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The Genomics of Hydrocarbon-Utilizing Bacteria

***Chunwafor, M.I., & Ogbonda, K.H.**
Department of Biology, Ignatius Ajuru University of Education, Port Harcourt, Nigeria

***Corresponding author email:** michunwafor@gmail.com

Abstract

Due to the increase in industrialization, environmental pollution by hydrocarbon is almost inevitable. Hydrocarbon-based products are major contaminants of soil and water. Hydrocarbon-based products are a major source of energy but contamination by hydrocarbon-based products is of great importance due to their toxic, mutagenic and carcinogenic nature and their ability to cause depression, low oxygen, poor food and water intake by plants leading to poor yield and hence food insecurity which affect man and other organisms adversely. However, bacteria can utilize hydrocarbon thereby bringing about their degradation. Though the process is complex, it depends on the nature, amount of hydrocarbon present and the environmental conditions at the time of contamination and remediation but the appropriate nutrients and environmental conditions must be put in place and maintained for the bacteria to achieve maximum results if they possess the needed enzymatic capabilities to catabolize hydrocarbon. Instead of the conventional mechanical and chemical methods which are expensive and can produce more toxic substances, biological methods involving the use of microorganisms that are natural and more sustainable are recently being employed. The use of 16S rRNA genes when extracted from the bacterial isolate, can be characterized and amplified with polymerase chain reaction (PCR) using different primer sets 27F: GAGTTTGATCCTGGCTCAG and 1492R: GGTTACCTTGTTACGACT. The genomic study is important when studying the bacterial community diversity and dynamics in oil-polluted ecosystems to fully describe the active microorganisms involved in hydrocarbon degradation. Bacteria like *Bacillus, Pseudomonas, Enterobacter, Klebsiella, Alcaligenes, Arthrobacter, Micrococcus* species and a lot others have been identified by several researchers to be capable of utilizing hydrocarbons thereby degrading them and thus resulting in bioremediation which is safe, easy, cheap and eco-friendly

Keywords: Genomics, Hydrocarbon, Bacteria, Industrialization, Environmental Pollution

Introduction

The pollution of the environment with crude oil has been a serious environmental issue all over the world. Crude oil is a complex mixture of hydrocarbon and non-hydrocarbon compounds that occur naturally and which at appropriate concentrations possess a measurable toxicity towards living systems. The toxicity of crude oil or petroleum products varies widely, depending on several factors which include their composition, concentration, environmental factors and the biological condition of the organisms at the time of the contamination (Ogbonna et al., 2020). Hydrocarbon, which is a component of crude oil, is known to cause pollution of air water and soil. Issues of pollution of soil like that of water and air have been and are increasingly of global interest since it is on the rise daily.

According to Yilei et al. (2020), crude oil causes drinking water pollution and a decline in water, air as well as soil quality. Hydrocarbon has also been gaining attention because of its toxic, mutagenic and carcinogenic effects on life in general (Clemente et al.,2001; Faggo et al., 2020). To bring to the lowest minimum or possibly put a stop to the problem of contamination by hydrocarbon from crude oil or other sources researchers came up with bioremediation, which is a solution different from the conventional chemical and mechanical method of control. According to Rao (2011), it has been demonstrated that when the regular carbon source in synthetic media (such as sucrose, glucose and others) is replaced by hydrocarbons such as paraffin, kerosene, gasoline or lubricating oils, the growth of certain microorganisms takes place indicating the ability of those microorganisms to utilize hydrocarbon. This paper therefore brings out the use of genomics (a method of identifying bacteria using the 16S rRNA gene) for identifying hydrocarbon-utilizing bacteria for bioremediation purposes.

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An Overview of Petroleum Hydrocarbon

Hydrocarbons which are a constituent of petroleum are organic compounds composed of two main elements- Carbon and Hydrogen. Apart from carbon and hydrogen, they also contain small quantities of molecules of nitrogen, sulphur, metals and oxygen (Vieira et al., 2007). Hydrocarbon is a component of petroleum products. In Latin, petroleum means "rock oil" which occurs as a dark, sticky, viscous liquid. Products of Petroleum such as Premium motor spirit (PMS), Automotive gas oil (AGO) and Dual purpose kerosene (DPK) popularly referred to as fuels are a complete mixture of organic compounds basically of Paraffinic, Olefinic and Aromatic hydrocarbon (Vieira et al., 2007; Singh & Lin, 2008; Mittal & Singh, 2009; Ogbonna, et al., 2020). According to Varjani et al. (2013), Faggo et al. (2020), and Ogbonna et al. (2020) one of the World's major environmental problems today is hydrocarbon pollution by petrochemical industries. Researchers have shown that they are degradable mechanically, chemically and also biologically by plants and microorganisms (bacteria, fungi and actinomycetes) which is an eco-friendly process. Crude oil which contains hydrocarbon as its major component continues to be used as the principal source of energy and plays an important role in global environmental pollutant considerations. This may be the reason hydrocarbon contamination of the environment remains and may continue to be a major environmental problem. On the other hand, crude oil will remain a major source of energy in the next several decades because no reliable alternative energy consumption has been substituted (Sephahi et al., 2008) and this may be a threat to issues of the environment and life in general. Hydrocarbons are generally not easy to degrade. However, some are more easy to degrade than others. Polycyclic aromatic hydrocarbons (PAHs) are more resistant to microbial attack than smaller aromatic hydrocarbons, when biodegradation sets in, this often continues through oxidation and ring cleavage by dioxygenases as reported by Eze et al. (2021).

Predisposing Factors to Hydrocarbon Contamination

Petroleum hydrocarbon can contaminate the soil in different ways, such as oil spills, urban wastewater treatment plants and run-off (Amala, 2008), gas flaring, disposal of used lubrication oils, washing from oil tanks, leakages from marine vessels, outboard engines, sabotage, seepages, refinery effluents, engineering errors and rupture of ill-maintained flow lines (Wokoma et al., 2020), as well as an increase in transportation, population growth, economic growth, urbanization, increase in use of energy and intensification of agricultural activities. Some areas are more prone to hydrocarbon contamination than others, like the rich Niger Delta region in Nigeria where exploration of oil takes place. The activity has predisposed the region to oil pollution from exploration and exploitation activities as well as the use and transport of crude oil and or its products. This statement of activities was also reported by Chikere and Ekwuabu (2014a) that the Niger Delta is among the ten most significant wetland and marine ecosystems in the world but unsustainable oil exploration activities have rendered the Niger Delta region one of the five most severely petroleum damaged eco-systems in the world. It is evident from the reports and findings of Amala (2018), Wokoma et al. (2020), and Chikere and Egwuabu (2014b) that some factors can predispose a region to hydrocarbon contamination than others. There is need for proper handling of petroleum products in other to reduce the rate of contamination in those areas that are at high risk.

That the people of the Niger Delta Region of Nigeria appear to be the most affected in the subject matter of oil pollution in Nigeria is also evident in the fact that most researchers from years ago have directed researches on ecosystem contamination with crude oil to the region and the environmental report issued of recent times about the region. This may be associated with the fact that over 80% of the country’s oil revenue comes from this region (Chikere et al., 2009) and the constant oil activities and spills, which go on in the oil-rich zone to meet the financial demand of the Nation. Besides meeting the nation's demand, several oil spillages in the area due to rupturing and explosion of some age-long oil pipes which may have expired, vandalism, illegal oil refinery and many other activities which go on in the area are also factors. Evidence of some of the activities mention above like pipe line vandalism, rupture of age long pipes and gas flaring which lead to contamination of the environment by hydrocarbon in the Niger Delta and other regions of the world where hydrocarbon contamination are prevalent and plates of some activities that have led to hydrocarbon contamination of environment in some Niger Delta Areas are presented in Plates 1-3.

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Plate 1: Picture of an oil-impacted site in Ahoada West L. G. A. of Rivers State **(**Earth Friends Magazine, 2020)

Plate 2: Impacted site with gas flaring in Ahoada West L. G. A. Rivers State. (Earth Friends Magazine, 2020)

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Plate 3: Picture of an impacted water body and soil surrounding a river in Goi community in Ogoni land in Rivers State. (Earth Friends Magazine)

Hazards/Effects of Petroleum Hydrocarbon Contamination

Petroleum hydrocarbon is a major source of energy for domestic and industrial purposes. Unfortunately, the activities of the oil-producing industries operating in some regions where hydrocarbon contamination is prevalent, provide potential sources of oil pollution of environment (air, agricultural land and domestic water) causing enormous damage to biodiversity, economic growth of farmers (animal and crop farmers), individual and national economic growth (Obidi et al., 2016). The oil pollution persists for a long time due to depletion of plants and microorganisms which could initiate the bioremediation process and this in turn has its own adverse effects (respiratory problems, mutation of genes, cancer etc.) on the overall life in the environment. Many substances known to have toxic properties are regularly introduced into the environment through human activities. These substances which include hydrocarbons range in degree of toxicity and danger to human, animal and plant health.

Hydrocarbon contamination has caused great challenges to humanity especially in the endangering of biota including the fauna and flora (Ogbonna et al., 2020). The contamination of these habitats creates public health and socio-economic problems. With the findings of many researchers on the effects of hydrocarbon on humans, animals and plants, one can summarize that the contamination is also directly or indirectly responsible for the increase in the cost of farm produce (animals and crops) thereby affecting the economic status of man, which is also a negative impact to man's existence. It is therefore true to say that the effects of hydrocarbon contamination on man and other organisms is hazardous and deadly. Much of the land and shorelines in the oil-producing communities are important agricultural resources and are under continuous cultivation. Any damage will affect the soil properties and plant communities due to associated changes in soil condition. At high concentrations of petroleum oil in the soil, most plants species suffer depression due to poor soil conditions, dehydration and impaired nutrient uptake by the roots created by the presence of crude oil (Anoliefo et al., 2003). This depression will also result in poor yield of crops bringing about high cost of farm produce since the farmer will have to make up for the investment and reduce or possibly eliminate loss. The domestic water source (underground water) which has been polluted for ages by contamination of petroleum hydrocarbon results in smell of petroleum products in most domestic water found in areas previously and periodically been contaminated by hydrocarbon which is a physical indication of hydrocarbon contamination of their underground water. This will make people in the area have to spend much more money in drilling water to a very far depth to reach the aquifer which may be free of hydrocarbon contaminants and safe for domestic use. This level of contamination

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has impacted the life of the common man living in those areas as they can no longer afford drilling of domestic water for drinking, cooking, bathing and other purposes.

The Genomics of hydrocarbon-utilizing Bacteria

The characterization of hydrocarbon-degrading bacteria populations within contaminated environments using culture-dependent techniques most likely provides tentative identities of the cultured isolates hence recent advances in microbiological studies render it possible to put together molecular and culture-dependent approaches in order to describe bacterial diversity and their degradative potential in any hydrocarbon polluted environment (Chikere & Ekwuabu, 2014a). This has led to the use of 16S rRNA rather than the culture method for the characterization of bacteria due to its sensitivity and reliability (Lima et. al., 2020). In genomic studies, a portion of the 16S ribosomal ribonucleic acid(16S rRNA) gene of the genomic DNA can be extracted from each bacterial isolate to be characterized and amplified with polymerase chain reaction (PCR) using the universal primer set 27F:GAGTTTGATCCTGGCTCAG and 1492R: GGTTACCTTGTTACGACT as reported by most researchers who have carried out studies using genes. Genomic DNA and the plasmid were extracted and obtained using the Zymo-Spin Research DNA extraction kit as reported by Ehis-Eriakha et al. (2020), though Lima et. al. (2020) used bacterial Genomic Miniprep Kit from Sigma Aldrich and then 16S rRNA gene fragment of strains were amplified by PCR amplification using forward primer 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1492r (5'-GGTTACCTTGTTACGACTT-3'). There are other types of PCR amplification machines with other primers. Other researchers like Yilei et al. (2020) also reported that they used soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) for extraction of soil bacteria DNA.

Although the process employed by Yilei was time intensive in obtaining results of identifying hydrocarbon degraders. Apart from the already mentioned DNA extraction kits, Eze et. al. (2021) however in their research report they used a different DNA kit from Germany. Common to most of the research was the use of enriched cultures from which the DNA genes were extracted. Several bacteria have been noted for their hydrocarbon-catabolic ability using genomic studies compared to the cultural and biochemical identification method. These bacteria are known to function effectively in the degradation of hydrocarbon-polluted environments due to their enzymatic activities. Functional degradation genes have also been reported in Plasmid and Chromosomal DNA of some hydrocarbon-utilizing bacteria by Ehis-Eriakha, et al. (2020). After amplification with the PCR machine, sequence identification is performed aligning with the GenBank's Basic Local Alignment Search Tool (BLAST) algorithm of National Centre for Biotechnology and Information (NCBI). However, similar bacteria isolates have been reported by researchers whose work was culture-dependent and those researchers whose research works were gene-based since it is very sensitive and reliable (Chikere & Ekwuabu, 2014b), though it is not very easy to identify bacteria using genomic studies (sequencing of their genes), due to the numerous procedures involved. Despite the mechanisms needed for the study and the many processes of gene or plasmid extraction, purification before the analyses, phenotypic and biochemical reactions are mostly carried out by researchers before proceeding to carry out gene sequencing. It is however worth the while as the results achieved are finite instead of tentative results usually obtained from the study of morphological and physiological characteristics, culturing and biochemical means of identification with comparism to Bergey’s Manual of Determinative Bacteriology (Lima et al., 2020).

Hydrocarbon-utilizing bacteria

Bacteria that were identified and characterized to be hydrocarbon-utilizing, were reported by researchers to possess certain enzymes that enable them to utilize hydrocarbon. Some of the enzymes are regarded to be responsible for the degradation of aliphatic hydrocarbons including alkane1-monooxygenase, long-chain alkane monooxygenase, cytochrome P450 CYP153 alkane hydroxylase, cyclopentanoldehydrogenase, cyclohexanone monooxygenase, gluconolactonase, alcohol dehydrogenase, and 6-hydroxyhexanoate dehydrogenase (Eze et al., 2021). The presence of high enzymatic capacity allows microbial communities to degrade complex hydrocarbons (aliphatics and polyaromatics). Some of the hydrocarbon-utilizing bacteria have similar functional genes which make them capable of degrading hydrocarbon. Ehis-Eriakha et al. (2020) reported that they used five specific genes to analyze the genetic diversity of the bacteria that were shown in their study to degrade hydrocarbon. The genes were alkB, Alma, C230, PAH-RHD(GP), and nahAC. Some of the bacteria were observed to possess more than one or two of these genes which may not be present in the genetic make-up of those bacteria that do not have hydrocarbon-degrading capability. The above-mentioned genes may be some of the genes which are present in the genome of the hydrocarbon-utilizing bacteria. In the research carried out by Lima et al. (2020), the detection of the alkane hydroxylase gene in bacterial isolates was carried which indicate that certain enzymes have to be present in the bacteria to enable them to degrade hydrocarbon as earlier reported by other researchers. The purified DNA was screened by PCR to detect two catabolic genes that encode enzymes involved in alkane degradation pathways. The alkB gene (group II) was amplified using the primers alk-2F (5’-GAGACAAATCGTCTAAAACGTAA-3’) and alk-2R (5’-TTGTTATTATTCCAACTATGCTC-3’) and the alkB gene (group III) was amplified using the primers alk-3F (5’-TCGAGCACATCCGCGGCCACCA-3’) and alk-3R (5’-CCG TAGTGCTCGACGTAGTT-3’). Enzymatic genes in bacteria however, play a significant role in the degradation of hydrocarbon of any class depending on the gene present in the bacteria. It is important to note that a few of the enzymes possessed by bacteria that make them able to degrade hydrocarbon are: 1-monooxygenase gene (alkB), which degrades short and middle-chain alkanes; long monooxygenase gene (Alma), which breakdown long chain alkane; ring cleavage catechol 2, 3, dioxygenase gene (C230), for breakdown of aromatic hydrocarbon; naphthalene dioxygenase gene (nahAC), which catabolize naphthalene; and PAH hydroxylating dioxygenase gene [PAH-RHD (GP)] aromatic hydrocarbon degrader. Bacteria like *Bacillus*, *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Alcaligenes*, *Arthrobacter*, *Micrococcus* species (plates 4-9) and so many others have been implicated in hydrocarbon degradation.

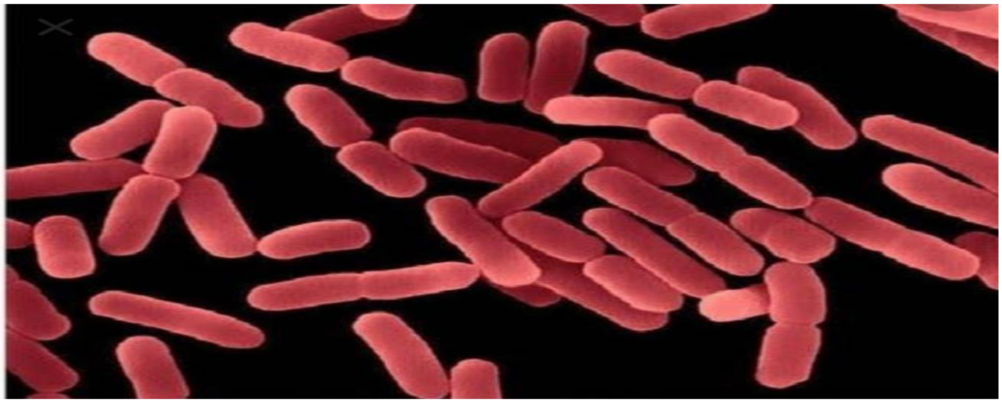

Plate 4:*Bacillus* species **(**George, 2019)

Plate 5: *Pseudomonas* species (CDCP, 2019)

Plate 6: *Klebsiella*species (Sciencephoto, 2010)

Plate 7: *Alcaligenes* species (Microbewiki, 2011)

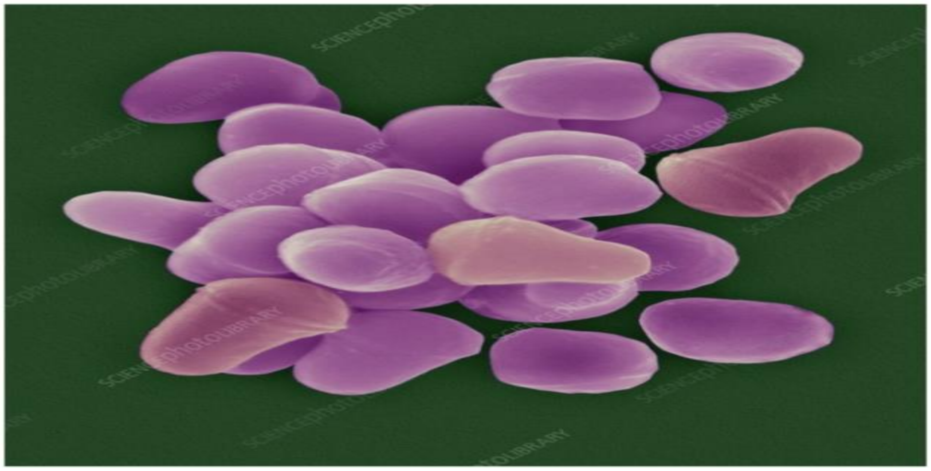

Plate 8: *Arthrobacter* species (Gómez, 2018)

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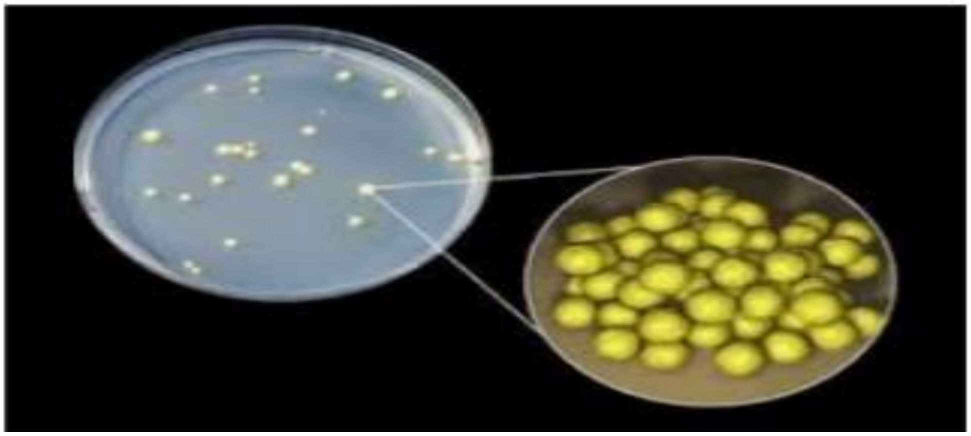

Plate 9: *Micrococcus* species, Britannica (2017)

Discussion

As a result of recurrent hydrocarbon contamination, it was discovered that the physical, mechanical and chemical methods of decontamination were not actually satisfactory as the methods were cumbersome while other methods produced some toxic substances and were quite expensive (Ogbonna et al., 2020). The toxic substances which the earlier mechanical and chemical methods released were of public health importance as the hazards and threats to humans, animals and the economy were and are adverse. Drastic action was required to help remedy the devastating effects of hydrocarbon contamination on the environment and its inhabitants. As the need for easy, cheap and ecofriendly methods of remediation rose, researchers discovered bioremediation as a tool. However, identification of hydrocarbon-utilizing bacteria was then only by culture-dependent techniques which included morphological and biochemical means but these methods had their limitations as they produced tentative identities of the cultured isolates. This, therefore, led to the recent need for advances in microbiological studies and it was discovered to be possible to put together molecular and culture-dependent approaches in order to describe bacterial differences and their degradative potential in any hydrocarbon polluted environment. The rise of molecular approaches of the use of 16S rRNA genes for bacterial identification bridged the gap of the limitations suffered by the cultural method. With advances in microbial molecular studies, different kits have been reported to be useful in genomic DNA and plasmid extraction. They include: The zymo-Spin Research DNA extraction kit (Ehis-Eriakha et. al., 2020), bacterial Genomic Miniprep Kit from Sigma Aldrich (Lima et. al., 2020), and Yilei et. al. (2020), also reported in their research that they used soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) for extraction of soil bacteria DNA and the availability of the enzyme capable of catabolizing the hydrocarbon contaminant that is present in the environment.

The microbial utilization of hydrocarbons depends on the chemical nature of the compounds within the petroleum mixture and on environmental determinant **(**Ekanem & Ogunjobi, 2017) as well as the enzymatic make-up of the organism though the indigenous microorganisms play a great role in the bioremediation of a polluted site (Varjaniet al.,2013). Some of the hydrocarbon utilizing bacteria which has been identified by16S rRNA genes are*Bacillus, Pseudomonas, Klebsiella, Alcaligenes, Arthrobacter,Micrococcus species.* Most of the bacteria are gram-negative indicating that most hydrocarbon-utilizing bacteria are gram-negative and possess the characteristics to survive stressful conditions (Pannapa&Suntornthiticharoen, 2018). A few of the enzymes possessed by bacteria that make them able to degrade hydrocarbon are: 1-monooxygenase gene alkB, Alma,C230, nahAC and PAH-RHD (GP). Ehis-Eriakha et al. (2020) and Lima et al. (2020) reported that *Bacillus*species has the highest degrading ability of all the hydrocarbon-utilizing bacteria they identified.

Conclusion

Due to recurrent hydrocarbon contamination, it was discovered that physical, mechanical, and chemical methods of decontamination were not satisfactory. These methods were cumbersome, while others produced toxic substances and were quite expensive. The toxic substances released by the earlier mechanical and chemical methods were of public health importance, posing hazards to humans, animals, and the economy. Drastic action was required to remedy the devastating effects of hydrocarbon contamination on the environment and its inhabitants. As the need for an easy, cheap, and eco-friendly method of remediation grew, researchers discovered bioremediation as a tool. However, the identification of hydrocarbon-utilizing bacteria was initially only possible through culture-dependent techniques, which included morphological and biochemical means. However, these methods had limitations as they produced tentative identities of the cultured isolates. This led to the recent need for advances in microbiological studies, and it was discovered that it was possible to combine sub-atomic and culture-subordinate methodologies to describe bacterial contrasts and their degradative potential in any hydrocarbon-contaminated environment. The rise of molecular approaches, such as the use of 16S rRNA genes for bacterial identification, bridged the gap of the limitations suffered by the cultural method.

Recommendations

1. Incorporate molecular methods alongside traditional techniques to enhance accuracy in identifying hydrocarbon-utilizing bacteria.
2. Evaluate bioremediation strategies in real-world settings to confirm their effectiveness and environmental impact.

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