BACTERIAL DEGRADATION OF CARBAZOLE AND MICROBIAL DIVERSITY OF HYDROCARBON-CONTAMINATED SOILS IN A TROPICAL ENVIRONMENT

By

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DEDICATION

This work is dedicated to my beloved wife and children and to my mentors (Academic and Religious) who gave me a clear insight about life and taught me the key ingredients to academic success.

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GLOSSARY OF TERMS

Amplification	The process by which copies of a particular fragment of DNA is multiplied by subjecting it to cycles of temperature changes in a machine called thermal cycler in the presence of the enzyme called DNA polymerase.
Angular Dioxygenation	A novel mode of dioxygenation with high regioselectivity and specificity for the angular position. In this atypical oxidative attack, the carbon attached to the carbonyl group in 9-fluorenone or to heteroatoms in other compounds, and the adjacent carbon in the aromatic ring are both oxidized.
Anthropogenic	Pollutants produced and released into the environment as a result of human activities such as fossil fuel burning, agricultural practices and so on.
Bioaugmentation	Addition of pregrown microbial cultures, either genetically modified or not, into contaminated compartments to enhance cleanup process.
Biodegradation	The breakdown of a complex organic substance through biological processes that can result in minor loss of functional groups, fragmentation into smaller constituents, or complete breakdown to carbon dioxide and minerals.
Bioremediation	A spontaneous or managed process in which biological (especially microbiological) catalysis acts on pollutants to either mineralize it to carbon dioxide and water or transform it to simpler organic compounds.
Clone Library	A large collection of clones (obtained through amplification of a target gene from a metagenomic DNA and sequencing of the PCR products) harbouring amplicons with different sequences.
Colony PCR	A PCR technique for screening bacterial colonies directly to check for correct DNA vector constructs.
Dioxygenase	An enzyme that catalyses the incorporation of both atoms of molecular oxygen to aromatic ring leading to hydroxylation of the ring; another class of dioxygenases cleave such rings.

Enrichment Technique	A primary isolation technique, where a medium with specific and known quantities that favour the growth of a particular microorganism of interest and inhibit the growth of others is designed.
***Meta* Cleavage**	The use of a non-haem Fe(II) by extradiol enzymes to cleave aromatic ring between a hydroxylated carbon and an adjacent non-hydroxylated carbon.
***Ortho* Cleavage**	The use of a non-haem Fe(II) by intradiol enzymes to cleave aromatic ring between two hydroxylated carbon atoms in the ring.

ABBREVIATIONS

CARDO	Carbazole 1,9a-dioxygenase
CFMM	Carbon-Free mineral medium
Cfu/mL	Colony forming unit per mililitre
CTAB	Cetyltrimethyl ammonium bromide
DMSO	Dimethyl sulfoxide
EDTA	Ethylene diamine tetraacetic acid
FID	Flame ionization detector
GC	Gas chromatography
HPLC	High performance liquid chromatography
HUB	Hydrocarbon utilizing bacteria
HUF	Hydrocarbon utilizing fungi
IPTG	Isopropyl-β-D-thiogalactopyranoside
LB	Luria Bertani
MS	Mass spectrometry
MSTFA	2,2,2-trifluoro-N-methyl-N-trimethylsilyl-acetamide
NSC	Native soil + carbazole
NSC1	Native soil + carbazole + strain SL1
NSC4	Native soil + carbazole + strain SL4
NSC6	Native soil + carbazole + strain SL6

PCR	Polymerase chain reaction
PTAH	m-(trifluoromethyl)-phenyltrimethylammonium hydroxide
SDS	Sodium dodecyl sulphate
SSC	Sterilized soil + carbazole
SSC1	Sterilized soil + carbazole + strain SL1
SSC146	Sterilized soil + carbazole + strains SLI, SL4, SL6.
SSC4	Sterilized soil + carbazole + strain SL4
SSC6	Sterilized soil + carbazole + strain SL6
TA	Total actinomycetes
TAE buffer	Tris-acetate/EDTA buffer
TBE buffer	Tris-borate/EDTA buffer
TE buffer	Tris-EDTA buffer
TEG buffer	Tris-EDTA/glucose buffer
THB	Total heterotrophic bacteria
THF	Total heterotrophic fungi
TNF	Total nitrogen fixers
UV/Vis	Ultraviolet/Visible
X-Gal	5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside

ABSTRACT

Carbazole, an N-heterocyclic aromatic hydrocarbon is of environmental concern due to its persistence, recalcitrance, mutagenic and toxic activities. Degradation of carbazole has been reported since early 1990s, to lead to production of dead-end metabolites and hydroxylated carbazoles, which are released into the environment. However, angular dioxygenation of carbazole reported in very few bacteria genera, with hydroxylation at C1 and C9a carbons produces anthranilic acid and catechol as major metabolites, which are completely mineralized. Four bacterial strains with extensive degradation abilities on carbazole were isolated from three hydrocarbon-contaminated soils (Abandoned Coal Power Plant, ACPP; Mechanic Workshop, MWO and NEPA Substation, UNILAG, NESU) in Lagos, Nigeria. Physicochemical analyses of the soil samples indicate gross pollution of the soils with a high hydrocarbon content (157 g/kg) and presence of heavy metals (lead, nickel, cadmium). Phylogenetic analysis of the four strains indicated that they were *Achromobacter* sp. strain SL1 (**AB646575.2**), *Pseudomonas* sp. strain SL4 (**AB646578.2**), *Microbacterium esteraromaticum* strain SL6 (**AB646579.2**) and *Stenotrophomonas maltophilia* strain B_A (**AB646574**). The rate of degradation of carbazole by the four isolates, after 30 days of incubation, were 0.057, 0.062, 0.036 and 0.050 mg l^{-1} h^{-1} for strains SL1, SL4, SL6 and B_A, respectively. Gas chromatographic analyses of residual carbazole, after 30 days of incubation, revealed that 81.3%, 85%, 64.4% and 76% of 50 ppm carbazole were degraded by strains SLI, SL4, SL6 and B_A, respectively. GC-MS and HPLC analyses of the extracts from the growing and resting cells of strains SL1, SL4 and SL6 cultured on carbazole revealed anthranilic acid and catechol, which were not detected in strain B_A under the same conditions. The three strains (SL1, SL4, SL6) degrade catechol via the *ortho* pathway producing *cis cis* muconic acid as established by UV-Vis spectroscopy. Microcosm experiments with sterile and native soils amended with 100 ppm carbazole indicated carbazole removal rates of 66.96%-82.16% in sterile soils and 19.19%-91.64% in native soils by the test organisms. All four strains utilized a wide range of polycyclic and heterocyclic aromatic hydrocarbons. Clone library analysis of 16S rRNA recovered four hundred and thirty seven clones from MWO soil that cut across thirteen different bacterial phyla as revealed by RDP-II and NCBI. The representative bacteria phyla identified from MWO soil, using clone library analysis, were *Proteobacteria, Bacteroidetes, Chloroflexi, Acidobacteria, Firmicutes, Actinobacteria, Verrucomicrobia, Planctomycetes, Chlorobi, Spirochaetes, Chlamydiae*, TM7 and OD1. The clone library coverage indicates 42% of the library is covered at the species delineation with Shannon index of 5.59. This study has established carbazole angular dioxygenation and mineralization by isolates from a tropical environment. It also revealed the presence of novel bacterial genera contributing to natural attenuation of hydrocarbon pollutants in soil through clone library analysis.