# PLASMID PROFILLING AND SIMILARITIES IN IDENTITIES OF PROBABLE MICROBES ISOLATED FROM CRUDE OIL CONTAMINATED AGRICULTURAL SOIL

Toochukwu Ekwutosi OGBULIE[*], Ann Ijeoma OGOAMAKA[**], Justina Ugochi UDENSI[*]

[*] *Department of Biotechnology, School of Science, Federal University of Technology Owerri (FUTO) Imo State Nigeria*
[**] *Department of Microbiologz, Faculty of Scrience, Imo State University Owerri (IMSU), Nigeria*
*Corresponding author: T.E. Ogbulie, Department of Biotechnology, School of Science, Federal University of Technology Owerri (FUTO) Imo State, Nigeria; phone: +2348035472379; e-mail: ogbulie_toochi@yahoo.com*

**Abstract.** Plasmid analysis of bacteria isolated from agricultural soil experimentally contaminated with crude oil was carried out and the resultant bands' depicting the different molecular sizes of the plasmid DNA molecules per isolate was obtained. There was no visible band observed for *Klebsiella* indicating that the organism lack plasmid DNA that confers degradative ability to it, possibly the gene could be borne on the chromosomal DNA which enabled its persistence in the polluted soil. Molecular characterization was undertaken to confirm the identities of the possible microorganisms that may be present in crude oil-contaminated soil. The result of the DNA extracted and amplified in a PCR using EcoRI and EcoRV restriction enzymes for cutting the DNA of the bacterial cells indicated no visible band for cuts made with EcoRV restriction enzyme showing that the enzyme is not specific for bacterial DNA of isolates in the samples, hence there was no amplification. By contrast though, visible bands of amplicons were observed using EcoRI restriction enzymes. The resultant visible bands of microbial profile obtained using the universal RAPD primer with nucleotide sequence of 5'—CTC AAA GCA TCT AGG TCC A---3' showed that only *Pseudomonas fluorescens* and *Bacillus mycoides* had visible bands at identical position on the gel indicating that both species possibly had identical sequence or genes of negligible differences coding for degradation of hydrocarbons as shown by similar values in molecular weight and positions in the gel electrophoresis field.

**Keywords:** Plasmid DNA; Polymerase chain reaction; crude oil; Agricultural soil, Nigeria.

## INTRODUCTION

A large number and size of areas in most developed and developing countries like Nigeria are contaminated with crude oil (hydrocarbon pollutants and heavy metals). Introduction of these pollutants into the environment may be naturally occurring (natural oil seeps) or anthropogenic as in the case of accidental or deliberate spills and leakages such as intentional or accidental bursting of pipelines [3, 18, 22, 23, 38]. In Nigeria, the terrestrial and aquatic environment of the oil-rich Niger Delta region and its adjoining areas are the main recipients of crude oil spills. This most times leads to enormous pollution of their ecosystem [11, 17, 22-24] resulting in loss of microbial communities, habitats of economically important fish species and other aquatic animals, damage to wetlands along the coast as well as areas of vegetation meant for agricultural purposes etc. These however, pose serious threat to public health [1, 19, 26].

Bioremediation is one of nature's prudent ways to purify the polluted environment. Although the term bioremediation may be recent, the process is not new as its origin relates to the origin of life when the first organism was stressed by certain compounds. These organisms however, according to Gupta *et al.,* [9] evolved the process to convert such compounds into less harmful forms by adopting certain detoxifying mechanisms in order to overcome the stress. In the past two decades, the application of bioremediation has been growing partly because of the better understanding of microbial process in soil. For instance, sites polluted with PAHs have been treated with some success [14, 27]. Recently, bioremediation technologies are based on the processes and potentials of almost all types of life form [9]. Such forms include plants (Phytoremediation), microorganisms (microbial remediation) and animals (Zooremediation), and in many cases, the most effective technique combines several techniques such as combination of plant and microbial action (Rhizoremediation) [14, 39].

Furthermore, the most important principle of bioremediation is based on the idea that all organisms remove substances from the environment to carry out growth and metabolism [35]. Bacteria, protista and fungi are very good at degrading complex molecules and incorporating the products of breakdown into their metabolisms [34]. The resultant metabolic waste that they produce is generally safe and somehow recycled into other organisms. Fungi, for instance, are good at digesting complex organic compounds that are normally not degraded by other organisms, although the ability to degrade a pollutant is dependent on presence of plasmid bearing genes coding for pollutant degradation [33] or enzymes produced by the organism [30]. In other words, petroleum can be degraded only by microbes with the ability to produce enzyme that selects petroleum as a substrate. However, bioremediation does not only involve the degradation of pollutants, it is sometimes, sufficient to remove the pollutants from the environment without degrading it. For instance, bacteria take up large amount of metals and minerals to ensure adequate resources for binary fission. Algae and plants also are very good at absorbing nitrogen, phosphorous, sulfur and many minerals and metals from the environment [12, 15, 39].

A variety of molecular methods have been developed however, to assay/monitor the presence of micro-organisms in samples and to profile for the possible presence of plasmid in them. Most recently, the method of choice to determine what micro-organisms are present in environmental sample is to amplify the conserved small subunit rRNA gene [8]. In this process, DNA is isolated from the soil using boiling method [20] and polymerase chain reaction (PCR) with gene-specific primers used to amplify the

specific gene from the sample. Through Randomly Amplified Polymorphic DNA (RAPD) profiling also, isolated DNA can be amplified at random and a semi unique profile can be gleaned from the resulting pattern.

This study was therefore designed to identify bacterial isolates present in the crude oil contaminated soil with a view to ascertain the presence of plasmid in these isolates and to confirm similarities in microbial identities.

## MATERIAL AND METHODES

### Microbial Characterization

Two (2) grams of soil collected within the rhizosphere of the test plants grown for 28 days on agricultural soil polluted with Bonny Light crude oil (Nigerian crude oil) was disolved in 10 ml of sterile water. The supernatant containing the organism was collected. Ten-fold serial dilutions was undertaken and an aliquot plated on Mueller Hinton agar, Sabouraud Dextrose agar and Luria agar medium. The samples were microbiologically analyzed using spread plate technique to isolate naturally existing microbial floras as well as persistent organisms after treatment as described by Cheesbrough [7]. Appearance of the bacterial and fungal genera on the media was examined for morphological details and identification was as described by Holt [10].

### Plasmid (Profile) Analysis

The plasmid of those that grew on the media was isolated by alkaline lysis method of Birnboim and Dolly [5]. This was carried out by centrifuging 1.5ml of overnight broth culture in a bench centrifuge and resuspended in 250µl lysis solution (sodium deodecyl sulphate, SDS) to lyse the bacterial cells. Then 250 µl high salt solution(3M NaAc, pH 5.2) was thereafter added and the mixture kept at $4^0$C for 30 min in a water bath. After short incubation, the tube containing the mixture was spinned at high speed of 8000 rpm in a centrifuge for 6 min, and the supernatant carefully transfered to a clean Eppendorf tube. Equal volume of isopropanol was then added and tube placed at $20^0$C for 20 min. The mixture was centrifuged again for 2 min and supernatant decanted. Pellets were redissolved in 100µl dilute NaAc (0.1M NaAc, pH 6). Equal volume of phenol:chloroform:isoamyl alcohol mixture was added to extract the DNA using high spin to separate the phases and enhance removal of traces of phenol. Thereafter, the aqueous layer was pipetted and transfered to a new tube.

Plasmid DNA was, however, recovered by precipitating the DNA through the addition of 400 µl of absolute ethanol and the tubes placed at -$20^0$C for 20 min and spin for 3 min. The DNA was further washed with 70% ethanol, spinned again for 3min to remove salt and proteins. Pellets were thereafter dried at room temperature for 20 min to remove all ethanol and pellets redissolved in 200µl of water. An aliquot was collected, placed into another Eppendorf tube, digested using EcoRI and crude lysates resolved by agarose gel electrophoresis [18] using λDNA HindIII marker. The bands were viewed on transilluminator. Distances moved were measured and molecular weight calculated on line using www.insilico.ehu.es.

### Similarities in Microbial Identities.

The similarities in identity of the microorganisms present in crude oil contaminated soil was determined as described by [32], by extracting the community DNA using boiling method of Sambrook *et al*., [31] and carrying out genomic polymorphism analysis by randomly amplified polymorphic DNA- polymerase chain reaction (RAPD-PCR) profiling using oligonucleotide primer P54 which have the following sequence, 5'—CTC AAA GCA TCT AGG TCC A---3'. Polymerase Chain Reaction (PCR) was carried out in 25µl reaction mixtures containing 50 pico mol of primer, each deoxynucleoside triphosphate at a concentration of 200µM, 2.5mM of $MgCl_2$ reaction buffer, 1U of *Taq* polymerase (Pharmacia Biotech) and 2µl of DNA. The amplification conditions therefore include 40 cycles that ran for 2 h 30 min with denaturation temperature of $94^o$C, annealing temperature of $45^o$C and extension temperature of $72^o$C.

## RESULTS

The prevalence of the isolates from the agricultural soil under study before and after pollution is as shown in Table 1. Before pollution of the soil sample, the presence of *Aspergillus fumigatus, Aspergillus niger, Penicillium* sp, *Candida albicans, Pseudomonas fluorescens, Acinetobacter baumanni, Bacillus mycoides, Klebsiella* sp., *Staphylococcus aureus* and *Escherichia coli* were observed. However after pollution, the disappearance of *S. aureus* and *E. coli* was observed.

**Table 1.** Prevalence of isolates from soil under study before and after pollution

| Isolates | Before pollution | After pollution |
|---|---|---|
| *Aspergillus fumigatus* | + | + |
| *Aspergillus niger* | + | + |
| *Penicillium* sp | + | + |
| *Candida albicans* | + | + |
| *Klebsiella* sp. | + | + |
| *Bacillus mycoides* | + | + |
| *Pseudomonas fluorescens* | + | + |
| *Acinetobacter baumani* | + | + |
| *Staphylococcus aureus* | + | - |
| *Escherichia coli* | + | - |

Legend: + = Present; - = absent

### Plasmid DNA of bacterial Isolates and its Molecular Weight

Plasmid analysis of the bacterial isolates was carried out and the resultant bands (fragments) are as shown in Fig. 1 depicting the different molecular weights of the plasmid DNA fragments per isolate. There was no visible band observed for *Klebsiella* showing that the organism do not have plasmid DNA

that confers degradative ability to it, instead the gene could be borne in the chromosomal DNA which enabled its persistence in the polluted soil.

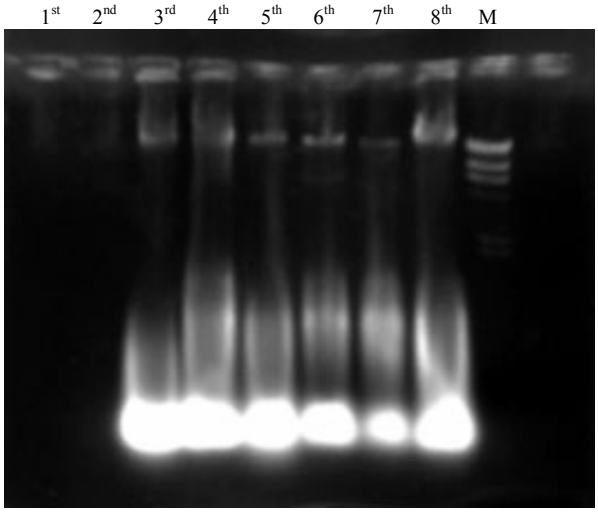


**Figure 1.** Bands of Plasmid DNA for *Klebsiella* sp (1st and 2nd well), *Acinetobacter baumanni* (3rdand 4th), *Bacillus mycoides* (5th and 6th well), *Pseudomonas fluorescens* (7th and 8th well) and the λDNA HindIII marker (9th well)

The molecular weights of λDNA marker include 23130bp, 9416bp, 6557bp, 4361bp, 2322bp, 2027bp and 564bp while Fig. 2 shows the distance covered by the isolates under study depicting there molecular weight in base pairs.

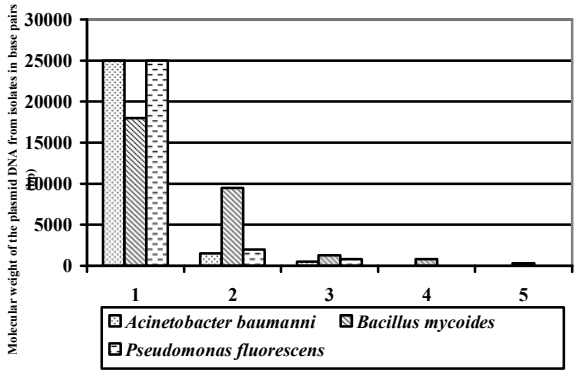


**Figure 2.** Mobility graph of the molecular weight of the plasmid DNA extracted from test isolates

**Molecular Characterization and Confirmation of Similarities in Microbial Identities.**

Molecular characterization/diversity was undertaken to confirm the identities of the micro-organisms present in crude oil-contaminated soil. The result of the community DNA extracted and amplified in a PCR using EcoRI and EcoRV as the restriction enzymes for cutting the DNA of the bacterial cells is as shown in Fig. 3 and 4. No visible band was observed using EcoRV restriction enzyme showing that the enzyme is not specific for bacterial DNA isolated from this study, hence there was no amplification. By contrast, however, visible bands of amplicons were observed using EcoRI restriction enzymes.

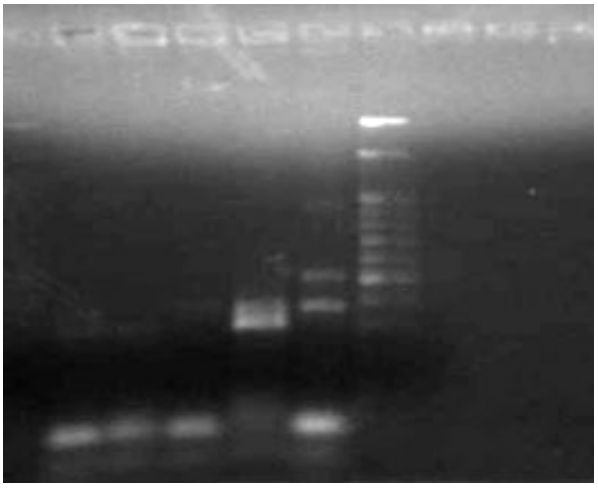

**Figure 3.** Bands of amplified cDNA of isolates using EcoRI restriction enzymes enzymes (*Acinetobacter baumanni* (1stand 2nd), *Bacillus mycoides* (3rd and 4th well), *Pseudomonas fluorescens* (5th and 6th well) and the 100kb pair marker (7th well)

**Figure 4.** Bands of amplified cDNA of isolates using EcoRV restriction enzymes (*Acinetobacter baumanni* (2ndand 3rd), *Bacillus mycoides* (4th and 5th well), *Pseudomonas fluorescens* (6th and 7th well) and the 100kb pair marker used as control (1st well)

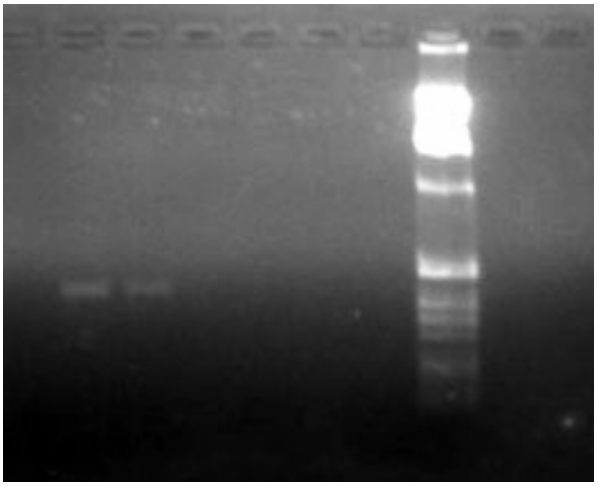


**Figure 5.** DNA fragment bands of isolates using RAPD-PCR fingerprinting *(P. Fluorescens* (2nd well) and *B. Mycoides* (3rd well))

The resultant visible bands of RAPD profile obtained using the universal RAPD primer with nucleotide sequence of 5'—CTC AAA GCA TCT AGG TCC A---3' is as shown in Fig. 5. Only *P. fluorescens* and *B. mycoides* showed visible bands indicating that both species had identical sequence or genes of negligible differences coding for degradation of crude oil as shown by similar values in molecular weight and positions in the gel electrophoresis field.

## DISCUSSION

The isolation of microbes as *Aspergillus fumigatus, Aspergillus niger, Penicillium* sp. and *Candida albicans* from the sample which persisted even after treatment with crude oil supports the findings of Saadoun [30] who studied on the isolation and characterization of bacteria from crude petroleum oil contaminated soil and the potentials of the isolated organisms to degrade diesel fuel; and Sutherland and da Silva *et al.* [36] who reported the degradation of polycyclic aromatic hydrocarbons (PAHs) by *Aspergillus niger* and *Penicillium janthinellum* among others. It also lend more weight to the studies made by Nkwelang *et al* [21] on the diversity, abundance and succession of hydrocarbon utilizing microorganisms in the tropical soil polluted with oil sludge. They also isolated bacterial as *Pseudomonas* sp., *Bacillus* sp., *Acinetobacter* sp. and fungal genera as *Aspergillus* sp, *Penicillium* sp, *Candida* sp., *Mucor* , *Rhizopus* sp., *Sporobolomyces*. The report also showed that *Pseudomonas* sp., *Bacillus* sp., *Aspergillus* sp., and *Penicillium* sp were present in the polluted soil throughout the experimental period [21]. The findings of this study is also in line with the report made by Ogbulie and Njoku [25] and Jyothi *et al*., [13] who isolated and identified hydrocarbon degrading bacteria as *Bacillus cerus, Staphylococcus aureus, Micrococcus luteus, Lactobacillus acidophilus* and others by Molecular characterization.

The resultant bands (fragments) of the plasmid analysis of the bacterial isolates carried out depicts the different molecular weight of the plasmid DNA fragments per isolate. There was no visible band observed for *Klebsiella* showing that the organism does not have plasmid DNA that confers degradative ability to it, instead the gene according to Watson *et al.* [37], be integrated in the chromosomal DNA which enabled its persistence in the polluted soil. Molecular characterization was undertaken to confirm the similarity in identity of the micro organisms present in crude-oil contaminated soil. The resultant visible bands of RAPD profile obtained using the universal RAPD primer with nucleotide sequence of 5'—CTC AAA GCA TCT AGG TCC A---3' showed that only *P. fluorescens* and *B. mycoides* had visible bands indicating that both species could have identical sequence or genes of negligible differences coding for degradation of crude oil as shown by similar values in molecular weight and positions in the gel electrophoresis field. This supports the result obtained by Amer *et al.* [2] who carried out research on biodegradation of monocyclic aromatic hydrocarbon and identified a new strain of *Pseudomonas* after genetic profiling with other known strains of *Pseudomonas* which showed similarity in both their bands and the 16S rDNA genes.

Indeed the findings of this study showed that rhizosphere microorganisms could be engineered in the future since plasmid can be identified in some of the isolates which can be incorporated into another organism to confer degradative ability to such organism for enhanced environmental clean up. This supports the findings of Lipthay *et al.* [16], who transferred a tfdA gene (encoding 2,4-dichloroPhenoxyacetic acid/2-oxoglutarate dioxygenase) in a plasmid to phenol-degrading bacteria (*Ralstonia eutropha* and *Pseudomonas sp*.) which has been reported to significantly increase their ability to degrade phenoxyacetic acid in sterile and non-sterile soil microcosms of a sandy loam soil. Recently, an endophytic microorganism *Burkholderia cepacia* of yellow lupine was genetically manipulated to enhance toluene degradation [28]. *B. cepacia* was transformed with a plasmid from a related strain containing genes that mediate toluene degradation. After infection of lupine with the modified strain, the resulting plants were more tolerant to toluene and volatilized less of it through the leaves. This was the first example of genetic modification of an endophyte for rhizoremediation.

In coming years, novel genes important for bioremediation will be searched from both plants and bacteria [28]. Further research is also needed to investigate different feedback mechanisms that select and regulate the plant-microbe interactions and microbial activity in the rhizosphere [6]. The use of molecular techniques to identify microorganisms (and their catabolic genes) is however, the current key tool to study bioremediation as well as rhizosphere ecology [4, 29].

**Acknowledgement.** The authors acknowledge the effort of the Mrs Fowora Muinah Alahaja of Nigerian Institute of Medical Research (NIMR) for her help during the laboratory analyses of this research.

## REFERENCES

[1] Abed, R.M.M., Safi, N.M.D., Koster, J., de Beer, D., El-Nahhal, Y., Rullkolter, J., Garcia-Pichel, F., (2002): Microbial diversity of a heavily polluted microbial mat and its community changes following degradation of Petroleum compound. Applied and Environmental Microbiology, 68(4): 1674-1683.

[2] Amer, R.A., Nasier, M.M., El-Helow, E.R., (2008): Biodegradation of monocyclic aromatic hydrocarbon by a newly isolated *Pseudomonas* strain. Biotechnology, 7: 630-640.

[3] Anderson, T.A., Guthrie, E.A., Walton, B.T., (1993): Bioremediation in the rhizosphere. Environmental Science and Technology, 27: 2630-2636.

[4] Barea, J.M., Pozo, M.J., Azcon, R., Azcon-Aguilar, C., (2005): Microbial co-operation in the rhizosphere. Journal of Experimental Botany, 56: 1761-1778.

[5] Birmboim, H.C., Doly, J., (1979): A rapid alkaline extraction procedure for screening recombinant DNA. Nucleic Acid Research, 7: 1513-1523.

[6] Chaudhry, Q., Blom-Zandstra, M., Gupta, S., Jones, E.J., (2005): Utilizing the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment. Environmental Science and Pollution Research, 12: 34-48.

[7] Cheesbrough, M., (1994): District laboratory practice in Tropical countries. Part 2. Cambridge University press, 479 p.

[8] Ford, S., Olsen, B.H., (1988): Methods for detecting genetically engineered micro organisms in the environment. Advaces in Microbial Ecology, 10: 45-79.

[9] Gupta, A.K., Yunus, M., Pandey, P.K., (2003): Bioremediation: Ecotechnology for the present century. Enviro News Archives International Society of Environmental Botanist 9(2), India http://isebindia.com/01_04/03-04-2.html, accessed 1 April, 2012.

[10] Holt, J.G., (1984): The Shorter Berger's Manual of Determinative Bacteriology. Williwms Wilkiins, Baltimore, 356 p.

[11] Ifeadi, C.N., Nwankwo, J.N., (1989): Oil spill incident in Nigeria Petroleum Industry: A critical analysis. Napetcor, 8: 11-45.

[12] Jemmings, W.J., Muttman, G.G., (2003): The use of algae to treat waste water in the Berkeley pit. Journal of Phycology, 39(1): 25.

[13] Jyothi, K., Babu, K.S., Nancy, C.K., Kashyap, A., (2012): Identification and Isolation of Hydrocarbon Degrading Bacteria by Molecular Characterization. Helix (2): 105-111.

[14] Kuiper, I., Lagendijk, E.L., Bloemberg, G.O., Lugtenberg, B.J.J., (2004): Rhizoremediation. A beneficial plant-microbe interaction. Molecular Plant-Microbe Interactions, 17(1): 6-15.

[15] Lai, K.M., Serimshaw, M.D., Lester, J.N., (2002): Biotransformation and bio concentration of steroid estrogens by *Chlorella organs*. Applied and Environmental Microbiology, 68(2): 859-864.

[16] Lipthay, J.R, Barkay, T., Sørensen, S.J., (2001): Enhanced degradation of phenoxyacetic acid in soil by horizontal transfer of the *tfd*A gene encoding a 2,4-dichlorophenoxyacetic acid dioxygenase. FEMS Microbial Ecology, 35: 75-84.

[17] Mentzer, E., Ebere, D., (1996): Remediation of hydrocarbon contaminated sites. *Proceedings of the International Seminar on Petroleum Industry and the Nigerian Environment,* Nigerian National Petroleum Corporation (NNPC), pp. 51-59.

[18] Meyers, J.A., Sanchez, D., Elwell, L.P., Falkow, S., (1976): Simple agarose gel electrophoresis method for identification and characterization of plasmid deoxyribonucleic acid. Journal of Bacteriology, 127: 1529-1537.

[19] Mishra, S., Jyot, J., Kuhad, R.C., Lal, B., (2001): Evaluation of inoculum addition to stimulate *in situ* bioremediation of oily sludge-contaminated soil. Applied and Environmental Microbiology, 67(4): 1675-1681.

[20] Madueno, L., Coppotelli, B.M., Alvarez, H.M., Morelli, I.S., (2011): Isolation and Characterization of indigenous soil bacteria for bioaugumentation of PAH contaminated soil of semiarid Patagonia, Argentina. International Biodeterioration and Biodegradation, 65: 345-351.

[21] Nkwelang, G., Kamga, H.F., Nkeng, G.F., Antai, S.P., (2008): Studies on the Diversity, abundance and succession of hydrocarbon utilizing microorganisms in tropical soil polluted with oily sludge. African Journal of Biotechnology 7(8): 1075-1080.

[22] NNPC, (2004a): Use of sophisticated alarm systems will deter pipeline vandalization NNPC News – *Official Monthly Newsletter of the Nigerian National Petroleum Corporation*, 25(6): 3.

[23] NNPC, (2004b): PPMC loses N34 billion to pipeline vandalization. NNPC News – *Official Monthly Newsletter of the Nigerian National Petroleum Corporation,* 25(8): 1-2.

[24] Olagbende, O.T., Ede, G.O., Inyang, L.E.D., Gundlach, E.S., Page, D.S., (1999): Scientific and clean up response to the Idoho-Qua Iboe Terminal oil spill, Nigeria Environmental Technology, 20: 213-222.

[25] Ogbulie, T.E., Njoku, H.O., (2011): Effects of Different Bioremediation Processes on degeradation of n-Alkanes in crude oil contaminated Agricultural soil. International Journal of Tropical Agriculture and Food Systems, 5(2): 99-105.

[26] Page, C.A., Bonner, J.S., McDonald, T.J., Autenrieth, R.L., (2002): Behaviour of chemically dispersed oil in a wetland environment. Water Research, 36: 3821-3833.

[27] Pitchard, P.H., Mueller, J.G., Rogers, J.C., Kremer, F.U., Glaser, J.A., (1992): Oil spill bioremediation: experiences, lessons and results from the *Exxon Valdez* oil spill in Alaska. Biodegradation, 3: 315-335.

[28] Pilon-Smits, E., (2005): Phytoremediation. Annual Review of Plant Biology, 56: 15-39.

[29] Puhler, A., Arlat, M., Bercker, A., Gottfert, M., Morrissey, J.P., O'Gara, F., (2004): What can bacterial genome research teach us about bacterial-plant interactions? Current Opinions in Plant Biology, **7**: 137-147.

[30] Saadoun, I., (2002): Isolation and Characterization of bacteria from crude petroleum oil contaminated soil and their potential to degrade diesel fuel. Journal of Basic Microbiology, 42(6): 420-428.

[31] Sambrook, J., Fritsch, E.F., Maniatis, T., (1989): Molecular cloning: a laboratory manual, second ed., vol. 1, Cold Spring Harbor, New York, pp. 29-31.

[32] Senesi, S., Celandroni, F., Tavanti, A., Ghelardi, E., (2001): Molecular characterization and identification of *Bacillus clausii* strains marketed for use in oral bacteriotherapy. Applied and environmental Microbiology, 67(2): 834-839.

[33] Singh, B.D., (2004): Genomics and bioinformatics. Biotechnlogy. Kalyani publishers, New Delhi, India, pp. 583-613.

[34] Sinha, U., Srivastava, S., (1983): An introduction to bacteria. Viska publishing house, New Delhi, India, pp. 25-26.

[35] Sivasubramaniam, L., Seshadri, M., Shah, N., (2005): Bioremediation: Biotransformations of toxic wastes to harmless products. Special Article Reviews, Career Development. Technical Free full text article. Katangulathur, Chennai, India, http://www.pharmainfo.net/reviews/ bioremediation-biotransformation-toxic-wastes-harmless-products, accessed 1 April, 2012.

[36] Sutherlan, K.P., da Silva, I., (2003): Fungal Diversity and use in decomposition of environmental pollutants. Critical Reviews in Microbiology, 31: 41- 97.

[37] Watson, J.D., Hopkins, N.H., Roberts, J.W., Steitz, J.A., Weiner, A.M., (1988): Molecular Biology of the Genes. Cummings Publishers, California, pp. 313-338.

[38] Young, L.Y., Cerniglia, C.E., (1995): Microbial transformation and degradation of toxic organic chemicals. John Wiley and Sons Inc. USA, pp. 77-117.

[39] Yee, D.C., Maynard, J.A., Wood, T.K., (1998): Rhizoremediation of trichloroethylene by a recombinant root–colonizing *Pseudomonas fluorescens* strain expressing toluene ortho-monoxygenase constitutively. Applied and Environmental Microbiology, 64(1): 112-118.

Received: 27 April 2012
Accepted: 17 February 2013
Published Online: 21 February 2013
Analele Universităţii din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433