



ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERISATION OF HYDROCARBON DEGRADING BACTERIA AND ITS ASSOCIATED GENES - A REVIEW

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ABSTRACT

Hydrocarbon is self-explanatory which means compounds of carbon and hydrogen only. Hydrocarbons play a key role in our daily life. The relatively sudden introduction of xenobiotic chemicals as well as the massive relocation of natural materials to different environmental compartments can often overwhelm the self-cleaning capacity of recipient ecosystems and therefore result in the accumulation of pollutant to problematic or even harmful levels. Hydrocarbons enter into the environment through waste disposal, accidental spills, as pesticides and via losses during transport, storage and use. Unfortunately, hydrocarbon contamination of agricultural lands that result from oil spillage kills vegetation and hence the biodiversity associated with it. Hydrocarbon substances particularly the polycyclic aromatic types have a deleterious effect on biota. The present review explores the purpose to investigate possible methods to enhance the rate of biodegradation of hydrocarbon in soil.

KEYWORDS: Bioremediation, Hydrocarbon, Carbon, Bacterial strain, Soil, Crude oil.



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INTRODUCTION

Hydrocarbons are the world's most widely used primary energy and fuel resources, due to the energy they produce. Apparently inevitable spillages, which occur during routine operations of crude oil production, refining, distribution and as a consequence of acute accidents, have generated continuous research interest in this field. Bioremediation functions basically on biodegradation, which may refer to complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell protein or transformation of complex organic contaminants to other simpler organic compounds by biological agents like microorganisms. Many indigenous microorganisms in water and soil are capable of degrading hydrocarbon contaminants. In addition, bioremediation technology is believed to be non-invasive and relatively cost-effective. Biodegradation by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment and is cheaper than other remediation technologies and the most efficient for environment safe depollution. The aim of the present review study was conducted to isolate and identify the bacterial population and assess their crude oil biodegradation potential under in vitro conditions. Degradation studies carried out with the most promising isolates at predefined set up to explore the biodegradation capability of hydrocarbon degrading bacterial strain.

REVIEW OF LITERATURE

Factors affecting the Bioremediation process

The microorganisms capable of degrading crude oil are present in any conceivable environment. Soil samples were contaminated with the hydrocarbon was estimated for total bacterial counts, total fungal counts (TFC), and total hydrocarbon utilizing bacterial counts (HYCUB). The following anions and cations were also determined in the soil samples; Ca^{2+} , Mg^{2+} , K^+ , Na^+ , NO_3^- , and NO_2^- . Bacterial species distribution from the soil samples are *Bacillus sp*, *Klebsiella sp*, *Pseudomonas sp*, *Proteus sp*, *Micrococcus sp*, *Arthrobacter sp*. The refined petroleum can have an increasing or decreasing effect on soil physico-chemical characteristics, and that some of these physico-chemical characteristics may have effect on microbial counts.¹ Kerosene

contamination soil characterize the microbes based on physicochemical properties such as pH, electrical conductivity, total nitrogen and phosphorus, microbial biomass and heavy metal content which are important indicators for assessing soil quality, fertility and productivity. The eight hydrocarbon degrading bacteria are *Alcaligen sp*, *Bacillus sp*, *Chromobacterium sp*, *Corynebacterium sp*, *Pseudomonas sp*, *Aeromonas sp*, *Serratia sp*, and *Flavobacterium sp*.²

Bioavailability of contaminants

The Elementary components of Hydrocarbons

Polyaromatic hydrocarbons degrading bacterial microbes are isolated using enrichment naphthalene (20 isolates), phenanthrene (25 isolates) or anthracene (6 isolates) as the sole source of carbon and energy. Three isolates, N6, A3 and P3, which were found to utilize naphthalene, phenanthrene and anthracene better than the rest of the isolates, were subjected for further analysis and characterization.³ Cycloparaffins are a sole source of carbon and energy that have proved unsuccessful. However, several hydrocarbon-utilizing bacteria oxygenated cycloparaffins to ketone derivatives that serve as the energy and carbon source for numerous other soil micro-organisms. The concerted attack of a mixed microbial population on cyclohexane has been demonstrated, suggesting that both co-metabolism and commensalism are associated with microbial degradation of cyclo-paraffinic hydrocarbons.⁴ Microorganisms represent an extremely reliable way to biodegrade petrol derived substances. The synthetic and semi-synthetic automotive lubricant oils took the longest time to completely change the color of DCPIP from blue to transparent; even with *Bacillus subtilis* inoculum.⁵ A study and isolation of *Cladosporium resinae* from soil and air sample showed that, strain of *C. resinae* grows in deep culture on C_{10} to C_{14} n-alkanes. Other strains grow on C_9 to C_{18} alkanes. It was interest to determine the range of hydrocarbons which *C. resinae* could use as sole source of energy and of organic carbon.⁶⁻⁷

Biodegradation

The cleaning up of petroleum hydrocarbons in the subsurface environment is a realworld problem. A better understanding of the mechanism of biodegradation has a high ecological significance that depends on the indigenous microorganisms to transform or mineralize the organic contaminants (Fig.1).⁸

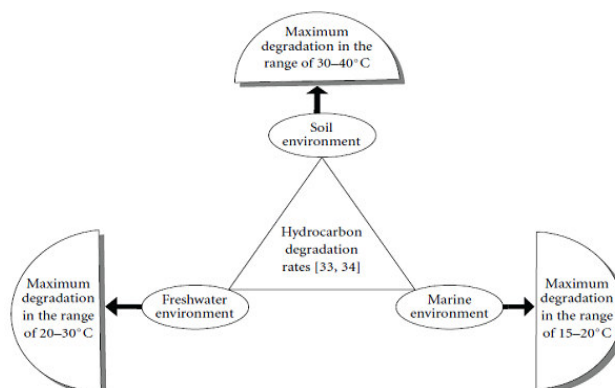


Figure 1
Hydrocarbon degradation rates in soil, fresh water and marine environments
Adopted from Review article (Nilanjana Das et al;2010)

Bioremediation Organisms

The utilization or degradation potential of three hydrocarbons of isolated bacteria from oil-polluted sites were performed in solid and liquid assay. In solid assay highest bacterial colony diameter was measured by *Chryseobacterium sp.*, and *Sphingobacterium sp.*, and in liquid assay highest support from bacterial growth was achieved by *Serratia odorifera* and *Enterobacter cloacae*. Solid and liquid medium enriched by gas oil (2%), toluene (1%), phenanthrene (0.05%).⁹ The increasing incident of oil spills as well as pollution of soil around filling stations demands the degradation of complex hydrocarbons. The isolated stains are *Bacillus sp.*, *Acinetobacter sp.*, *Clostridium sp.*, and *Pseudomonas sp.*, were selected for the study based on the efficiency of hydrocarbon naphthalene or anthracene in methanol utilization.¹⁰ The morphological and biochemical characterization of the petroleum contaminated soil has bacterial isolates *Bacillus megaterium*, *Corynebacterium*, *Pseudomonas fluorescence* and *Streptomyces griseus*. These isolates were able to grow on hydrocarbon as the sole source of carbon and energy when screened for hydrocarbon utilization.¹¹ Hydrocarbon utilizing fluorescent *pseudomonas* species are not only isolated from hydrocarbon contaminated sites, but also from normal soil environments. The isolates were grown well on diesel and subsequently produced the extracellular enzymes protease and urease at significant levels when compared to their production in the absence of diesel. Optimum temperature and pH the increasing growth by four isolates were found to be 37°C and pH 8.0 indicating the maximum utilization of diesel.¹²

Enrichment Techniques

The bacteria were isolated from three different types of samples on nutrient agar medium. Isolates are *Bacillus sp.*, *Micrococcus sp.*, *Corynebacterium sp.*, and *Neisseria sp.*, The O.D readings based on the turbidity of MSM broth at regular intervals of 2 days give the degradative activity on hydrocarbons by bacteria. The results demonstrated that *Micrococcus luteus* and *Bacillus megaterium* have the greatest ability to degrade petrol while *Bacillus megaterium* and *Corynebacterium xerosis* demonstrated the greatest ability to degrade diesel. Hydrocarbon degradation by bacteria reveal that catechol 2, 3 dioxygenase is the one of the enzyme that involved in hydrocarbon degradation.¹³ Many bacteria have the ability of biodegradation of oily pollutants.

These organisms are widely found in soil or watery ecosystem. Identified different bacterial genera by using biochemical tests, *Bacillus sp.*, *Corynebacterium sp.*, *Staphylococcus sp.*, *Streptococcus sp.*, *Klebsiella sp.*, *Escherichia*, *Acinetobacter sp.*, *Alcaligenes sp.*, *Shigella sp.*, the basis of degraded, number of bacterial to the total of isolated strain; it was detected that *Bacillus* with 69% was the best hydrocarbon degrading bacteria and camphor with 34% showed highest rate of degradation.¹⁴ Microorganism can be characterized by biochemical analyses and it can be isolated by serial dilution method and agar plating methods culture was purified by streaking technique. Purified cultures were characterized for the various staining and biochemical activities and were compared with Bergey's manual. The maximum oil degradation abilities were gram positive, *Bacillus sp.*, in hydrocarbon bioremediation. Preliminary screening of purified culture was also done by recovering oil from the flask and estimating the amount of oil left after degradation.¹⁵ The microbial degradation of oil has been shown to occur by attack on aliphatic or light aromatic fractions of the oil, with high molecular weight aromatics, resins and aliphatics. The mechanisms of adaption include both selective enrichment and genetic changes resulting in the increase in the number of hydrocarbon utilizing, organisms, threshold concentration for microbial degradation of hydrocarbons may apply also to soil ecosystems.¹⁶ The studies on the soil sample collected from 10 different sites shows the isolates on the microorganisms using different substrates kerosene, petrol, diesel and engine oil. *Pseudomonas*, *Streptococcus* and *Bacillus* species were not found to utilize all the different substrates tested. *Staphylococcus* species and *Micrococcus* species did not utilized engine oil, but utilized petrol, kerosene and diesel. *Klebsiella* species and *Mycobacterium* species utilized petrol and diesel, but did not utilize kerosene and engine oil. *Pseudomonas sp.*, *Streptococcus sp.*, and *Bacillus sp.*, could be harnessed for use in bioremediation of land polluted with petroleum and petroleum products.¹⁷ Hydrocarbon-contaminated environments are characterized by low or elevated temperature, acidic or alkaline pH, high salt concentrations or high pressure. The degradation of a wide range of hydrocarbons, including aliphatic, aromatic, halogenated and nitrated compounds has been shown to occur in various extreme habitats (Fig. 2).¹⁸

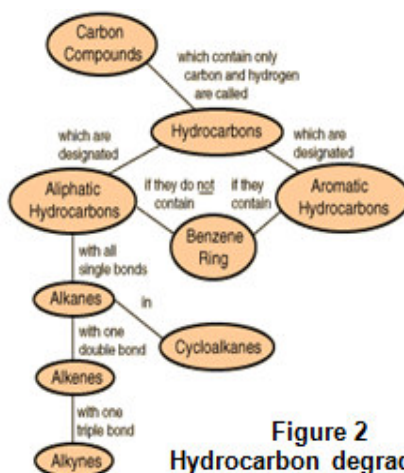


Figure 2
Hydrocarbon degradation

Adopted from Review article (Nilanjana Das et al;2010)

The bacterial strains degrade crude oil under in-vitro conditions *Pseudomonas* strain PS-1 could degrade alkanes (70.69%) and aromatics (45.37%). Alkanes and aromatic fractions separated by column chromatography were analyzed by gas chromatography. This clearly indicated that *Pseudomonas* strains PS-I, PS-II, and PS-III were comparatively better and potent aromatic hydrocarbon degraders. Out of these *Pseudomonas*

strains PS-I was almost comparable with standard strain of *Acinetobacter calcoaceticus*.¹⁹ Aerobic bacteria from soil contaminated with industrial xenobiotic compounds using enrichment technique containing phenol as sole source of carbon and energy was isolated in pure culture and selected for their ability to degrade phenol (Fig-3).²⁰

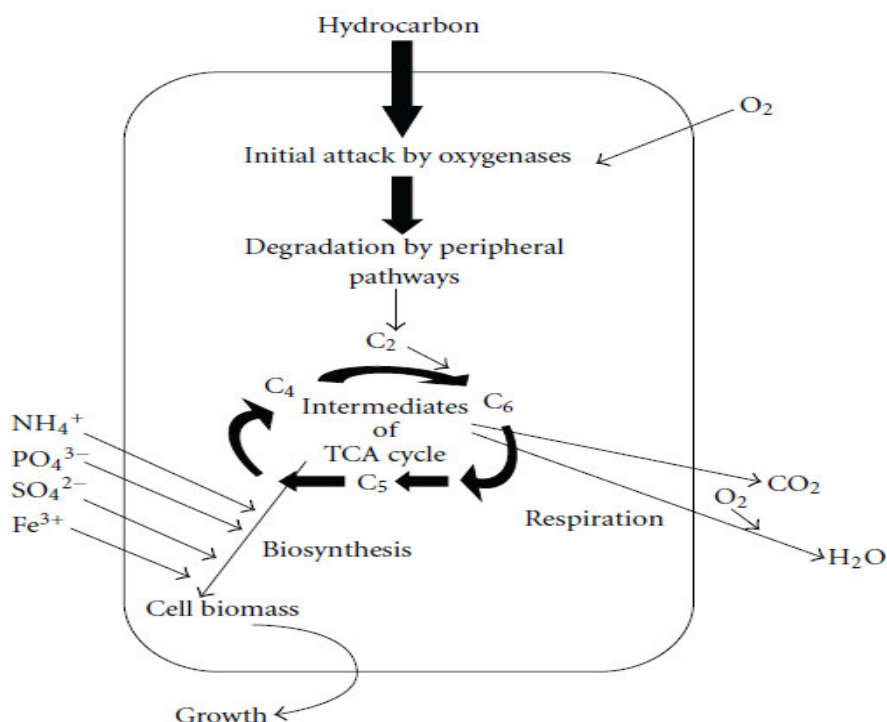


Figure 3

Main principle of aerobic degradation of hydrocarbons by microorganisms
Adopted from Review article (Nilanjana Das et al;2010)

Biosurfactant

The effect of surfactant Brij (1%) on biodegradation of various polycyclic aromatic hydrocarbons (PAHs) by *Pseudomonas* sp, PSS6 was evaluated. The degrading capability of the test isolates was determined by Bushnell-Haas agar and sprayed-plate method. *Pseudomonas* sp, PSS6 degraded PAHs forming an intensive clear zone in Bushnell-Haas agar. A modified mineral medium was employed for PAHs degradation along with Brij 35. The degradation of PAHs namely naphthalene, fluoranthene, phenanthrene and anthracene was influenced by 1% Brij 35.²¹ Oil degrading bacterial cultures, shows isolates from oil contaminated soil samples *Bacillus* sp, IOSI-7, *Corynebacterium* sp, BPS2-6, *Pseudomonas* sp, HPS2-5, and *Pseudomonas* sp, BPSI-8. The mixed bacterial consortium showed more growth and degradation than did individual strains. At 1% crude oil concentration, the mixed bacterial consortium degraded a maximum of 77% of the crude oil. This was followed by 69% by *Pseudomonas* sp, BPSI-8, 64% by *Bacillus* sp, IOSI-7, 45% by *Pseudomonas* sp, HPS2-5, and 41% by *Corynebacterium* sp, BPS2-6. The percentage of degradation by the mixed bacterial consortium decreased from 77 to 45% as the concentration of crude oil was increased from 1 to 12%. Temperature of 35°C and pH 7 were found to be optimum for maximum degradation of crude oil.²² Microbial and physicochemical properties of the soil samples varied

with the different plots and at different periods of remediation. Mineral salt medium to which crude oil had been added was used as a sole source of carbon and energy to isolate hydrocarbon utilizers from the sample collected from different plots of the contaminated site. Five genera of bacteria *Lactobacter* sp, *Arthrobacter* sp, *Bacillus* sp, *Pseudomonas* sp and *Micrococcus* sp were isolated and identified.²³ Isolated microbes were enriched in Bushnell-Haas medium containing 100ppm filter sterilized potassium cyanide which are less toxic products such as ammonia, CO₂, formate and formamide. Three distinct groups of bacteria were regarding the pH, maximum concentration of cyanide, dry weight of microbes and presence of ammonia of the stimulated effluent were optimized favoring the growth of cyanide degrading bacteria. Morphological, cultural, biochemical and 16S rDNA characterization of isolated bacterial cells has shown that *Pseudomonas* sp, *Brucella* sp and *Ochrobacterium* bacterial species are responsible for degrading cyanide.²⁴ Soil sample from abandoned coal power plant soil isolates were identified as belonging to the genus *Pseudomonas*; *Pseudomonas putida* G1 exhibited strong degradative ability on crude oil, kerosene, fair ability on engine oil, brake oil and petrol and a weak ability on pyrene, while *Pseudomonas aeruginosa* K1 had corresponding values of 0.21 days⁻¹ and 3.31 days. The amount of crude oil degraded by strain G1 and K1 after 21 day incubation period were 92.05% and 90.89% respectively.²⁵

Analyses through Molecular Approach

Analyze of physico-chemical, microbial characterization and identification of potential microorganisms capable of bioaccumulation and biodegradation of heavy metals were discussed in detail. pH, temperature, moisture, nutrients; Nitrogen, phosphorus and sulphur respectively. The biological parameters studied indicate Dissolved oxygen, biological oxygen demand, chemical oxygen demand. Microbial consortium was sequenced and compared using BLAST, Clustal W, and PHYLIP. The 16S rRNA sequence and phylogenetic tree characterized the organisms as *Klebsiella pneumoniae*. It's confirmed by biochemical tests. The potential microorganism identified by BLAST technique can be used for remediation of the heavy metal from contaminated environment.²⁶ Two bacterial species from a paddy soil microcosm that had been artificially contaminated with diesel oil to which extrinsic *Pseudomonas aeruginosa*, strain WatG, has been added exogenously PCR amplification of 16S rRNA genes. Total petroleum hydrocarbons were analyzed by gas-chromatography. Autochthonous isolates and / or bacterial consortia would be more useful and effective inoculants in situ bioremediation.²⁷ Isolated microbial consortium and were tested for their ability to grow on mineral salt agar plates supplemented with one of the following model n-alkanes or aromatic hydrocarbons: hexane, heptanes, paraffin, benzene, toluene, naphthalene and kerosene. They are degraders with broader abilities to grow on both types of hydrocarbons, good production of glycolipids and emulsifying activity.²⁸ Two hydrocarbon degrading bacteria *Bacillus sp*, and *Corynebacterium sp*, isolated from tannery effluent indicated that *Bacillus sp*, has a greater potential to degrade hydrocarbon when grown in a medium containing used engine oil. Degradation potential of *Bacillus sp*, was tried to increase by including mutation by UV radiation and the mutants were identified by performing RAPD.²⁹ Studies were carried out on evaporation of the hydrocarbons. Evaporation must be considered in estimates of oxidation. Mineralization of [¹⁴C] hexadecane can be equated with the total number of petroleum-degrading bacteria and the percentage of the total heterotrophic population, which they represent.³⁰ Isolates of two strains WatG and Hok M which were identified as new strains of *Pseudomonas aeruginosa* and *Serratia marcescens* species. Its high capacity and wide spectrum to degrade the hydrocarbons in gasoline, kerosene, diesel, lubricating oil. In mineral salt medium 90-95% of excess amount of total diesel oil and kerosene was added as a sole carbon source degraded by WatG within 2-3 weeks. Lubricating oil 60% degraded within 2 week. Strain Hok M was more capable than WatG in degrading aromatic compounds in gasoline. This strain could also degrade kerosene, diesel, and lubricating oil with a capacity of 50-60%. Thus, these two isolates have potential to be useful for bioremediation of sites highly contaminated with petroleum hydrocarbons.³¹ Bacteria with limited genomic cross-hybridization were isolated from soil contaminated with C₅+, a mixture of hydrocarbons identified by partial 16S rRNA sequencing. Filters containing denatured genomic DNA's used in a reverse sample genome probe analysis of the effect of the easily degradable compound and highly recalcitrant

compound.³² Chemical screening of hydrocarbon pollutants to gas chromatography a mixture source of biogenic and petrogenic hydrocarbons but the biogenic and hydrocarbons were stronger than the petrogenic hydrocarbons. The average count of heterotrophic bacteria in water samples as 10⁴ CFU/ml, while in sediment samples was 10⁵ CFU/ml. The average density of crude oil utilizing bacteria in water samples was 10³- 10⁴ MPN/100ml, while in sediment samples was 10⁵ MPN/100ml. The average of hydrocarbon degrading bacteria within the average of heterotrophic communities was fluctuated between 0.2% to 2.1%.³³ Isolated *Pseudomonas aeruginosa* from petroleum-contaminated soil had surface properties of the culture supernatants of the isolate, using water-soluble and water-insoluble carbon sources as substrates are described in terms of surface tension and emulsification index. Water-soluble substrates showed better surface activity than water-insoluble substrates. The surface tension of the culture supernatants was lowest with glucose, sucrose, glycerol, fructose, hexadecane and paraffin oil. The culture supernatants showed emulsification index of between 52% and 85% and the emulsion formed remained stable during an extended ageing period of 30 days. The bio surfactants formed by the isolate retained surface active properties after exposure to high temperature (100°C), a wide range of pH (4-12) and high salinity (16%).³⁴ The microorganisms survive in contaminant habitat because they are metabolically capable of utilizing its resources and can occupy a suitable niche. Contaminants are often potential energy sources of microorganism.³⁵ The bioremediation achieves contaminant decomposition or immobilization by exploiting the existing metabolic potential in microorganisms with catabolic functions derived through selection or by introducing of genes encoding such functions.³⁶ The ability of microbes to degrade organic contaminants into harmless constituents has been explored as a means to biologically treat contaminated environments.³⁷ The response of the growth and anatomy of *Amaranthus hybridus* in provoked crude oil contaminated soil was investigated using 1-4% v/w crude oil and a control (0%). For growth parameters like plant height, leaf area, plant fresh weight and plant dry weight, the mean values obtained were higher for control (0%) and progressively decreased from 1-4%.³⁸

Bioremediation of Crude oil

The bioremediation of crude oil hydrocarbon polluted mangrove swamps using cow dung as source of limiting of nutrients. In a 70 days study, the cow dung treated polluted soil had its total culturable hydrocarbon utilizing bacterial / fungi, heterotrophic bacterial and fungal counts increased progressively from the 28th day to the 78th day. The conductivity values of cow dung decreased progressively. In the cow dung treatment option, the nitrate concentration decreased from 35.44mg/kg to 14.28mg/kg. Phosphate concentration of cow dung option decreased from 25.41mg/kg to 9.31mg/kg. The control had the nitrate decreased from 8.42mg/kg to 6.98mg/kg. Percentage total organic carbon in the cow dung option decreased from 4.06% to 0.96%. Control experiments had the % TOC decreased from 3.32% to 2.99%.³⁹ The microbes are highly efficient in degrading hydrocarbons in producing surface active

bio agents that can stimulate the uptake of hydrocarbon substrates for better biodegradation.⁴⁰ The successful use of microbial inoculum in soils requires that the microorganisms contact the contaminant and the physical absorption to the soil practices or filtration through small pores may limit the transport of organisms.⁴¹ A soil sample was contaminated with two different crude oils so as to isolate and identify the bacteria capable to acclimatize and degraded the crude oil contaminants present. The bacteria strains in a serial diluted solution were inoculated on nutrient agar plate incubated at 37°C. The isolates were characterized for gram reaction, cell morphology and various biochemical / enzymatic analysis tests. The result revealed the presence of *Pseudomonas aeruginosa* and *Proteus vulgaris* which are highly adapted bacteria with great potential to biodegrade total petroleum hydrocarbons from crude oil contaminated soil.⁴² A need arose to ascertain the impact of crude oil spillage on soil and food production in Ogba/Egbema/Ndoni Local Government Area of Rivers State. A set of structured questionnaires were distributed to one hundred and twenty respondents randomly selected from three communities in the area. The data obtained were analyzed using simple percentage. The result of the findings shows that oil spillage has a negative effect on the physical, chemical and biological properties of soil and also creates a negative impact.⁴³ An effort at developing an active indigenous bacterial consortium that could be of relevance in bioremediation of petroleum contaminated system in Nigeria, documented four hydrocarbon degrading bacteria strains were isolated. Partial sequencing of the 16s rDNA of the isolates are all strains of *Pseudomonas aeruginosa*. Amount of crude oil biodegraded in 15 days ranged significantly ($p < 0.05$) from 4.9% to 29.6%.⁴⁴

rRNA Sequencing

Crude oil degrading bacteria isolated from deep-sea sediment of Sulu-Sulawesi Sea and the diversity of potential degraders was determined. Deep-sea sediments were collected using Smith McIntyre Grab. A total of 21 strains were identified as positive oil degraders from 42 isolates obtained from the sediments. Identification of positive degraders was done using 16S rRNA cloning and DNA partial sequencing method. A set of forward (27F) and reverse (1492R) universal primers were used. The DNA sequences of 16S rDNA were aligned and matched using online DNA database to determine the type and phylogenetic diversity of crude oil degrading bacteria. According to the BLAST analysis, it was found that the degraders belong to 7 different phylotype.⁴⁵ The degradation of Total Petroleum Hydrocarbons (TPH) in soil was evaluated. In addition, the number of diesel-degrading 12 weeks of incubation, all three treatments showed differing effects on the degradation of light (C12 – C23) and heavy microorganisms present and microbial activity as indexed by the dehydrogenate assay were monitored.⁴⁶ Biodegrading activities on hydrocarbons as the sole carbon source using enrichment medium and the microbial growths were determined using calorimeter blanked at 595 nm. The identified bacteria were screened for the presence of one of the hydrocarbon degrading enzyme catechol 2, 3 dioxygenase by DNA isolation, PCR amplification of gene using specific

primers and sequencing of gene (C23O). The organisms with catechol 2, 3 dioxygenase enzymes were identified as *Bacillus megaterium*, *Bacillus cereus*, *Micrococcus luteus* & *Lactobacillus acidophilus* with a 216 bp amplification using C23O specific primers. The sequence has similarity about 90% to catechol 2, 3 dioxygenase gene.⁴⁷ The samples of water were collected from the port of Gdynia and the Gulf of Gdansk (Southern Baltic Sea), and were artificially covered by oil film and stored at a constant temperature. It indicates a greater rate of degradation for the film which covered port water than water from the Gulf of Gdansk. It was revealed that light decreases the rate of biodegradation of an oil film.⁴⁸ An oil-degrading microorganism from contaminated (scale) soil were isolated, *Nocardia simplex* is the best degrader, among all the isolates, on both used and unused lubricating oil. Significance and impact of the presence of *Nocardia simplex* W9 in scale soil enables iron to be recycled by biodegradation.⁴⁹ Three lipase-producing thermophilic bacteria (AK-P1, AK-P2, and AK-P3) were isolated from the Taptapani hot water spring in Orissa, India. Strain AK-P3, exhibited the highest lipolytic activity of 5.5U/mL was identified as *Porphyrobacter* sp., The lipolytic activities of strains AK-P1 and AK-P2 were 4.5U/mL and 3.5U/mL, respectively. Strains AK-P1 and AK-P2 were identified as *Acinetobacter* sp, and *Brevibacillus* sp.⁵⁰ Microorganisms inhabiting subterranean oil fields have recently attracted much attention. Since intact groundwater can easily be obtained from the bottom of underground oil storage cavities without contamination by surface water, studies on such oil-storage cavities are expected to provide valuable information to understand microbial ecology of subterranean oil fields. DNA was extracted from the groundwater obtained from an oil-storage cavity situated at Kuji in Iwate, Japan, and 16S rRNA gene (16S rDNA) fragments were amplified by PCR using combinations of universal and Bacteria-specific primers. The sequence analysis of 154 clones produced 31 different bacterial sequence types.⁵¹ 16S rRNA gene sequencing was used to analyze the bacterial content of the Vostok drilling fluid sampled from four depths in the borehole. Six phylotypes were identified in three of four samples studied. The two dominant phylotypes recovered from the deepest and comparatively warm borehole horizons were from within the genus *Sphingomonas*, a well-known degrader of polyaromatic hydrocarbons.⁵² Biodegradation of crude oil and 7 different distillation products by *Ralstonia picketti* and *Alcaligenes piechaudii* was investigated. Crude oil degradation was above 80% after incubation of 20 days for both strains and their mixture. Removal of short/long alkanes investigated was different, from 97% to 22%. Hexadecane, pristane and cyclohexane were degraded with high efficiency between 72-91%. Among toluene was degraded very well by both strains and their mixture.⁵³ The effect of oil pollution in the environment which has led to a global interest in the microbial biodegradation of pollutants in the recent years. Oil-degrading bacteria associated with oil-polluted lands in River State, Nigeria. Crude oil was supplied to the enrichment culture as a sole carbon and energy source. The 16S rRNA analysis revealed that the isolates belonged to the genera *Citrobacter* sp, *Enterobacter* sp, *Klebsiella* sp, *Aeromona* sp, *Ewingella* sp and *Pseudomonas* sp. The percentages of *Proteobacteria*

and *Firmicutes* in soil were greater than those in the water samples.⁵⁴ One-hundred and fifty different thermophilic bacteria isolated from a volcanic island were screened for detection of an alkane hydroxylase gene using degenerated primers developed to amplify genes related to the *Pseudomonas putida* and *Pseudomonas oleovorans* alkane hydroxylases. Ten isolates carrying the alkJ gene were further characterized by 16s rDNA gene sequencing. Nine out of ten isolates were phylogenetically affiliated with *Geobacillus* species and one isolate with *Bacillus* species.⁵⁵ The anaerobic growth on crude oil was observed in enrichment cultures and pure cultures of sulfate-reducing bacteria. Several alkyl benzenes and n-alkanes were specifically consumed with concomitant reduction of sulfate to sulfide; consumed hydrocarbons together accounted for up to approximately 10% of the crude oil.⁵⁶ Soil from the Niger Delta region of Southern Nigeria, contaminated with kerosene by 10% artificial stimulation to determine the attendant effect associated with the soil physicochemical properties and microbiological composition. Typical generation times vary between 0.64 and 1.09 d, 0.97 and 3.03 d, 0.88 and 2.97 d respectively for kerosene, diesel and naphthalene. All the isolates utilized the hydrocarbons as sole carbon and energy sources equally well. The study revealed for the first time, the types of microorganisms that are associated with Nigerian bitumen deposit and their potential to degrade oil. Bio-deterioration of crude oil and oil fuels is a serious economic and an environmental problem all over the world. It is impossible to prevent penetration of microorganisms in oil and fuels both stored in tanks or in oil fields after drilling. Both aerobic and anaerobic microorganisms tend to colonize oil pipelines and oil and fuel storage installations. Complex microbial communities consisting of both hydrocarbon oxidizing microorganisms and bacteria using the metabolites of the former form an ecological niche where they thrive.⁵⁷

Fermentation assay

The potential biodegradation of crude oil was assessed based on the development of a fermentative process with a strain of *Pseudomonas aeruginosa* which produced 15.4 g/L rhamnolipids when cultured in a basal mineral medium using glycerol as a sole carbon source. However, neither cell growth nor rhamnolipid production was observed in the comparative culture system using crude oil as the sole carbon source instead.⁵⁸ A preliminary study was undertaken to determine the optimal conditions for the biodegradation of a crude oil. Among 57 oil-degrading bacterial cultures isolated from oil contaminated soil samples, IOS1-7, *Corynebacterium sp*, BPS2-6, *Pseudomonas sp*, HPS2-5 and *Pseudomonas sp*, BPS1-8 were selected for the study based on the efficiency of crude oil utilization. The mixed bacterial consortium showed more growth and degradation than did individual strains.⁵⁹ Hydrocarbon utilizing microorganisms are ubiquitously distributed in the environment following oil spills. 36 bacterial isolates capable of utilizing crude oil as a carbon source were isolated from various contaminated soils using the enrichment technique. Five of them were characterized based on morphological, cultural and biochemical tests. Further, the screening was done by estimation of whole cell protein and growth in terms of turbidity when crude

oil is supplied as the sole source of carbon.⁶⁰ Two hydrocarbon degraders isolated from an abandoned coal power plant soil in Lagos, Nigeria, were tested. The isolates were identified as belonging to the genus *Pseudomonas*. *P. putida* G1 exhibited strong degradative ability on crude oil, kerosene, fair ability on engine oil, brake oil and petrol and a weak ability on pyrene, while *P. aeruginosa* K1 had corresponding values of 0.21 days⁻¹ and 3.31 days. The amount of crude oil degraded by strain G1 and K1 after a 21 day incubation period were 92.05% and 90.89% respectively. Data obtained from gas chromatographic analysis of oil recovered from culture fluids of G1 and K1 confirmed a near-disappearance of aliphatic fractions and a significant reduction of aromatic fraction in the hydrocarbon mixture.⁶¹ Oil contamination in Iran due to high application of oil compounds, is one of the most dangerous pollution factors. Bioremediation is one of principal strategies for remediation, Hydrocarbon degrading efficiency of some isolated bacteria from oil polluted sites were *Pseudomonas stutzeri*, *Pseudomonas alcaligenes*, *Pseudomonas aeruginosa*, *Pantoea sp*, and *Chryseobacterium sp*, were determined in a solid and liquid medium enriched by gas oil, toluene and phenanthrene.⁶² The bacterial flora of soils contaminated with used oil in Keffi town was studied. The bacterial species isolated were *Pseudomonas sp*, *Streptococcus sp*, *Escherichia coli*, *Staphylococcus sp*, *Klebsiella sp*, *Bacillus sp*, *Mycobacterium sp*, *Enterobacter aerogenes*, *Salmonella sp*, and *Micrococcus sp*. The hydrocarbon substrates (petroleum products) used was petrol, kerosene, diesel and engine oil. *Pseudomonas sp*, *Streptococcus sp*, and *Bacillus sp*, were found to utilize all the four petroleum products as their sole source of carbon and energy. *Staphylococcus sp*, and *Micrococcus sp*, utilized petrol, kerosene and diesel, while *Klebsiella sp*, and *Mycobacterium sp*, utilized only petrol and diesel.⁶³ Remediation by Enhanced Natural Attenuation (RENA) was employed to remediate an oil contaminated site in the Gokana Local Government Area of Rivers State, Nigeria between January and September 2006. Two fungi, *Articulosporium inflata* and *Zoopagemitospora* as well as five genera of bacteria, *Lactobacter sp*, *Arthrobacter sp*, *Bacillus sp*, *Pseudomonas sp* and *Micrococcus sp* were isolated and identified.⁶⁴

CONCLUSION

The need to develop strains that could be useful in the bioremediation of hydrocarbon polluted sites cannot be overemphasized. This review of study reveals that bacterial species isolated from contaminated soil can be harnessed in an attempt at developing strains that will be useful in environmental bioremediation of contaminated sites. Many indigenous microorganisms that are capable of degrading hydrocarbon contaminant can be readily isolated from contaminated sites as shown in this study. Models and strategies which will enhance the removal of hydrocarbon contaminants from oil impacted sites need be promoted.

CONFLICT OF INTEREST

Conflict of interest declared none.

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