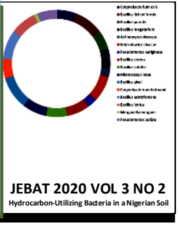
HIBISCUS PUBLISHER

JOURNAL OF ENVIRONMENTAL BIOREMEDIATION AND TOXICOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JEBAT/index



JEBAT 2020 VOL 3 NO 2
Hydrocarbon-Utilizing Bacteria in a Nigerian Soil

Distribution of Hydrocarbon Utilizing Bacterial Population Receiving Hydrocarbon Micro-seepage in Hydrocarbon Exploration Sites of Kukawa North-East Nigeria

Ibrahim Alkali Allamin[1]*, Gambo Ismail[1,2], Haruna Yahaya Ismail[1], Usman Bukar[1], Hussaini Shettima[1] and Amina Umar Faruk[1]

[1]Department of Microbiology, Faculty of Sciences, University of Maiduguri, P.M.B. 1069,
Maiduguri, Borno State, Nigeria.
[2]Northeast Zonal Biotechnology Centre University of Maiduguri,
Borno State, Nigeria.

*Corresponding author

Ibrahim A. Allamin
Department of Microbiology,
Faculty of Science,
University of Maiduguri,
Borno State,
Nigeria.
Emel: ibnallamin@gmail.com / ibnallaminmcb@unimaid.edu.ng

HISTORY

Received: 13th Nov 2020
Received in revised form: 4th Dec 2020
Accepted: 20th Dec 2020

KEYWORDS

hydrocarbon
bacteria
prospecting
Kukawa
micro-seepage

ABSTRACT

The search for hydrocarbon has been of great priority for alternative source of energy and economy to many countries. Different types of studies are being conducted to trace specific reservoirs of hydrocarbons. The used of geologic and seismic surveys which are environmental unfriendly and costly requires alternate such as that of microbial survey. The use of culture based method to isolate bacteria with ability to utilize hydrocarbon in survey areas was conducted. Soil sample were collected randomly from all the 13 sample sites from different depths for microbiological analysis. Mineral salt agar supplemented with diesel was used to isolate hydrocarbon utilizing bacteria (HUB). Nutrient agar was used to isolate total heterotrophic bacteria (THB). Soil samples were collected randomly from sample sites at various depths (10, 20 and 30 cm) the bacterial populations at 10 cm depth for HUB was 129×10^5 to 269×10^5 CFU/g at Kukawa (KW) and Baga (BG), THB was 122×10^5 to 266×10^5 CFU/g at Bundur (BD) and Baga (BG), HUB was 128×10^5 to 282×10^5 CFU/g at Kukawa (KW) and Baga (BG), THB was 110×10^5 to 220×10^5 CFU/g at Alagarno (AL) and Bawarti (BW) in 20 cm depth. The HUB count at 30 cm depth was 128×10^5 to 285×10^5 CFU/g at Kukawa (KW) and Baga (BG), THB counts was 72×10^5 to 147×10^5 CFU/g at Alagarno (AL) and Kauwa (KU). Bacillus, Enterobacter, Actinomycetes and Pseudomonas are the dominant bacterial genera in the sample sites. *Bacillus subtilis, Bacillus cereus, Bacillus pumilis, Bacillus megaterium, Bacillus licheniformis, Bacillus lentus, Bacillus azotofomans* and *Bacillus alvei* are among the dominant species of Bacillus in the sample sites. The isolation of these bacterial species in high population projects directly a guaranteed discovery of hydrocarbon reservoir and could be a relatively inexpensive and environment friendly method for hydrocarbon prospecting.

INTRODUCTION

Soil environment receiving upward micro-seepage of hydrocarbon was reported to directly stimulate the abundance, biodiversity and distribution of microbes in an environment [1]. The possibility to isolate large population of certain hydrocarbon utilizing bacteria from soil and sediments is generally taken as confirmation that those bacteria used hydrocarbon as their carbon source for their metabolism in that microenvironment [6]. Compelled by reservoir force, some of the gaseous constituents from oil and gas reservoirs can vertically infiltrate the concealment above and rise to the surface of the earth. Consequently, these gaseous and volatile constituents of hydrocarbon will lead to the mobilization of specific bacteria with the capacity to use such constituents to the near surface soil and they will colonize that particular habitat [9]. The fact that those bacteria utilize the gaseous compounds as growth substrates makes it possible for them to colonize those specific microenvironments where larger deposits of those carbonic constituent occur naturally [11], and the larger the population the denser the reservoirs may be [16].

The critical component of this kind of survey is that it does away with negative long wasted survey, where prospecting area will be prioritized according to the presence of large quantities of the hydrocarbon in particular reservoirs [11]. The ability to utilize hydrocarbon substrates as a carbon source is exhibited by a widespread and diverse microbes [8] widely distributed in oil reservoirs in soil and sediments [8]. Using culture dependent isolation techniques, different bacterial genera have been characterized from these relevant environments [16]. The hydrocarbon utilizing bacteria isolated from the soil were species of *Bacillus, Enterobacter, Arthrobacter, Pseudomonas, Micrococcus, Seratia,* and *Corynebacterium* [2]. The present study has been conducted to detect and enumerate the indicator bacterial population with specific interest to those utilizing petroleum hydrocarbons as their source of carbon in their metabolism in the hydrocarbon exploration sites soil samples.

MATERIALS AND METHODS

Study Area

The study area is Kukawa, one of the 27 local government areas in Borno State, Nigeria. Kukawa is located in the northern part of Borno bordering four other local government areas including Abadam, Monguno, Guzamala and Marte. It also has international border with Chad with geographical coordinates $12^0 55'33''$ North and $13^0 34'12''$ East (**Fig.**, 1). It has an average elevation/altitude of 277 meters. The majority populations are into farming and fishing with estimated population of over 25,000. Kukawa is considered one of the potential crude oil prospecting areas by the Nigerian National Petroleum Corporation (NNPC).

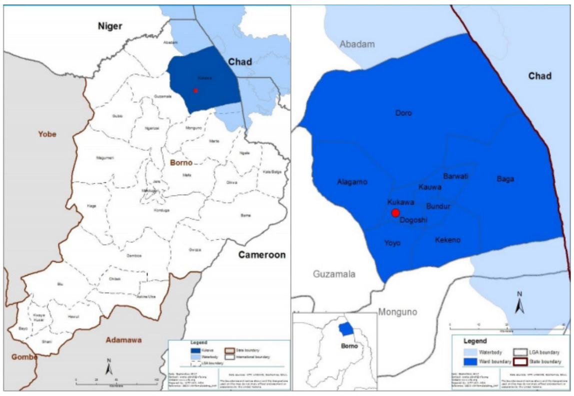


Fig. 1. Map of Borno Showing Kukawa (Right) and Kukawa showing the 10 ward of AL (Alagarno), BG (Baga), BW (Barwati), BD (Bundur), DR (Doro), DG (Dogoshi), KU (Kauwa) KW (Kukawa), KK (Kekeno), and YY (Yoyo) (Left) [17].

Microbiological analysis of the soil samples

Enumeration of total aerobic heterotrophic and hydrocarbon utilizing bacteria

Representative one-gram soil was sieved and weighted under aseptic condition and a tenfold serial dilution was performed as described by [4]. Nutrient agar was prepared according to manufacturer's specification and used for the isolation of total aerobic heterotrophic bacteria while the Mineral salt medium supplemented with 5% per liter of diesel as oil agar (OA) was prepared and used for the isolation of hydrocarbon utilizing bacteria. Aliquot from the 10^5 dilution was spread on the prepared plates and were incubated at room temperature (30 ± 2 °C) for 18 hours and 5 days for NA and OA, respectively. Bacterial colonies, which developed on the plates were counted and recorded as colony forming units per gram (CFU/g) of soil [5]. Pure cultures of the isolates were obtained by repeated sub-culturing on media used for primary isolation. The pure isolates were maintained on agar slants for further characterization and identification [4].

Characterization and Identification of the hydrocarbon utilizing bacteria

Bacterial isolates were characterized and identified after studying their Gram reaction as well as cell morphology. Bacterial biochemical identification was performed using the Bergey's Manual of Determinative Bacteriology the tests were performed according to the methods described by [4].

RESULTS AND DISCUSSION

The abundance of hydrocarbon utilizing bacteria in soil samples was calculated by observing and counting the developed colonies. The hydrocarbon utilizing bacterial colonies and total heterotrophic bacterial colonies in 10cm observed on plates are shown in **Fig.** 2, with Kukawa (KW) indicating the least with 129×10^5 CFU/g and Baga having the highest count of 269×10^5 CFU/g for HUB. THB count was recorded highest at Baga (BG) 129×10^5 CFU/g and least count was recorded at Bundur (BD) 106×10^5 CFU/g.

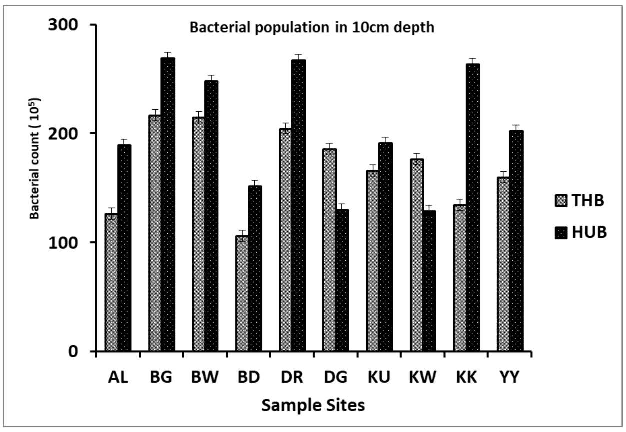


Fig. 2. Distribution of total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) counts at the various sample sites from the depth of 10cm. **Keys**: AL (Alagarno), BG (Baga), BW (Barwati), BD (Bundur), DR (Doro), DG (Dogoshi), KU (Kauwa) KW (Kukawa), KK (Kekeno), and YY (Yoyo).

The count of HUB at 20 cm depth in the various sample sites shows highest count at Baga (BG) 282×10^5 CFU/g and least count at Kauwa 128×10^5 CFU/g. The count of THB was highest at Bawarti (BW) 220×10^5 CFU/g and least count was 110×10^5 CFU/g at Alagarno (AL). Similarly HUB count at 30 cm depth in the various sampling sites shows highest count at Baga (BG) 285×10^5 CFU/g and the least count at Kauwa (KW) 128×10^5 CFU/g. The count of THB was highest at Kauwa (KU) 147×10^5 CFU/g and the least count at Alagarno (AL) 72×10^5 CFU/g.

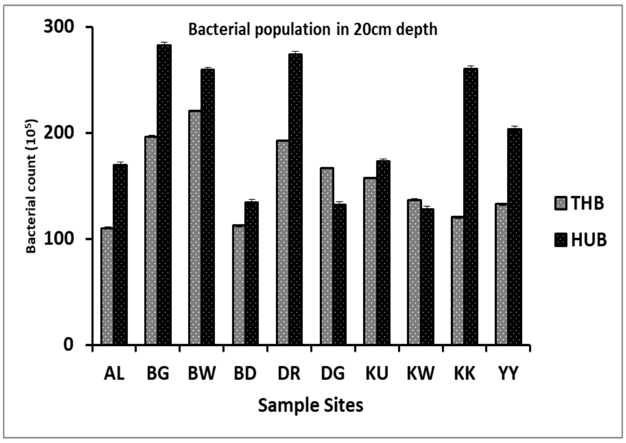


Fig. 3. Distribution of total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) counts in the various sample sites in the depth of 20cm **Keys**: AL (Alagarno), BG (Baga), BW (Barwati), BD (Bundur), DR (Doro), DG (Dogoshi), KU (Kauwa) KW (Kukawa), KK (Kekeno), and YY (Yoyo).

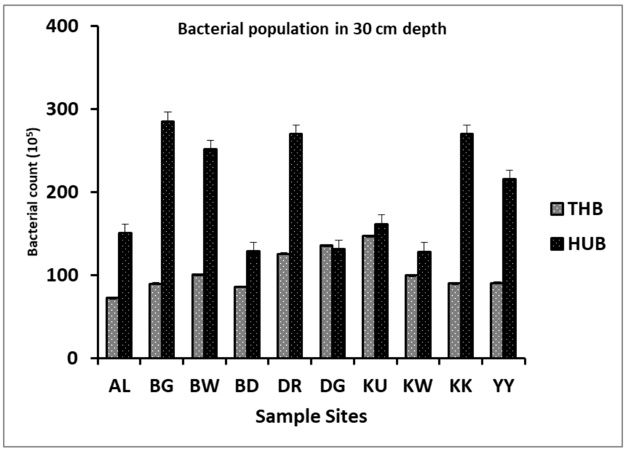


Fig. 4. Distribution of total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) counts in the various sample sites in the depth of 30cm **Keys**: AL (Alagarno), BG (Baga), BW (Barwati), BD (Bundur), DR (Doro), DG (Dogoshi), KU (Kauwa) KW (Kukawa), KK (Kekeno), and YY (Yoyo).

The diversity of hydrocarbon utilizing bacteria in the sampling sites shows various genera and species, among the most occurring genus, Bacillus is dominating the sampling sites followed by Enterobacter, Actinomycetes.

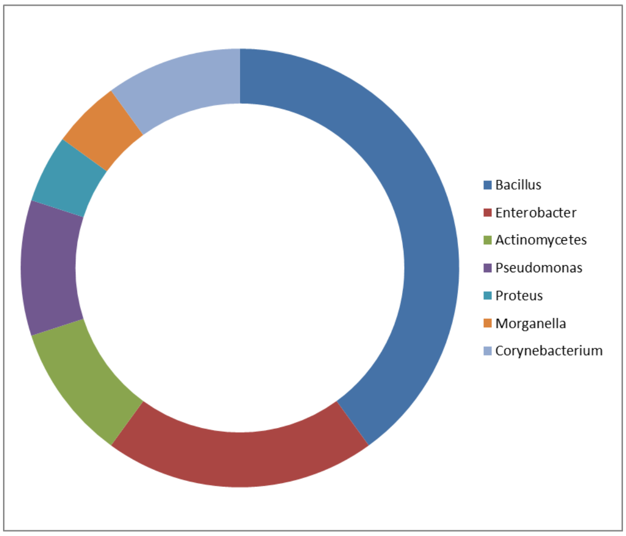


Fig. 5. Distribution of hydrocarbon utilizing bacterial genera in the soil of Kukawa hydrocarbon exploration site in Northeast Nigeria.

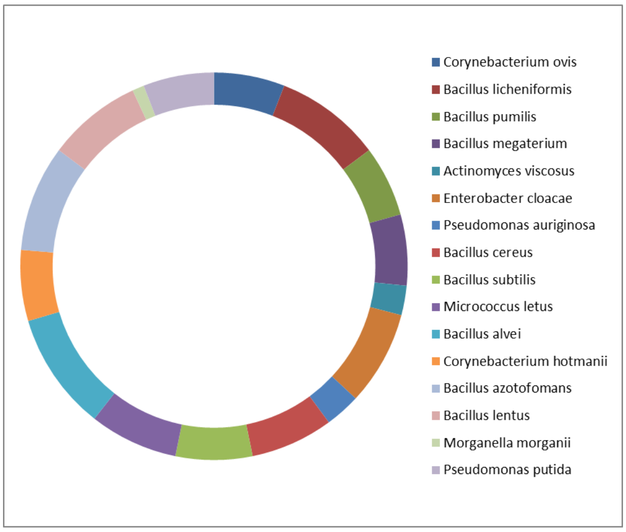


Fig. 6. Distribution of hydrocarbon utilizing bacterial species in the soil of Kukawa hydrocarbon exploration site in Northeast Nigeria.

The prospect of discovering hydrocarbon (Fossil fuel) reservoirs in soil and sediments by biological method is tinted by the fact that the hydrocarbon utilizing bacteria range between 10^3 and 10^5 CFU/g in soil/sediment receiving hydrocarbon micro-seepages depending on the ecological conditions [13]. The soil from relative higher depth indicates low bacterial count in view of oxygen deficient conditions at sea bed [14]. In the present study area, Baga shows higher population of hydrocarbon utilizing bacteria range between 269×10^5 and 285×10^5 CFU/g of soil sample, which is significant and proves the seepage of lighter hydrocarbon accumulations from oil and gas reservoirs. Other geologic and physical prospecting studies suggest that hydrocarbon seepage of subsurface origin is present in the Baga offshore study area and indicate that the area has encouraging prospects for petroleum exploration.

In most cases the main dominant genera bacteria in soils receiving upward seep of hydrocarbon are *Bacillus, Pseudomonas, Actinomycetes, Micrococcus, Corynebacterium* and *Proteus* [2,3]. In the present study, the most dominant occurring bacterial genus was *Bacillus*. Bacillus was reported to be the predominated genus, especially in the hydrocarbon polluted soils [1]. This may be because Bacillus species are tolerant bacteria to harsh conditions due to their ability to produce spores, which may defend them from the toxic effects of the hydrocarbons [10]. Although, hydrocarbon utilizing bacteria may be expected to be readily isolated from a petroleum-polluted environment, the same degree of expectation may be anticipated for a total unrelated environment [8,11]. The rate of hydrocarbon seepage is rapid as shown in the higher increase of hydrocarbon utilizing bacteria in the subsurface soil specifically 10 cm depth. This may be due to the fact that the microorganisms in the soil have efficient ability in utilizing the residual hydrocarbon as a source of carbon and energy. Indigenous and adapted microorganisms are more efficient for biodegradation of oil pollutant.

CONCLUSION

The results of this study revealed that hydrocarbon-utilizing bacteria are abundant and widely distributed in the soils Kukawa where crude oil exploration is underway; the diverse species of bacteria that are using hydrocarbon micro-seepage as their source of carbon in their metabolism are isolated in higher population indicating the positive prospect of the sites.

REFERENCES

1. Afuwale, C and Modi, HA. Study of bacterial diversity of crude oil degrading bacteria isolated from crude oil contaminated sites. Life Sci Leaflet. 20126:13-23.
2. Ajayi, A O, Balogun S A, and Adegbehingbe, K. Microorganisms in the crude oil-producing areas of Ondo State Nigeria. Acad J Sci Res Essay. 2008;3(**5**):174-179.
3. Antai, S P. Biodegradation of Bonny light crude oil by *Bacillus* sp. and *Pseudomonas* sp. Waste Management 1990;10: 61-64
4. Allamin, I A, Ijah, UJJ, Ismail, H Y and Riskuwa, M L. Occurrence of Hydrocarbon Degrading Bacteria in Soil In Kukawa, Borno State. Int J Environ. 2014;3(2):36-47.
5. Bashir, AH. Diversity and morphology of bacterial community characterized in topsoil samples from the Gaza Strip, Palestine. Res J Mirobiol 2012;7(6): 309-318.
6. Bayode, OJ, Adewunmi, EA, and Odunwole, S. Environmental implications of oil exploration and exploitation in the coastal region of Ondo State, Nigeria: A regional planning appraisal. J Geo Region Plan. 2011;4(3):110-121.

7 Bergey’s Manual of Determinative Bacteriology. 9th edition, Holt, JG Williams Wilkins CO, Baltimore Advances in Applied Science Res, 1994;3(**5**):2789-2795.

8. Chikere, CB, Okpokwasili, GC, and Chikere, BO. Bacterial diversity in a tropical crude oil-polluted soil undergoing bioremediation. Afr J Biotechnol. 2009;8(**11**):2535-2540.
9. Klusman, R and Saeed, M. Comparison of light hydrocarbon microseepage mechanisms, in: Hydrocarbon Migration and Its near Surface Expression, edited by: Schumacher, D and Abrams, M, AAPG Memoir, Tulsa, 1996;157–168.
10. Latha, R, Kalaivani, R. Bacterial degradation of crude oil by gravimetric analysis. J Microbiol Biotechnol. 2012;6:34-39.
11. Leifer, I, and Judd, AG. Oceanic methane layers: the hydrocarbon seep bubble deposition hypothesis Terra Nova 12002;4: 417–424.
12. Oboh, BO, Ilori O, Akinyemi O, and Adebusoye SO. Hydrocarbon Degrading Potentials of Bacteria Isolated from a Nigerian Bitumen (Tarsand) Deposit. Nature Sci. 2006;(**4**)3:51-57.
13. Onifade, OA, and Abubakar, FA. Characterization of hydrocarbon-degrading microorganisms from isolated from crude oil contaminated soil and remediation of the soil by enhanced natural attenuation. Res J Microbiology. 2007;2(**2**);149-155.
14. Rasheed, MA, M Lakshmi, M S Kalpana, D J Patil, A M Dayal and S V Raj. Evaluation of hydrocarbon charge using microbial and light gaseous hydrocarbon anomalies in the Seabed Sediment Samples of Gulf of Mannar, Kerala-Konkan Offshore Basin. Nat Resour. 2012;Res DOI: 101007/s11053-012-9189-x
15. Tucker, J, Hitzman, D. Detailed microbial surveys help improve reservoir characterization Oil and Gas J 92. 1994;(**23**): 65–69.
16. Waheed SM, Sangal P, Shukla S. Microbiological analysis for hydrocarbon exploration. Environ Risk Assess Remed. 2018;2(3):1-7.

1.7 World Food Programme (WFP). Rapid food security Assessment in Doro Baga, Baga, Kukawa and Cross Kauwa (Kukawa LGA). 2018.