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Physiological behavior of newly isolated Bacterium CB1 and *Stenotrophomonas maltophilia* strain CB2 on Chrysene, Pyrene, Naphthalene and Fluoranthene

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Abstract

The biodegradability of some polycyclic aromatic hydrocarbons were studied in liquid culture media using bacterial strains (Bacterium CB1 and Stenotrophomonas maltophilia strain CB2) isolated from a former industrial site contaminated with organic and inorganic contaminants. The enrichment experiment was done with chrysene. The partial 16S rRNA gene analyses of Bacterium CB-1 possessed 100% similarity to an uncultured bacterium clone nbt020a while Stenotrophomonas maltophilia strain CB2 had 99% similarity to Paenibacillus sp. Y412MC10. Bacterium CB1 and Stenotrophomonas maltophilia strain CB2 degraded naphthalene between the range of 27% and 42%, chrysene 47% and 12%, fluoranthene 5% and 16%, pyrene 12% and 17% respectively. The strains utilized the test compounds as sole source of carbon and energy. As anticipated, the controls (abiotic and killed) losses were insignificant. The residual PAH obtained in some cases correlated to an increase in cell number indicating that our strains were responsible for the degradation.

Keywords: 16S r RNA gene, biodegradation, PAHs, Bacterium CB1, *Stenotrophomonas maltophilia* strain CB2.

1. Introduction

As a result of the massive industrial revolution, many former industrial sites have been extensively contaminated with polycyclic aromatic hydrocarbons (PAHs). The United States Environment Protection Agency (1) reported that contaminated lands abound around the world. It has been estimated that contaminated site treatment costs may approach 1.7 trillion dollars over the next 30 years. Consequently, effective strategic development plans towards reclaiming of former contaminated industrial sites is one of the modern challenges facing developed and developed societies around the world (2). PAHs are non-polar organic compounds which are persistent in the environment due to their negative resonance energy, high melting and boiling points; low water solubility and vapor pressures (3). The number of aromatic rings present influences the environment fate of PAHs. PAHs impact negatively upon the soil functioning and human health due to carcinogenic and mutagenic potential, thus; making it a serious concern to the scientific communities (4-6). It has been noted, that high molecular weight compounds endure relatively unaltered for years than the low molecular weight. For instance,

the half of life a three ring PAH (phenanthrene) could last for 16-126 days while for a five ring PAH (benzo (a) pyrene is 4years (7-8). Traczweska and Zeng et al., demonstrated that low molecular weight PAHs (LMW PAHs) could co- exist with high molecular weight PAHs in the environment and as such pose serious risk to human health and the environment (9-10). In addition, some of the metabolites of LMW PAHs arising from incomplete degradation processes have serious toxic and mutagenic properties. In the environment, the main sources of PAHs are mainly anthropogenic. These include but not limited to the following activities: pyrolysis, petroleum refining and wood treatment processes. Natural sources of PAHs include volcanic activity, plant and bacterial reactions. PAHs are ubiquitous having impacts upon sediments, ground waters and the atmosphere. PAH contamination could impair the health of living organisms and ecological systems. Reports on the effect of PAH on mammals, birds, invertebrates, plants, amphibians, fish, and humans have been documented. For instance in humans and mammals, exposure could occur by inhalation, dermal contact, and ingestion (11). In humans, PAHs tend to be stored in kidneys, liver, and adipose tissues. Smaller amounts could be stored in the spleen, adrenal glands, and ovaries. Plants absorb PAHs from soil, especially low molecular weight PAHs, and translocate them to above-ground tissues. Fishes when exposed to PAH contamination exhibit fin erosion, liver abnormalities, cataracts, and immune system impairments that lead to increased susceptibility to disease (11-12). It has been accepted that microorganisms degrade environmental pollutants in various matrices and environments. Thus, the disappearance of PAHs from soils is mediated by biotic and abiotic processes. These processes include: volatilization, photodecomposition, leaching and microbial biodegradation (13). Photodecomposition, leaching and volatilization are not considered as major pathways for removal of sorbed PAHs from the soil environment rather biological transformation (4). Despite the chemical stability and hydrophobicity of PAHs, several PAH compounds are amenable to microbial degradation especially those of two or three benzene rings. Microorganisms including bacteria, fungi, and algae have been isolated and identified as being capable of utilizing PAHs as carbon and energy sources, reducing PAH toxicity and co-metabolic substrates (14-15). The influence of number of ring on PAHs greatly controls the variety of bacteria that could degrade PAH (16). PAHs utilization by bacteria occurs predominantly by aerobic conditions but relatively in anaerobic conditions (17-19). For effective resolution of PAH contamination issue at any particular site, there is need for strategic development of cost efficient biologically based treatments that exploit the degradative ability of microorganisms that are associated with the soil environment. In addition, a greater understanding of the processes involved, and those that limit the degradation of high molecular weight PAHs may be required. In view of the fact that PAH clean-up of contaminated sites are environment specific, local microorganisms must be isolated and studied for the benefits of bioremediation. Thus, preliminary laboratory studies which involve physiological characterization and determination of degradation efficiency are obligatory towards increasing the successes of field trials in developing and improving bioremediation technology. In this communication report, we report the isolation of natural occurring isolate capable of aerobic growth and sole use of naphthalene, chrysene and partial utilization of pyrene and fluoranthene as carbon and energy source.

2. Materials and Methods

Soil Sampling

Soil samples were taken from the PAH- contaminated sites in McDoel switchyard, Bloomington, Indiana. The total solid dry matter was 79.05% and moisture content was 20.95%. The soil samples were collected via a two stage sampling. The soil samples were placed in sterile jars and transported back to the laboratory at ambient temperatures.

Chemicals

Naphthalene, fluoranthene, pyrene and chrysene of analytical grades were obtained from Sigma Aldrich Corp. (St Louis, MO. USA). Sodium benzoate (99+ % purity), 2,2,4,4,6,8,8-heptamethylnonane (HMN), and all other organic solvents were obtained from Fisher scientific co.(Springfield, NJ,USA). Hexane, a high purity solvent for GC- chromatograph was obtained from EMD chemicals Inc. Merck. The PAH analytical standards were procured from AccustandardInc (New Haven, CT 06513).

Stock solutions and media

Chloride free minimal salts (MS) medium as described by (20-23) was used for the enrichment and degradation experiments. The chloride free media consisted of (g) 0.5 (NH4)2SO4, 0.1 MgSO4•7H2O, 0.076 Ca(NO3)2.4H2O and 1.0 mL each of trace metal and vitamin solutions per liter of 40 mM phosphate buffer (pH 7.25). The naphthalene stock solution were prepared in 2,2,4,4,6,8,8-heptamethylnonane, a non-degradable carrier to provide an initial concentration of ca. 117 ppm. This concentration represents the total mass in both the aqueous and HMN phases, divided by the aqueous volume. The chrysene, fluoranthene and pyrene stock solutions were prepared by dissolving the weighted test compounds in acetone respectively. The different stock solution of the test compounds (fluoranthene, chrysene and pyrene) were added into the different balch tubes using a Hamilton gas-tight syringe in 250 µL aliquots. This was to provide the test compound concentration of ca. 92 ppm for fluoranthene, ca. 62 ppm for chrysene and pyrene ca. 94 ppm in the final medium. Solid MS medium was made by the addition of 1.8% Bacto-agar (Difco Laboratories, Detroit, MI, USA). The naphthalene solution was added with Hamilton gas tight syringe 250 µL aliquots into the balch tubes to provide test compound concentration of 117 ppm in the final medium. The MS medium was amended with the test compound to achieve an experiment dependent concentration. Prior investigations were carried out using MS medium amended with HMN as the sole carbon and energy sources to determine that HMN did not serve as growth substrate. The cultures were incubated at ambient temperature on a shaker table to allow for slow mass transfer of the naphthalene into the aqueous phase.

Isolation of chrysene degrading bacteria

Conventional enrichment was carried out by adding 5.0 g of the soil samples into 160 mL serum bottles mixed with 30 mL of sterile Minimal salt (MS) medium. As a result of PAH sorption and limited bioavailability, PAH-chrysene was amended in enrichment bottles as complement for carbon and energy source. All the slurry bioreactors were crimp-sealed with teflon-coated, butyl rubber stoppers to prevent losses due to volatilization and/or sorption. All the slurry bioreactors were set up in triplicates. These were incubated horizontally on an orbital shaker table 200 rpm at ambient temperature. Re-aeration of the headspace of the bioreactors were done biweekly and periodic transfers made by using about 15% inoculums into new MS medium supplemented with chrysene. The procedure was repeated for seven consecutive times. Pure cultures from chrysene-enriched media were isolated by directly plating aliquots (0.2 mL) of highly-enriched cultures onto MS agar (24).

Assay for solid hydrocarbon degradation

The MS agar medium was supplemented with chrysene to maintain selective pressure. The chrysene was added to the medium using the spray plate technique as described by Kiyohara et al., (25). Immediately after spread-plating the 0.2 mL aliquot of enrichment culture, an ethereal solution of chrysene was uniformly sprayed onto the surface of the agar. The plates were sealed with parafilm film and incubated for 1 week at 30°C. Chrysene-degrading microorganisms were identified by cleared zones around an individual colony. The colonies were purified on MS agar sprayed with chrysene and sustained on solid MS plates containing 2.5 mM chrysene.

Phylogenetic analysis

The 16S rRNA from the genomic DNA isolates of overnight cultures growing on 2.5mM benzoate were isolated using an UltraClean Microbial DNA Isolation kit (Mo Bio Laboratories, Solana Beach CA,USA). The genomic DNA was amplified using the set of three eubacterial PCR primers; 8F (AGAGTTTGATCMTGGTCAG) and the reverse primers 926R (CCGTCAATTCCTTTRAGTTT) and 1387R (GGGCGGWGTGTACAAGGC). The PCR products were purified and sent to be sequenced using an Applied Biosystems 3730 automated sequencing system (Applied Biosystems, Inc., Foster City, CA, USA). The resultant sequences were edited and aligned using CodonCode Aligner v. 2.0.6 (CodonCode Corporation, Dedham, MA, USA). Sequences were submitted to the GenBank database to align with the deposited sequences in GenBank database using the BLAST. The accession numbers are JN983821 for CB-1 and JN 624746 for CB-2.

Growth on different carbon and energy sources

The utilization of naphthalene, fluoranthene, pyrene and chrysene as sole source of carbon and energy was carried out in MS medium supplemented with each PAH tests compound as sole carbon source. These experiments were conducted in crimp-sealed tubes. For quality assurance and controls, the tubes used for this study were baked in muffle furnace at 500°C to remove organic contaminants. In addition, all the stock solutions were aseptically prepared before use. Each tube was added with 10 ml of MS medium, the tested PAH, inoculum, and approximately 15 ml air headspace to maintain aerobic conditions for the growth and degradation studies. The different tubes were supplemented with different PAHs respectively. For naphthalene, it was added from an HMN stock solution at a concentration of ca. 117 ppm and inoculated with 105 cells/ml of phosphate buffer (pH 7.25) washed cells pre-grown in 2.5 mM benzoate. Fluoranthene, chrysene and pyrene were added from the stock solution into the balch tubes to provide test compound concentration ca. 92 ppm for fluoranthene, ca. 62 ppm for chrysene, and pyrene ca. 94 ppm in the final medium. The tubes were incubated horizontally on a shaker table at (120 rev/ min) at ambient temperature. The growth fluxes were monitored by counting the cells numbers using replicate tubes via epifluorescence microscopic examination. The cells were stained with acridine orange stain after fixation with 50 µl of glutraldehyde. Visual examinations in concurrence with periodic GC analyses to measure the test compound disappearance was done. For statistical evaluation of the cell count, at least 10 microscopic fields were randomly selected and a minimum of 1000 cells counted. The data are presented as the mean cell numbers ± the SEM. In this study, growth was regarded as positive when there is an increase in turbidity greater than the killed or abiotic control.

Degradation of PAH compounds – naphthalene, fluoranthene, pyrene and chrysene experiments.

The degradation study of other PAHs including naphthalene, fluoranthene, pyrene and chrysene were likewise conducted in the Balch tubes. The tubes were inoculated with the respective bacterial cultures crimp sealed and incubated horizontally on the shaker table at ambient temperature. The degradation reactions were stopped after 14 days for naphthalene while experiments with fluoranthene, pyrene and chrysene were stopped after 21 days using hexane. The hexane and aqueous extracts were separated and collected for further analysis. The hexane extracts were stored in target vials with a headspace of 1mL and crimp sealed using an 11mm Teflon rubber stopper and stored at 4°C prior to analysis.

Analytical methods

Gas Chromatography-Flame Ionization Detector and statistical analysis

PAHs extracts were analyzed on an HP 5890 Series II gas chromatography GC (Hewlett Packard Co., Palo Alto, CA, USA) fitted with an HP 3396 series II integrator and equipped with a flame ionization detector (FID). Hexane extracts (5 µL injection volume) were injected through a 30 m HP-5 megabore fused-silica capillary column (J & W Scientific,

Folsom, CA, USA; 0.32 mm id, 0.25 μm film thickness). The GC utilized Helium (He) as the carrier gas and was programmed at an initial temperature of 50°C; this was held for 5 min then ramped at 30°C/min to 180°C for 2 min, then ramped to 300°C at 40°C/min for 4 min. The analytical standards of the PAHs were prepared in hexane. Typical coefficients of correlation for standard curves were 0.98-0.99.

Statistical tests were performed using the Prism 4.0 computer software programme (Graph Pad Software, San Diego, CA, USA).

3. Results and discussion

Isolationof chrysene- degrading bacteria.

Ten different discrete colonies were selected from the MS agar plates upon initial enrichment on chrysene. The colonies were later screened down to two following growth on MS salicyclic acid, MS benzoate and microscopic view. The colony morphology of the isolates observed under the fluorescent microscope showed non-spore-forming uniform bacillary rods. The colonies had a smooth and glistening margin. The 16S rRNA sequencing of CB-1 had 100% identity to Uncultured bacterium clone nbt02a0 consequently, it was named as Bacterium CB-1 with base pair length of 861. The CB-2 was named *Stenotrophomonas maltophilia* strain CB2 (base pair length of 1207) with 99% identity as Paenibacillus sp. Y412MC10 under GenBank database.

Chrysene degradation assay and Growth of CB-1 and CB-2 on chrysene

Strains CB-1 and CB-2 potentials to degrade chrysene were evaluated by comparing the GC peak areas of the initial day time (0) and the final time (t). Strain CB-1 and CB-2 were able to utilize chrysene to some extent. Within the incubation period of 21 days Fig. 1a, our strains CB-1 and CB-2 utilized 47 and 12% of the chrysene respectively. The mean biodegradation rate of strain CB-1 was 1.519 ± 0.069 mg l^{-1} day^{-1}. The strain CB-2 mean biodegradation rate was 0.386 ± 0.209 mg l^{-1} day^{-1}. There was increase in the cell numbers that correlates to the reduction in the chrysene. The growth pattern of the strains on chrysene is as illustrated in (Fig 1b). In the growth curve, strain CB-1 showed no lag period. Evidently, the cell numbers continued to increase until after 14days following which the organisms entered the decline phase. Contrary to the growth behavior of strain CB-1, strain CB-2 showed an initial decline in the cell numbers this may possibly be due to synthesis of the required enzymes for chrysene degradation and possible evolution of different metabolic pathway. Obviously, the two bacterial species exhibited possible pathways for chrysene degradation.

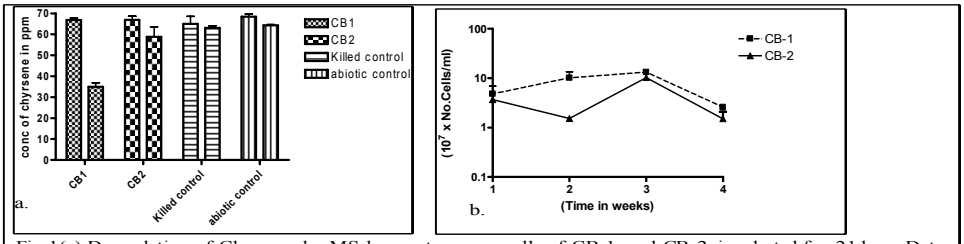

Fig 1(a).Degradation of Chrysene by MS-benzoate grown cells of CB-1, and CB-2, incubated for 21days. Data represent the mean and standard deviation of triplicate determination of initial and final concentration respectively. The error bars (Stand. Dev.) were due to differential response of cells in triplicate tubes. (b) Chrysene dependent growth and cell numbers distribution of Strains CB-1 and CB-2, in Chrysene incubated for 21 days. Data represent the mean of replicates tubes for initial time (0) cell density represented as (1) and final time (21 days) represented as (4) respectively. The x-axis value range was chosen as such to allow for even spread of the growth curve. The error bars (Stand. Dev.) were due to differential response of cells in triplicate tubes.

Fluoranthene degradation assay and Growth of CB-1 and CB-2 on Fluoranthene

The capacity of strains CB-1 and CB-2 to degrade fluoranthene a tetracyclic aromatic hydrocarbon was studied by using washed, benzoate – grown cells. The fluoranthene served as sole carbon and energy sources. After an incubation period of 21 days, the net percentage reductions in the total fluoranthene content were 5 and 16 % respectively for strain CB-1 and CB-2 (Fig. 2a). The mean biodegradation rate of strain CB-1 on fluoranthene was 0.246 ± 0.064 mg l^{-1} day^{-1} while strain CB-2 consumed fluoranthene at the rate of 0.859 ± 0.306 mg l^{-1} day^{-1}. Although, both strains grew on the fluoranthene, but the growth proceeded without a noticeable lag period within the first week of incubation (Fig. 2b). Subsequently, the strains showed a decline in cell numbers after the second week this continued until the third week. From the third week, an increase was noticed in the growth profile, however; the flux changes were not significant when compared to the logarithmic growth experienced at the onset of this investigation. Thus, it might be proposed that the strains showed similar growth pattern when exposed to fluoranthene in liquid culture medium.

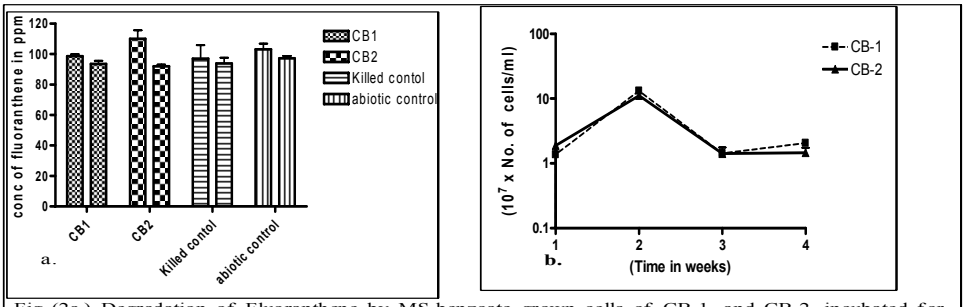

Fig (2a.) Degradation of Fluoranthene by MS-benzoate grown cells of CB-1, and CB-2, incubated for 21days. Data represent the mean and standard deviation of triplicate determination of initial and final concentration respectively. The error bars (Stand. Dev.) were due to differential response of cells in triplicate tubes. (b) Fluoranthene dependent growth and cell numbers distribution of Strains CB-1 and CB-2, in Fluoranthene incubated for 21 days. Data represent the mean of replicates tubes for initial time (0) cell density represented as (1) and final time (21 days) represented as (4) respectively. The x-axis value range was chosen as such to allow for even spread of the growth curve. The error bars (Stand. Dev.) were due to differential response of cells in triplicate tubes.

Naphthalene degradation assay and Growth of CB-1 and CB-2 on naphthalene

Elevated competence was observed for naphthalene utilization by strain CB-2 when compared to strain CB-1 (Fig. 3a). The MS-benzoate washed cells of strains CB-2 showed an increase in cell numbers from 7.8 x 10^6 - 15.14 x 10^7after 14 days of incubation, with corresponding decrease in the net percentage of the naphthalene to 42%. Strain CB-1 had a reduction in the cell numbers from 6.9 x 10^6 to 2.6 x 10^6 with reduction of 27% of naphthalene (Fig. 3a). From the growth profile study in (Fig. 3b), the strains exhibited similar growth pattern, nonetheless after one week CB-1 showed a decline in the cell number that lasted until the end of this study. On the contrary, strain CB-2 had a continuous increase in the cell numbers that continued until the end of the experiment. The initial concentration of naphthalene at time zero was (~117 ppm) and the observed final values obtained for strain CB-2 was ~ 68 ppm while for CB-1 was 90 ppm. The mean biodegradation rate of strain CB-1 on naphthalene was 2.44 ± 0.812 mg l^{-1} day^{-1}, while strain CB-2 mean biodegradation rate of 3.49 ± 0.279 mg l^{-1} day^{-1}

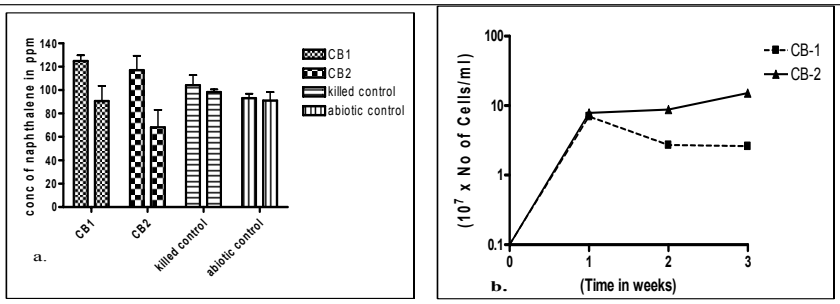

Fig.(3a).Degradation of Naphthalene by MS-benzoate grown cells of CB-1, and CB-2, incubated for 14 days. Data represent the mean and standard deviation of triplicate determination of initial and final concentration respectively. The error bars (Stand. Dev.) were due to differential response of cells in triplicate tubes. (b) Naphthalene dependent growth and cell numbers distribution of Strains CB-1 and CB-2, in Naphthalene incubated for 14 days. Data represent the mean of replicates tubes for initial time (0) cell density represented as (1) and final time (14 days) represented as (3) respectively. The x-axis value range was chosen as such to allow for even spread of the growth curve. The error bars (Stand. Dev.) were due to differential response of cells in triplicate tubes.

Pyrene degradation assay and Growth of CB-1 and CB-2 on pyrene

The MS-benzoate washed cells of strainsCB-1 and CB- showed different growth patterns in the degradation of pyrene. In (Fig. 4b), strain CB-1 showed a continuous but slow increase until after the third week. The net percentage reductions in the total content of pyrene were 12 and 17% respectively for strain CB-1 and CB-2 (Fig. 4a). The mean biodegradation rate of strain CB-1 on pyrene was 0.534 ± 0.127 mg l^{-1} day^{-1} . Strain CB-2 exhibited a decline in cell numbers after the 1st week of incubation then entered into a stationary phase until the end of the experiment (Fig. 4b). The mean biodegradation rate of strain CB-2 on pyrene was 0.836 ± 0.050 mg l^{-1} day^{-1}

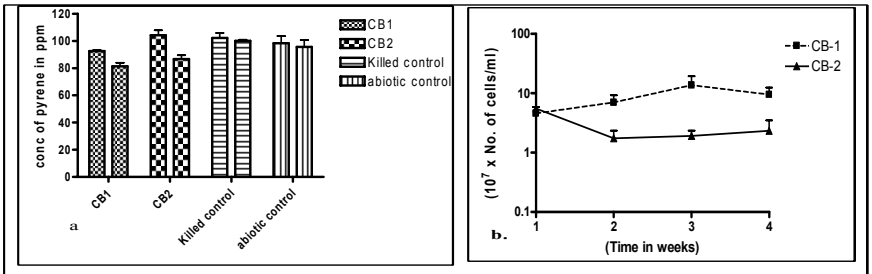

Fig. (4a).Degradation of Pyrene by MS-benzoate grown cells of CB-1, and CB-2, incubated for 21 days. Data represent the mean and standard deviation of triplicate determination of initial and final concentration respectively. The error bars (Stand. Dev.) were due to differential response of cells in triplicate tubes. (b) Pyrene dependent growth and cell numbers distribution of Strains CB-1 and CB-2, in pyrene incubated for 21 days. Data represent the mean of replicates tubes for initial time (0) cell density represented as (1) and final time (21 days) represented as (4) respectively. The x-axis value range was chosen as such to allow for even spread of the growth curve. The error bars (Stand. Dev.) were due to differential response of cells in triplicate tubes.

PAH bioremediation, especially in contaminated matrices is judged to be a complex phenomenon partly due to issues of toxicity, hydrophobic nature of contaminants to soil composition and heterogeneity of microbial environment. Conversely, microbial communities especially the bacteria species holds a considerable potential for the remediation of PAH contaminated sites around the world (26). Several PAH bacteria species have been studied with their primary metabolic pathways elucidated (27). In spite of these achievements, it is expedient to determine new bacterial systems required for effective cleanup of catastrophic contaminated sites around the world. In this study, we report for the first time chrysene enriched bacteria species isolated from former industrial site in McDoel switchyard,

Bloomington, Indiana. In many occasions, microbial strains are capable of degrading only specific hydrocarbons compounds. In this study, we were able to isolate indigenous bacteria species with unique capabilities in utilizing broad range of PAH as carbon and energy sources. For effective application of remediation strategies using bacteria species, it is crucial that we understand the behavioral responses of these bacterial species in response to cases where a single PAH serves as sole carbon and energy sources. This we have achieved by isolating and investigating our bacteria species capabilities from a site inundated both organic and inorganic pollutants. Mishra et al.,demonstrated the importance of using indigenous microorganisms in degrading PAHs (28). They reiterated its importance by revealing that microorganisms have capacity to the degrade PAH and its constituents and as well express a higher tolerance to PAH toxicity that may decimate the allochthonous species. Normally, the indigenous bacteria communities that have been exposed to various pollutants may favor evolution of species with the necessary survival plasmids. Data from the degradation of chrysene further illustrate notable catabolic properties exhibited by strains CB-1 and CB-2. Strain CB-1 degraded 47% chrysene while CB-2 utilized 12%. This difference may be that strain CB-1 had more oxidative ability on chrysene where it used oxygen as an electron acceptor to break down chrysene, a high molecular weight PAH that consist of four fused benzene ring. Willson, reported about none of their organisms in pure culture were able to degrade chrysene as a sole source of energy and carbon (29). Strain CB-1 and CB-2 utilized fluoranthene but minimally. Strain CB-2 degraded about 16% of fluoranthene over strain CB-1 that could degrade (5%). Conversely, each of the strains exhibited a decline in their growth profile after the 7^{th} day of incubation. It may be due to membrane toxicity and possibly non – possession of the relevant enzymes that could break down the hydrophobic nature and stereochemistry of fluoranthene. Zeng and co-workers showed that different results could be obtained especially for fluoranthene degradation. In their findings, they reported that fluoranthene could be readily utilized by their organisms when sprayed on a solid media than in a liquid culture (10). This possibly may provide an explanation for the minimal degradation result obtained when the catabolic versatility of our strains CB-1 and CB-2 were assessed in fluoranthene. According to Nwanna et al., there are possibilities of lipophilic hydrocarbon accumulating around the peptidoglycan thus affecting the structural and functional properties of these membranes (30). In naphthalene degradation assay, (a 2 ring PAH), the strains were able to utilize it as a sole source of carbon and energy. Strain CB-2 degraded naphthalene than strain CB-1 as shown in (Fig.3a) where percentage utilization of CB-2 > CB-1. Our obtained data showed similar trend with organisms obtained by (31), however the organisms obtained by Pepi and co-workers showed more catabolic efficiency than Bacterium CB1 and *Stenotrophomonasmaltophilia* strain CB2. This could be that our organisms may have utilized a different catabolic pathway during the attack on naphthalene. From the range of PAHs that our organisms showed potentials to degrade, there possibilities that our organisms may have acquired a broad substrate range having been domiciled in a PAH- contaminated environment over decades. Several workers have also shown that PAH-degrading bacterial strains may act on chemical analogues of their growth substrates producing fortuitous oxidative reactions. For instance, the fluorine degrading strain *Bulkholderiacepacia* F297 was shown to grow on creosote-PAHs utilizing some of the components as a carbon source while transforming others to a number of aromatic ketones and carboxylic acids that accumulated in the medium (32). Expectedly, these processes occur in soils naturally (33). During the naphthalene degradation assay, we experienced a yellow color in the liquid culture tubes; these may possibly suggest the strains utilized the meta-cleavage pathway in the degradation of naphthalene. In the biodegradation and growth fluxes on pyrene, our strains were able to use minimal concentration of pyrene. This correlated to the pseudo-logarithmic increase in their cell numbers that we experienced.

This pseudo-logarithmic phenomenon may be because of the hydrophobic nature ofpyrene on the bilayer of the cell membranes of our strains. Of particular significance in this physiological study is *Stenotrophomonas maltophilia* strain CB2. This organism's family has been known to be associated with nosocomial infections. The ability of this strain to utilize the selected PAHs was an interesting phenomenon. This further validates the various reports made by several workers about *Stenotrophomonas maltophilia* potential to utilize xenobiotics (34-35). From these findings, it is topical to note that bacterial cells could detect temporal changes in the concentrations of specific chemicals and respond behaviorally to these changes. As a result, the bacterial cells adapt to new concentration by possible plasmid mediation and evolution of catabolic versatility using the adequate enzyme activity.

4. Conclusion

In conclusion, we have presented evidence of the potential of Bacterium CB1 and *Stenotrophomonas maltophila* strain CB2 to utilize the selected PAHs. Taken together, the growth and degradation assay results, our strains may be excellent candidate to exhibit the slow rate of some bacterial species in utilization of organic pollutants. Also, there are possibilities of prospects in the application of these strains in the bioremediation of industrial sites contaminated with organic pollutant.

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