingomonas* sp. ZP1 and *Tistrella* sp. ZP5. *Journal of Haradous Materials*, 152(3), 1293-13.