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CHARACTERIZATION OF HYDROCARBON UTILIZING BACTERIA IN SOIL SAMPLES COLLECTED FROM VARIOUS SITES IN PORT HARCOURT (NIGER-DELTA, NIGERIA)

[1]Stanley, H. O., [2]Amakiri, M.A. & [1]Okerentugba, P.O.
[1]Department of Microbiology, Faculty of Biological Science, College of Natural and Applied Sciences, University of Port Harcourt, P.M.B. 5323 Port Harcourt, Rivers State, Nigeria
[2]Department of Forestry and Environment, Faculty of Agriculture, Rivers State University of Science and Technology, Nkpolu Port Harcourt, Rivers State, Nigeria

ABSTRACT
In this study, the hydrocarbon utilizing bacteria present in soil samples collected from various sites in Port Harcourt (Niger-Delta, Nigeria) was characterized. The soil samples collected from the six locations namely; Agricultural soil (AGS) in UNIPORT, Forest soil (FOS) in UNIPORT, Pine vegetation soil (PVS) in UNIPORT and RSUST respectively, Mangrove soil (MGS) in RSUST and Melina vegetation soil (MVS) in RSUST were analysed for their microbiological qualities using standard culture-dependent techniques. Some physicochemical parameters of the soils were also determined using standard methods. Soil pH, moisture, nitrate, phosphate, potassium and total organic carbon ranged from 5.9 to 6.8, 10.2% to 24.8%, 13.3mg/kg to 20.7mg/kg, 11.3mg/kg to 26.3mg/kg, 5.9mg/kg to 10.4mg/kg and 2.5% to 9.7% across the sample locations, respectively. Total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) populations ranged from 6.2 x 10^7 CFU/g to 2.5 x 10^7 CFU/g and 2.4 x 10^3 CFU/g to 1.2 x 10^3 CFU/g across the sample locations, respectively. The total heterotrophic bacteria isolated and identified belonged to the following genera; *Alcaligenes, Arthrobacter, Bacillus, Corynebacterium, E. coli, Erwinia, Flavobacterium, Gordonia, Mycobacterium, Norcadia, Pseudomonas, Rhodococcus, Salmonella, Shigella and Staphylococcus*, with *Bacillus* been the predominant THB across the sample locations with a frequency of 14.40%. The hydrocarbon utilizing bacteria isolated and identified belonged to the following genera; *Arthrobacter, Bacillus, Corynebacterium, Flavobacterium, Gordonia, Mycobacterium, Norcadia, Pseudomonas and Rhodococcus*, with *Gordonia and Rhodococcus* been the predominant HUB across the sample locations with a frequency of 20.20% and 16.30% respectively. Species of these bacteria isolates are known hydrocarbon degraders and it is assumed that the genera identified from these soils may have the catabolic ability to use petroleum hydrocarbons as source of carbon. Therefore, these soils may harbour important bacteria genera that may have beneficial applications in petroleum microbiology.

KEYWORDS: hydrocarbon, bacteria, petroleum microbiology.

INTRODUCTION

The increase in demand for petroleum as a source of energy and a primary raw material for chemical industries in recent years has resulted in an increase in world production (Gutnick and Rosenberg, 1977). This dramatic increase in production, refining and distribution of crude oil has brought with it an ever increasing problem such as terrestrial and aquatic pollution (Pepper *et al.*, 1996; Odu *et al.*, 1997; Atlas and Barther, 1992). Nigeria still depends largely on crude oil for income earnings. Crude oil which is abundantly located in the Niger Delta region of Nigeria is mainly spilled on soil due to pipeline destruction (Nwilo and Badejo, 2005). Previous studies on crude oil pollution in soil had revealed its adverse effects on the physicochemical and biological properties of soil (Ewetola, 2013; Nwachukwu and Ugorji, 1995; Okpowasili and Odokuma, 1990; Udo and Fayemi, 1975). Soil is a major component of the environment and is inhabited by a wide range of microorganisms, including bacteria, fungi, algae, viruses and protozoa. Microorganisms are found in large numbers in the soil- usually between one and ten million microorganisms are present per gram of soil - with bacteria and fungi being the most prevalent. Soil bacteria are the primary digestive system of the soil and their activity is responsible for almost 90% of all biological and chemical actions. The toxicity of crude oil or petroleum products varies widely, depending on their composition, concentration, environmental factors and on the biological state of the organisms at the time of the contamination. Biodegradation of hydrocarbons by natural population of microorganisms represents one of the primary mechanisms of eliminating petroleum pollution from the environment (Leahy and Colwell, 1990). The ability to degrade and/or utilize hydrocarbon substrates is exhibited by a wide range of bacteria and fungi (Kiyohara *et al.*, 1992; Johnson *et al*., 1996; Antai, 1990; Bhattacharya *et al.*, 2002). The ability to isolate these hydrocarbons degraders from a pristine or oil-polluted environment is commonly taken as evidence that these microorganisms may be the active degraders of hydrocarbon pollutants in the environment (Okerentugba and Ezeronye, 2003). The aim of this study was to characterise hydrocarbon utilizing bacteria (HUB) isolated from various sites in the University of Port

Harcourt (UNIPORT) and Rivers State University of Science and Technology (RSUST) respectively located in Port Harcourt, Nigeria.

MATERIALS & METHODS

Collection and Determination of Physicochemical Parameters of Soil Samples

Composite soil samples ranging from 0 – 15cm depth were collected using soil auger. These soil samples were collected from various locations which included Agricultural soil [AGS] in UNIPORT (Nigeria); Forest soil [FOS] in UNIPORT (Nigeria); Pine vegetation soil [PVS] in UNIPORT (Nigeria); Mangrove soil [MGS] at RSUST in Port Harcourt (Nigeria); Melina vegetation soil at RSUST in Port Harcourt (Nigeria)and Pine vegetation soil [PVS] at RSUST (Nigeria). After collection, soil samples were separately put in sterile polyethylene bags and immediately taken to the laboratory for physicochemical and microbiological analyses. Soil parameters such as pH, moisture content, nitrate, phosphate, potassium and total organic carbon (TOC) were determined using the methods from APHA (1998).

Enumeration and Isolation of Bacterial

Total heterotrophic bacterial (THB) count was determined using the spread plate method on nutrient agar (Sigma-Aldrich) according to APHA (1998). Soil suspensions were prepared by 10 fold serial dilutions with 1 g of soil and then 10^{-5} dilution was spread on the plates in triplicates. The colony forming unit (CFU) of heterotrophs was counted after incubation at 28°C for 24hrs. Hydrocarbon utilizing bacteria (HUB) were enumerated as adopted from Hamamura *et al*. (2006) using mineral salts medium with crude oil as the sole source of carbon. Isolated colonies were further purified by sub-culturing and identified using biochemical tests and microscopy (Holt *et al.*, 1994).

Statistical analysis

Two-way ANOVA test was used to determine whether there was a significant difference between the population of total heterotrophic bacteria (THB) and the population of hydrocarbon utilizing bacteria (HUB) with respect to the variation in sample location and culture medium, respectively.

RESULT & DISCUSSION

Physicochemical Analysis

The pH of soil samples ranged from 5.9 to 6.8 across the various locations. The highest soil pH (6.8) was recorded in the Mangrove soil (RSUST) while the lowest soil pH (5.9) was recorded in the Pine vegetation soil (RSUST). Soil moisture content ranged from 10.2% to 24.8% across the sample locations.

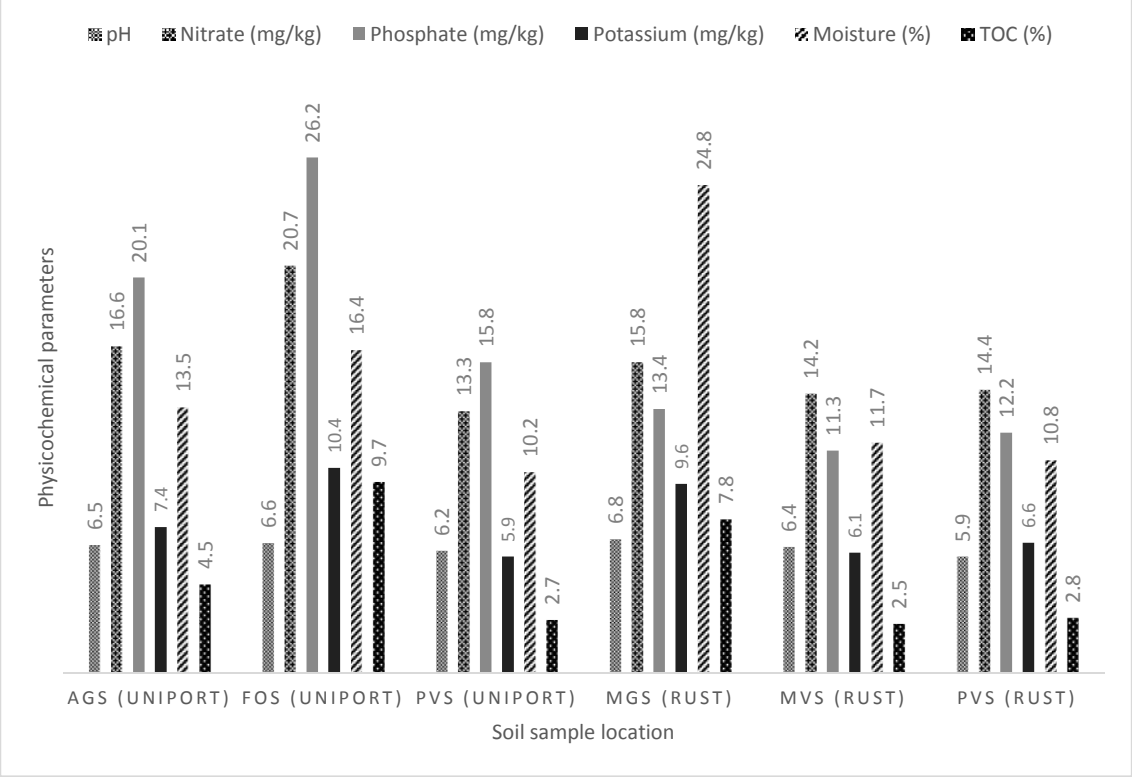

FIGURE 1: Physicochemical properties of the soil samples collected from various locations in the University of Port Harcourt (UNIPORT) and Rivers State University of Science and Technology (RSUST), respectively.

The highest soil moisture (24.8%) was recorded in the Mangrove soil (RSUST) while the lowest soil moisture (10.2%) was recorded in the Pine vegetation soil (UNIPORT).Nitrate concentration ranged from 13.3mg/kg to 20.7mg/kg across the sample locations. The highest nitrate concentration (20.7mg/kg) was recorded in the Forest soil (UNIPORT) while the lowest nitrate concentration (13.3mg/kg) was recorded in the Pine vegetation soil (UNIPORT). Phosphate concentration ranged from 11.3mg/kg to 26.3mg/kg across the various

sample locations. The highest phosphate concentration (26.3mg/kg) was recorded in the Forest soil (UNIPORT) while the lowest phosphate concentration was recorded in the Melina vegetation soil (RSUST). Potassium concentration ranged from 5.9mg/kg to 10.4mg/kg across the sample locations. The highest potassium concentration (10.4mg/kg) was recorded in the Forest soil (UNIPORT) while the lowest potassium concentration (5.9mg/kg) was recorded in the Pine vegetation soil (UNIPORT). Total organic carbon (TOC) ranged from 2.5% to 9.7% across the sample locations. The highest TOC (9.7%) was recorded in the Forest soil (UNIPORT) while the lowest TOC (2.5%) was recorded in the Melina vegetation soil (RSUST) [See Fig 1].

Bacteriological Analysis

Total heterotrophic bacteria (THB) population ranged from 6.2 x 10^7 CFU/g to 2.5 x 10^7 CFU/g across the various sample locations. The highest THB count (6.2 x 10^7 CFU/g) was recorded in the Forest soil (UNIPORT) while the lowest THB count (2.5 x 10^7 CFU/g) was recorded in the Melina vegetation soil (RSUST). Hydrocarbon utilizing bacteria (HUB) population ranged from 2.4 x 10^3 CFU/g to 1.2 x 10^3 CFU/g across the sample locations. The highest HUB count (2.4 x 10^3 CFU/g) was recorded in the Mangrove soil (RSUST) while the lowest HUB count (1.2 x 10^3 CFU/g) was recorded in the Melina vegetation soil (RSUST).The presence of hydrocarbon utilizing bacteria (HUB) in soil samples across these locations may be an indication of the presence of some form of hydrocarbon in the soils [See Fig 2].

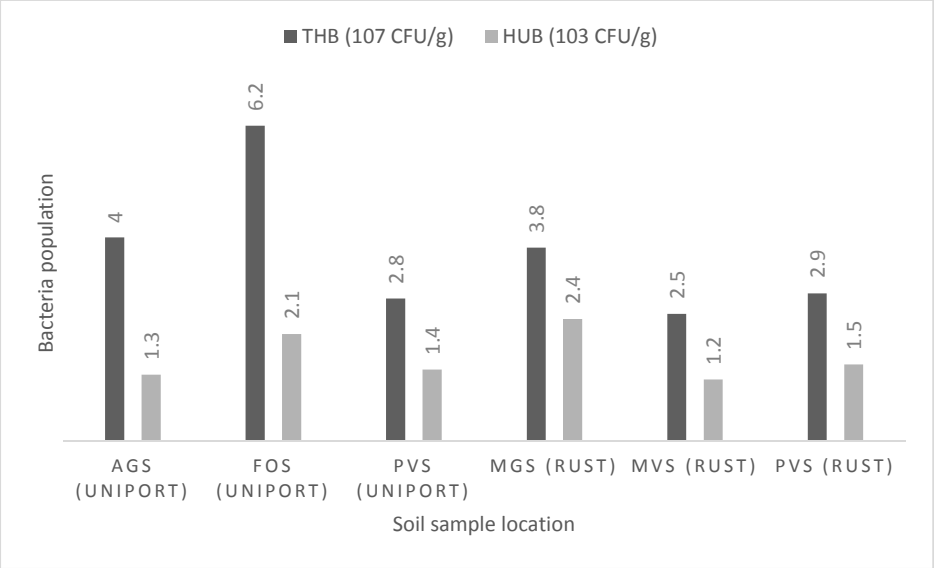

FIGURE 2: Population of total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) across the sample locations in the University of Port Harcourt (UNIPORT) and Rivers State University of Science and Technology (RSUST) respectively.

Statistical analysis suggested that there was a moderately positive correlation (60.1%) between the population of THB and the population of HUB across the sample locations. This is suggesting that the population of the HUB contributed positively to the population of THB to some degree across the sample locations and "vice versa". Two-Way ANOVA suggested that there was a significant difference [$P < 0.05$] between the population of THB and the population of HUB however; this difference was not as a result of variation in the sample locations. It may have been due to the variation in their culture media [See Table 1].

TABLE 1: 2-Way ANOVA of THB and HUB Populations with Respect to Variation in Soil Sample Location (SSL) and Culture Medium (CM)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
CM	4.62E+14	5	9.24E+13	1.000086ns	0.499964	5.050329
SSL	4.11E+15	1	4.11E+15	44.44599**	0.001146	6.607891
Error	4.62E+14	5	9.24E+13			
Total	5.03E+15	11				

Most of the total heterotrophic bacteria (THB) isolated and identified across the sample locations belonged to genera *Alcaligenes, Arthrobacter, Bacillus, Corynebacterium, Erwinia, Flavobacterium, Gordonia, Mycobacterium, Norcadia, Pseudomonas, Rhodococcus, Salmonella, Shigella and Staphylococcus*. *E. coli was also identified as part of the THB across the sample locations. Bacillus* predominated across the sample locations with a frequency

of 14.40% while *Flavobacterium* was the least with a frequency of 3.20%across the sample locations [See Fig 3]. This result seemed to agree with the results of other researchers who also reported higher percentage occurrence of Bacillus in various soil samples (Chikere *et al.,* 2009a; Lawson *et al.,* 2013; Ekhaise and Nkwelle, 2011).

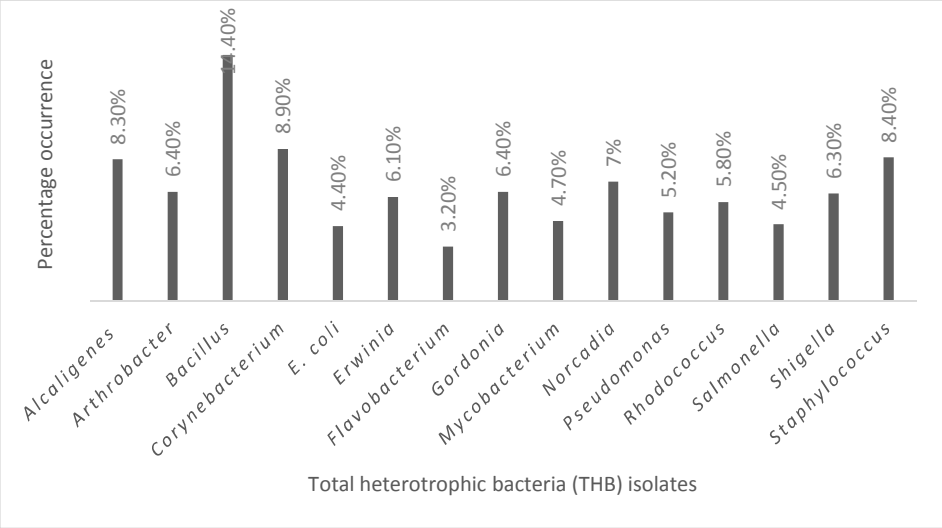

FIGURE 3: Percentage occurrence of total heterotrophic bacteria (THB) isolated from soil samples collected from various locations in the University of Port Harcourt (UNIPORT) and Rivers State University of Science and Technology (RSUST).

Most of the hydrocarbon utilizing bacteria (HUB) isolated and identified across the sample locations belonged to genera*Arthrobacter, Bacillus, Corynebacterium, Flavobacterium, Gordonia, Mycobacterium, Norcadia, Pseudomonas and Rhodococcus. Gordonia and Rhodococcus* predominated across the sample locations with a percentage occurrence of 20.20% and 16.30% respectively, while *Flavobacterium* was the least with a percentage occurrence of 5.50% across the sample locations [See Fig 4]. Other studies such as Chikere *et al.*, 2009b; Lawson *et al.,* 2013 and Ekhaise and Nkwelle, 2011 have also implicated some bacteria species belonging to these genera in hydrocarbon degradation (*i.e.*, hydrocarbon utilization as sole source of carbon and energy). It is well documented that bacteria genera like *Gordonia, Mycobacterium, Norcadia, Pseudomonas Rhodococcus* and *Bacillus* which are known hydrocarbon degraders could be cosmopolitan (Margesin *et al.,* 2003; Hamamura *et al.,* 2006; Van Beilen and Funhoff, 2007). They are of considerable environmental and biotechnological importance because of their wide catabolic abilities, resilience in harsh environmental conditions and ability to produce bio-surfactant (Arenskotter *et al.,* 2004; Larkin *et al.,* 2005; Van Beilen and Funhoff, 2005; Hamamura *et al.,* 2006; Van Beilen and Funhoff, 2007).

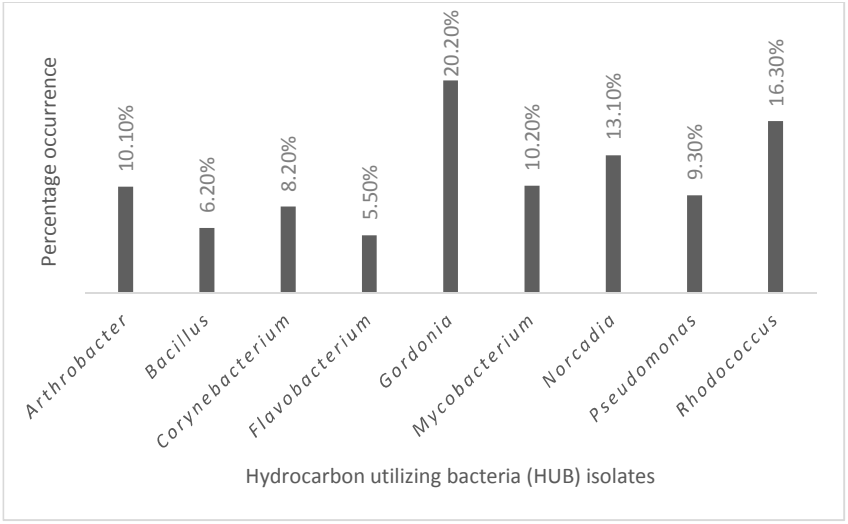

FIGURE 4: Percentage occurrence of hydrocarbon utilizing bacteria (HUB) isolated from soil samples collected from various locations in the University of Port Harcourt (UNIPORT) and Rivers State University of Science and Technology (RSUST).

The result of the present study revealed that the soil in the University of Port Harcourt (UNIPORT) and Rivers State University of Science and Technology (RSUST), both in the Niger Delta region of Nigeria, may harbour hydrocarbon degraders which may be useful in bioremediation of oil polluted soils in the region (Chikere *et al.,* 2009a, 2009b; Lawson *et al.,* 2013 and Ekhaise and Nkwelle, 2011).The biases associated with culture-dependent microbial enumeration techniques limited the full description of the bacterial diversity in these soil samples. It is an established fact that more than 90% of micro-organisms in the environment may not be culturable and as such, can only be detected with molecular methods used in the field of metagenomics (Macnaughton *et al.,* 1999; Edlund and Jansson, 2006). Application of genomic technologies in conjunction with more conventional biochemical and microbial community analysis will help in providing exciting opportunities for increasing our understanding of the vast microbial diversity in soils across UNIPORT and RSUST which are located in the Niger Delta region of Nigeria.

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