MOLECULAR AND PHYSIOLOGICAL STUDIES ON BACTERIAL DEGRADATION OF POLYNUCLEAR AROMATIC HYDROCARBONS

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A thesis submitted in partial fulfillment of the requirements for the award of the Degree of Doctor of Philosophy in Microbiology in the Department of Biological Sciences, School of Natural and Applied Sciences, College of Science and Technology, to the School of Post-Graduate Studies, Covenant University, Ota, Nigeria.

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DECLARATION

I, NWINYI, Obinna Chukwuemeka, hereby declare that this thesis is a product of my own unaided research work. It has not been submitted, either wholly or in part, to this or any other institution for the award of any degree, diploma, or certificate. All sources of scholarly information that were used in this thesis were duly acknowledged.

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NWINYI, Obinna Chukwuemeka

CERTIFICATION

We certify that this thesis entitled "**Molecular and Physiological Studies on Bacterial Degradation of Polynuclear Aromatic Hydrocarbons"** is an original research work carried out by NWINYI, Obinna Chukwuemeka (CUGP050150) of the Department of Biological Sciences, Covenant University, Ota, Nigeria under the supervision of Prof. O.O. Amund and Dr F.W. Picardal. We have examined and found the research work acceptable for the award of a degree of Doctor of Philosophy in Microbiology.

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DEDICATION

This work is dedicated to my Lord, my Saviour, Jesus Christ, the Son of the Ever Living, Almighty God to whom I owe completely my survival to this day and also the grace and inspiration to complete this work that was very daunting.

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GLOSSARIES OF TERMS

Amplification: a process of multiplying a fragment of DNA by subjecting it to different cycles of temperature using a thermal cycler in the presence of an enzyme called DNA polymerase.

Anthropogenic: sources of pollution that occur as a result of human activities.

Bioaugmentation: the direct introduction of microorganisms into a contaminated environment to enhance the cleanup of such an environment.

Biodegradation: the breakdown of a complex chemical by microorganisms, resulting in a minor loss of functional groups, fragmentation into larger constituents or complete breakdown to carbon dioxide and minerals.

Bioremediation: the use of biological agents to clean soils and waters polluted by substances hazardous to human health or the environment.

Teratogenicity: the extent to which a substance causes damage, reflected in the reproductive organs or abnormality of embryo and offspring.

Biodegradable: undergoing a biological transformation.

Persistent: not undergoing biodegradation in a certain environment.

Recalcitrant: resisting biodegradation in a wide variety of environments.

ABBREVIATIONS

ANT	Anthracene
AODC	Acridine orange direct counting method
BLAST	Basic local alignment search tool
BMB	Bead mill bioreactor
DHHS	Department of Health and Human Services
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulphoxide
GC-FID	Gas chromatography – flame ionization detector
GC-MS	Gas chromatography – mass spectrometry
HPLC	High pressure liquid chromatography
HMN	Heptamethylnonane
HMW	High molecular weight
IRT	Inhibitor removal technology
LMW	Low molecular weight
MS	Minimal salt
NAPLs	Non-Aqueous Phase Liquids
NRCC	National Research Council of Canada
PAH	Polycyclic aromatic hydrocarbons/polynuclear aromatic hydrocarbons
PCR	Polymerase chain reaction.
PYRQ	pyrene-4,5–dione
PYRdHD	cis-4,5-dihydroxypyrene
RNA	Ribonucleic acid
SDS	Sodium dodecyl sulphate
SSU	Small subunits
SIM	Selected ion monitoring
SOM	Sorbent organic matter
UDS	unscheduled DNA synthesis
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

PHYSICAL SYMBOLS

cm	centimeter
^{o}C	degree celsius
g	gram
g/cm^2	gram per centimetre square
g/mol	gram per mole
µg/ml	microgram per millilitre
ml	milliliter
mmHg	millimetre mercury
mM	millimolar
MHz	megahertz
ppm	parts per million
psi	pound per square inch
µL	microlitre
µM	micromolar
nm	nanometer
%	percentage
rpm	revolutions per minute
ng/m^3	nanograms per cubic meter
ng/l	nanograms per liter
ppb	parts per billion
s	seconds

ABSTRACT

McDoel Switchyard, an old industrial site in Bloomington, Indiana, US inundated with extensive levels of organic pollutants, was screened for the presence of Polynuclear aromatic hydrocarbons (PAHs) degrading bacteria. The incidence of the PAHs and other organic pollutants was evaluated using the US EPA methods 8270 and 3546. The technique of continual enrichment of selected PAHs- naphthalene, chrysene, pyrene, fluoranthene and anthracene yielded eleven unique bacterial isolates tentatively named OC-1, OC-2, OC-3, OC-4, FB-1, FB-2, FB-3, CB-1, CB-2; PB-1 and PB-2. Following the isolation of pure bacterial strains, each of the bacterial strains was screened against four different PAHs. Their degradative abilities on the PAHs were determined using a GC- FID HP 5890 series II gas chromatograph connected to an HP 3396 Series II Integrator. Epifluorescent microscopy was used to measure the cell numbers via a PAH-dependent growth study. For the molecular characterization of the bacterial strains, genomic DNA was extracted. The polymerase chain reaction was carried out using 8FM as forward primer, and as reverse primers 926R and 1387R to amplify the 16S rDNA. The amplified fragments of 16S rDNA were sequenced with an ABI 3730 sequence machine. The results were analyzed using the following bioinformatic tools: Stuffit Expander 2009, Chromas Lite 2.0, BioEdit,7.0.9, Codon Aligner and BLAST algorithm. The bacterial strains evolutionary relatedness were performed on the basis of 16S rDNA gene analysis by comparison of the obtained sequences data with known sequences in the GenBank.

From the environmental audit carried out at the site of study, two- to three-ring PAHs which were seven in number were recorded. From the values obtained, the 4-ring PAHs Surface Benzo (a) anthracene had the highest incidence of about 63,000 μg/kg with minimum and maximum values between 13.35-63000 μg/kg while the lowest recorded PAH was Sub surface dibenz(a,h) anthracene with values between 1-1249 μg/kg. Following the screening on the different PAHs, all the strains showed an ability to utilize a broad spectrum of the different PAHs as carbon and energy sources. They have also shown an ability to utilize the PAHs under anaerobic conditions. The biodegradation and PAH-dependent studies showed rapid exponential increase in cell numbers in some PAHs with about 99% disappearance of some of the PAHs at different volume biodegradation rates. The 16S rDNA analysis classified the organisms (OC-1, OC-2, OC-3 and OC-4) as species of uncultured bacterium OC-1, Bacterium OC-2 with about 99% homology to the type strain of *Pseudomonas putida*, strain OC-3 as *Pseudomonas* sp. strain OC3 and OC-4 as

Pseudomonas putida strain OC4. Furthermore, strains of FB-1 FB-2 and FB-3 were identified as *Lysinibacillus* sp. FB1, Bacterium FB2 with 99% homology to the type strain of *Paenibacillus* sp. and *Lysinibacillus fusiformis* strain FB3 respectively. Strains CB-1 and CB-2 were identified as Bacterium CB1 with 100% homology to the type strain of an uncultured bacterium and *Stenotrophomonas maltophila* strain CB2 respectively. Strains PB-1 and PB-2 were identified as *Pseudomonas plecoglossicida* strain PB1 and *Pseudomonas* sp. strain PB2. The obtained 16S rDNA gene sequences have been deposited at the GenBank with the accession numbers issued.