

Polycyclic Aromatic Hydrocarbons and Their Importance in Bioremediation- A Review

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Abstract - Polycyclic aromatic hydrocarbons are carcinogenic to humans, animals, and plants. Polycyclic aromatic hydrocarbon concentrations have been steadily growing in the environment, including water, soil, air, sediments, marine water, and vegetables. The excessive use of polycyclic aromatic hydrocarbon-containing petrochemical compounds for globalisation and industrialization, as well as the polycyclic aromatic hydrocarbon's resistance to microbial degradation, are major factors contributing to higher polycyclic aromatic hydrocarbon concentrations in the environment. A sustainable method will be the formation of consortia of efficient polycyclic aromatic hydrocarbons degraders for the total removal of polycyclic aromatic hydrocarbons from polluted locations. The consortia formed by efficient polycyclic aromatic hydrocarbons digesting bacteria with new plasmids is effective for polycyclic aromatic hydrocarbon bioremediation.

Keywords - Polycyclic aromatic hydrocarbons, Consortia, Bioremediation

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INTRODUCTION

The term "polycyclic aromatic hydrocarbons" (PAHs) refers to non-polar chemical molecules with two or more fused benzene rings that are organised linearly, angularly, or in clusters. They are extremely stubborn and persistent in the environment, and they naturally occur in coal, crude oil, and gasoline. Additionally, fossil fuel-derived goods including coal tar pitch, creosote, and asphalts include PAHs. Aqueous solubility of PAHs diminishes logarithmically as molecular mass increases, and the majority of PAHs are not soluble in water, which restricts their solubility in the environment (Bayoumi, 2009). High molecular weight PAHs have limited water solubility, making these compounds less accessible for biological uptake and degradation (Maliszewska-Kordybach, 1999), in contrast to two and three ring PAHs, which dissolve in water and become available for biological uptake and destruction.

In well-established animal model studies, PAHs have been connected to stomach, bladder, skin, lung, and liver cancer. Heart problems and inadequate prenatal development have also been connected to PAH exposure. Many PAHs have teratogenic effects on both humans and animals, are mutagenic, genotoxic, and carcinogenic (Marston et al., 2001). The amount of aromatic rings in PAHs affects how mutagenic they are. The dispersion of these pollutants in the environment and human exposure to them have been of particular concern since the US-EPA (United States Environmental Protection Agency) has designated 16

PAHs as priority pollutants, some of which are thought to be potential human carcinogens (Harvey, 1996).

Human activity is the primary source of pollutants like PAHs. Manufacturing of aluminium, iron, and steel, coal gasification, tar distillation, hale oil extraction, production of coke, creosote, carbon blades, calcium carbide, and asphalt, production of rubber tyres, production of or use of metalworking fluids, and coal activity in natural gas power plants are some of the industrial processes that generate and disperse PAHs. Since the turn of the century, PAH concentrations have steadily increased due to the growth of anthropogenic sources, atmospheric deposition from natural sources, and the persistence of PAHs. Reducing anthropogenic activity can help reduce the dangers of PAH, but as industrialization became more important, petrochemical chemicals were used to the fullest extent possible. As long as the concentration of these substances is not too high, nature is capable of eliminating these pollutants in limited quantities. Various physical, chemical, and biological mechanisms may be able to eliminate the PAHs discharged into the environment.

Volatilization, photo-oxidation, chemical oxidation, and adsorption are some of the physical and chemical techniques utilised to remove PAHs from the environment. These methods, nevertheless, are expensive and risk incomplete pollutant destruction. Nitrogenated, halogenated, hydroxylated, and oxygenated PAHs can be produced as a result of

abiotic breakdown in the atmosphere and upper layers of surface water. Some of these substances have higher levels of toxicity, water solubility, and mobility than their parent PAHs. In situ bioremediation research has been sparked by efforts to remove significant amounts of PAHs from polluted locations. In situ PAHs bioremediation by microorganisms is a more affordable, ecologically sound, and cost-effective remediation option than non-biologic remediation, depending on particular microbiological, chemical, and hydro-geological restrictions. In the environment, PAHs are transformed and degraded mostly biologically by microorganisms (Ahn et al., 2005).

The PAHs molecule's ring number has an inverse relationship with the biodegradation processes. The higher molecular weight PAHs with four or more fused rings are more slowly destroyed than the lower molecular weight PAHs (Mrozik et al., 2003). Microorganisms can easily break down the majority of monoaromatic and low-molecular-weight PAHs; however, the high-molecular-weight PAHs-degrading microbial community is less suited and its rate of PAHs degradation in nature is modest (Yamada et al., 2003). The high molecular weight PAHs are often not a suitable substrate for microbial digestion.

Pseudomonas, *Mycobacterium*, *Achromobacter*, *Arthrobacter*, *Burkholderia*, *Rhodococcus*, *Micrococcus*, and *Sphingomonas* are among the genera of PAH-degrading bacteria that have been isolated from soil contaminated with PAHs (Lee et al., 2005). Species of PAH-degrading bacteria that have been isolated from marine environments are from the *Cycloclasticus* and *Moraxella* (Dagher et al., 1997).

The components of hydrocarbons, especially polycyclic aromatic hydrocarbons (PAHs), are very poisonous, mutagenic, and carcinogenic, posing a major risk to the health of both people and the environment. Numerous remediation procedures, including both physical and chemical ones, have been developed to reduce the risks posed by PAHs. Although the majority of physico-chemical remediation approaches are successful in removing a variety of PAH pollutants, they are quite costly and occasionally harmful to the soil's structure and texture. As an alternative, biodegradation or bioremediation has emerged as a desirable option for the remediation of hydrocarbon-contaminated soil due to its affordability, effectiveness, and environmental friendliness. The capacity of microorganisms to convert organic pollutants like PAHs into non-toxic byproducts like water and carbon dioxide is known as biodegradation (Alrumman et al., 2015).

It was recently revealed the genome sequence of the bacterium *N. pentaromativorans* US6-1, which can use PAHs as its only carbon source. However, the genes that code for the proteins necessary for the biodegradation of PAH have not yet been fully annotated, and up until now, little is known about how they work. In the biodegradation gene area of plasmid pLA1 and contig 58 of the chromosome, two potential clusters were discovered that may include the genes required for the breakdown of aromatic chemicals. In this work, the differential expression of biodegradative genes was investigated using a proteome analysis of

N. pentaromativorans US6-1 grown in the presence of three distinct PAHs (Yun et al. 2014).

Decontamination of PAH-polluted locations is critical since many PAH chemicals are poisonous, mutagenic, or carcinogenic. The United States Environmental Protection Agency (EPA) has designated 16 PAH chemicals as priority pollutants based on their prevalence and toxicity (Isiodu et al., 2016). Because it potentially offers benefits like the complete destruction of the pollutants, lower treatment cost, greater safety, and less environmental disruption, biological treatment of soils contaminated with PAH should be an efficient, economical, and versatile alternative to physicochemical treatment. Therefore, the biodegradation of PAHs has received a lot of attention from researchers. It was discovered that microorganisms either destroy PAHs by metabolism or cometabolism. The breakdown of PAH combinations and high-molecular-weight PAHs depends on cometabolism (Habe & Omori, 2014). The US EPA has identified the chemical structures of 16 priority PAH compounds which can be observed in the following figure (I. Keuper, 2010):

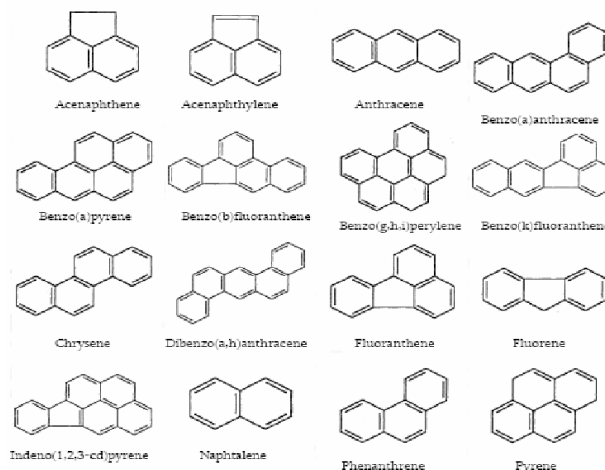


Figure 1: priority PAH compounds

Although PAH is degraded by monocultures of PAH-degrading microorganisms in situ or at the laboratory level, the process of degradation is sluggish. Use of efficient PAH degrader consortia in the right mix and ratio can significantly speed up the degradation process. More importantly, the initial attack on PAH is made by one member of the consortium, and the partially metabolised compound will be used by other consortium members through peripheral catabolic pathways. The members of bacterial consortia degrade PAH synergistically, and even degradation metabolites toxic to one species are utilised by other species.

The plasmids, a unique distinguishing feature of the PAH-degrading bacteria, are in charge of PAH degradation. The genetic make-up of consortia, including specific plasmids, affects how well they degrade PAHs. These distinct plasmid-bearing bacteria are employed not only to form consortia but also to create superbugs with enhanced PAH biodegradation potential. Since these plasmids have distinctive genes, such as *nidA* and *phnAc*, as well as distinctive sizes, restriction sites, genes, and gene sequences. For characterisation research,

plasmids from effective high molecular weight PAHs-degrading bacteria can be obtained.

PAH BIOREMEDIATION: It appears that the main method for eliminating PAHs from soil is biodegradation (Sims and Overcash, 1983; Wilson and Jones, 1993). Microorganisms like bacteria and fungus have the ability to transform PAHs into other organic molecules or inorganic end products like CO₂ and H₂O. (Cerniglia, 1984; Cerniglia, 1992; Gibson, 1993). The latter phase is known as mineralization. PAHs may be utilised as a carbon and energy source by some PAH-degrading microorganisms, mostly bacteria, allowing the pollutants to be transformed into compounds that may enter the organisms' primary metabolic pathways (Cerniglia, 1984; Cerniglia, 1992). Other bacteria may digest PAHs and continue to live on a shared substrate. Co-metabolism does not necessarily lead to the formation of microbes; in this situation, the PAH is simply changed into another molecule with no apparent benefit to the organism. Incomplete breakdown may occur if the metabolite's conversion-capable enzyme is absent (Gibson, 1993). The importance of co-metabolic breakdown mechanisms grows with the number of rings in the PAH-molecule since far fewer bacteria can utilise HMW PAHs as carbon and energy sources.

PAH BACTERIAL DEGRADATION: Numerous genera of Gram-positive and Gram-negative bacteria have been found and reported as having the ability to utilise and breakdown PAHs. A list of bacterial species that may break down PAHs is provided in Table 1.3. The majority of bacterial PAH degradation occurs by aerobic oxygenase-mediated catabolism (Kanaly and Harayama, 2000). There are metabolic routes, enzymes, and genes for the aerobic metabolism of PAHs, which has undergone extensive study and characterization. In the absence of molecular oxygen, nitrate, iron, and sulphate ions were shown to act as an alternate electron acceptor for oxygen during various PAH breakdown processes (Suthersan, 1999).

A concentration gradient from the environment helps passive diffusion, which is how PAHs enter cells (Johnsen et al., 2005). Another crucial element in this process is the concentration and bioavailability of PAHs. The efficiency of microbial PAH breakdown may be diminished by passive diffusion of PAHs into bacterial cells at low concentrations (Johnsen et al., 2005). This constraint has been overcome by certain bacteria by the development of biosurfactant excretion, direct contact with solid phase PAHs, and a high affinity PAH absorption mechanism without the need of biosurfactants, as demonstrated in the cells of *Mycobacterium* sp. strain LB501T. (Wick et al., 2002; Chauhan et al., 2008). High concentrations of dangerous PAHs can be swiftly broken down by bacteria, but this might result in PAH accumulation in the cell wall and compromise cell viability. Bacteria can rapidly degrade dangerous PAHs in large amounts, however this can cause PAH accumulation in the cell wall and damage protein and cell membrane functions (Sikkema et al., 1995).

TOXICITY ASSESSMENT OF PAHs BIODEGRADATION END PRODUCTS: The main goal of any remediation method is to eliminate or detoxify a contaminant from a specific environment. The bulk of field studies tended to measure treatment by the amount of parent pollutant removal or transformation, but they did not include the possibility of producing more dangerous degradation metabolites. At the end of the treatment procedure, it is crucial to make sure that the pollutant or contaminant is thoroughly detoxified into inconvenient byproducts (Mendonca and Picado, 2002; Lundstedt et al., 2003; Sihag et al., 2014). Lundstedt et al. (2003) found that oxy-PAHs are produced during the biodegradation of PAHs. Oxy-PAHs are more dangerous than parent PAHs molecules. Therefore, it's crucial to monitor the metabolites of remediation processes, especially for dead-end items (Sihag et al., 2014).

MICROBIAL DIVERSITY IN PAHs CONTAMINATED ECOSYSTEMS: In recent years, several different microbes that metabolise PAHs have been discovered and described. Numerous bacterial species, such as *Sphingomonas*, *Stenotrophomonas*, *Martelella*, *Mycobacterium* (Hennessee and Li, 2016), *Pseudomonas* (Singh and Tiwary, 2017), *Pseudomonas* (Zhu et al., 2017), *Aspergillus*, *Phanerochaete*, *Pleurotus*, *Trametes*, *Penicillium*, *Rhizopus*, *Trichoderma*, *Scopulariopsis*, *Candida*, *Yarrowia*, and *Pichia* are just a few of the many yeast and fungal taxa that have a reputation for being efficient hydrocarbon degraders (Chaillan et al., 2004; Wang et al., 2008; Balaji et al., 2014; MirTutusaus et al., 2014; Mao and Guan, 2016; Praveen Kumar et al., 2016; Hussain et al., 2017). In particular, low-molecular-weight (LMW) chemicals including naphthalene, anthracene, and phenanthrene were shown to degrade these microbial species. But little is known about the microorganisms that can break down HMW PAHs with five rings or more, such as pyrene, benzo[a]pyrene, and benz[a]anthracene.

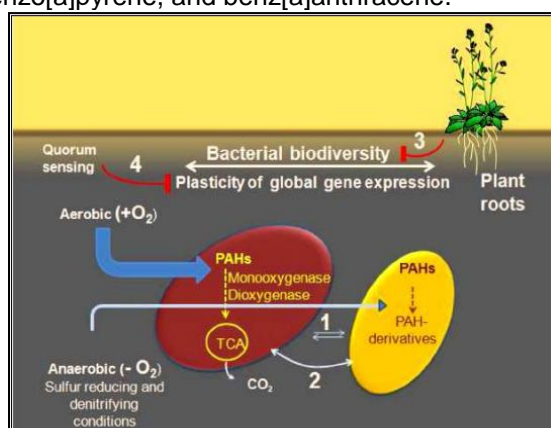


Figure 2: Bacterial biodiversity and ecology of PAH catabolism in a polluted environment
MOLECULAR DIVERSITY OF PAHs DEGRADING BACTERIAL STRAINS IN CONTAMINATED ECOSYSTEMS: A thorough understanding of soil biodiversity and dynamics of microbial communities that might digest contaminants is needed to analyse the long-term sustainability of soil pollution and to

create bioremediation solutions (La Rosa et al., 2006; Patel et al., 2016). It is important to consider the diversity of microorganisms because their ability to adapt, grow, and survive in a variety of environments aids in the maintenance of ecological equilibrium (Sarethy et al., 2014). Different environmental factors frequently have an impact on them since traditional methods of diversity study tend to concentrate largely on physical attributes (Govindaraj et al., 2015). However, molecular markers can sample the variety of the genome. Pérez-Loada et al. (2007) and Govindaraj et al. (2015) shown that these indicators are unrestricted and unaffected by environmental influences. They increase the precision and breadth of genetic diversity both within and between bacterial populations.

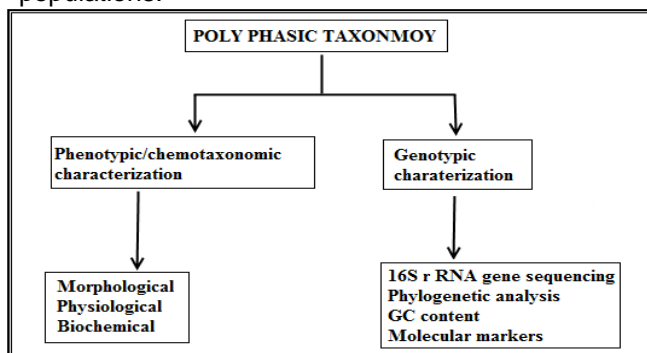


Figure 3: Techniques used for studying microbial diversity

BIODEGRADATION OF PAHS BY BACTERIA: Bacteria, fungus, and algae are the main degraders of PAHs in soil and water. Bacteria are microorganisms that have been discovered to breakdown PAHs from polluted areas, especially low-molecular-weight substances like naphthalene, phenanthrene, and anthracene. Less is known about bacteria that may utilise PAHs with five or more rings as a source of carbon and energy, such as benzo[a]pyrene and benz[a]anthracene (Peng et al., 2008).

MECHANISM OF PAHS DEGRADATION/TRANSFORMATION IN BACTERIA:

The primary mechanism by which bacteria degrade PAHs is metabolism or co-metabolism (Cerniglia et al., 1994). Both aerobic and anaerobic settings can result in the breakdown of PAHs, however research on the aerobic metabolism of PAHs and the enzymatic and genetic control of the degradation process has attracted the greatest interest (Cerniglia et al., 1993). Energy is generated during the bioremediation process via a redox reaction inside the microbial cells. The bulk of these reactions include metabolic processes like respiration, which are important for maintaining and regenerating cells. An electron donor or energy source (petroleum hydrocarbons, chlorinated solvents, reduced inorganic compounds, fatty acids, etc.), an electron acceptor (oxygen, nitrate, manganese, iron (III), iron oxides, nitrate and sulphate, etc.), and additional nutrients are often needed (Adams et al., 2015). Most aromatic compounds, including polyaromatics, are known to metabolise

protocatechuates and catechols, which are ring-cleaved intermediates. The results of central routes are subsequently exposed to ortho- and meta-cleavage pathways. These essential intermediates are transformed into intermediates by the Tricarboxylic Acid Cycle (TCA) (Cerniglia et al., 1994).

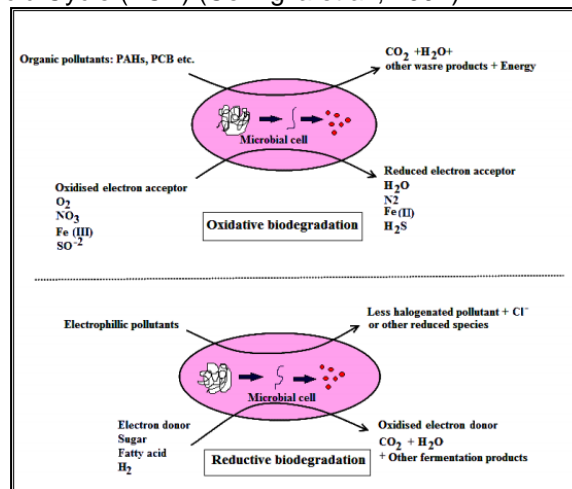


Figure 4: General process of biodegradation of organic contaminants

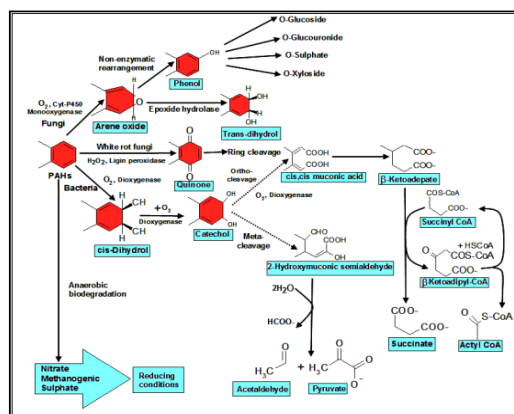


Figure 5: Current knowledge of the pathway for microbial catabolism of polycyclic aromatic hydrocarbons

PAHS BIODEGRADATION PATHWAYS IN BACTERIA:

The conversion of 1,4-naphthaquinone to benzoic acid and ultimately to catechol, an intermediate with an aromatic ring cleaved, was described by Hadibarata et al. (2013). Salicylate is often transformed to catechol by decarboxylation, which is subsequently metabolised through ortho meta and routes. Another research by Fuenmayor et al. found that salicylate is further hydroxylated into gentisate by the enzyme salicylate -5-hydroxylase (1998). The enzyme dioxygenase typically starts the degradation of phenanthrene (3 ring PAHs) by bacteria (Fig. 2.6), resulting in cis-3, 4-dihydroxy-3,4-dihydrophenanthrene, which is subsequently broken down by the enzyme dehydrogenase into 3,4-dihydroxyphenanthrene (Seo et al., 2007). Samanta et al. reported that salicylic acid was converted into catechol (1999).

Numerous bacteria, including Mycobacterium, have the ability to mineralize pyrene, a four-ring PAH, by ring oxidation and ring fission (Pandey et al., 2016). Due to the availability of other metabolites including 4 cis- and trans -4,5- dihydrodiols, many mechanisms for the initial oxidative assault on the pyrene structure (degrade multiple PAHs including phenanthrene, pyrene, and benzo(a)pyrene) have been postulated. Mrozik et al. (2003); Pandey et al. (2016).

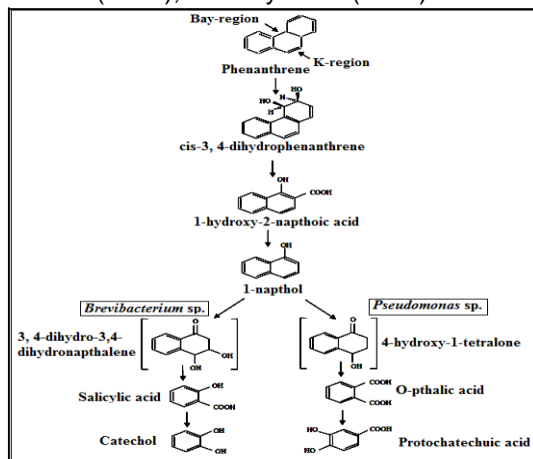


Figure 6: The proposed pathway for phenanthrene oxidation by Brevibacterium, pseudomonas and citrobacter species

MICROBIAL ENZYMES AND GENES INVOLVED IN PAHs BIODEGRADATION: The microbial degradation of pollutants, which transforms pollutants into less hazardous or non-toxic compounds, involves several metabolic processes mediated by a variety of enzymes. Numerous microbial enzymes, such as oxidoreductases, oxygenases, hydrolases, dehydrogenases, and dehalogenases, have been shown to be involved in both aerobic and anaerobic PAH breakdown processes. Some bacterial species, such as Mycobacterium, may oxidise PAHs to create transdihydrodiols with the aid of cytochrome P450 monooxygenase (Bamforth & Singleton, 2005). Multicomponent enzymes called microbial dioxygenases transport two oxygen atoms from molecular oxygen to a variety of organic substrates (Arora et al., 2009). Cis-dihydrodiols are a primary by-product of the multi-component enzyme system known as dioxygenase, which catalyses the opening of the benzene ring in organic molecules. This enzyme system uses a variety of coenzymes and metal ions as cofactors (Peng et al., 2008). The uses in the environment are to treat aromatic hydrocarbons since dioxygenases mostly oxidise aromatic compounds. Intradiol cleaving enzymes, which use Fe³⁺, and extradiol cleaving enzymes, which use Fe²⁺ and Mn²⁺, are the two types of dioxygenase enzymes (Fig. 2.8). (Mittal & Singh, 2009).

FACTORS AFFECTING BIOREMEDIATION OF PAHs: It is quite challenging to maintain appropriate bioremediation conditions within and/or outside of the lab. Before implementing any remediation technique, some restrictions must be properly taken into account.

In general, biotic and abiotic parameters (Figure 6) that differ from site to site, such as soil properties, nutrient availability, oxygen, and the bioavailability of pollutants, impact the effectiveness and speed of bioremediation treatments (Ghosal et al., 2016). The key elements influencing the viability of bioremediation are described in the sections that follow.

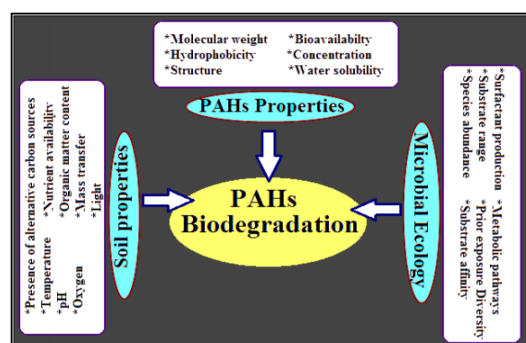


Figure 7: Factors affecting biodegradation of PAHs in soil

CONCLUSION

Polycyclic aromatic hydrocarbons are substantial environmental pollutants that are exceedingly hazardous, mutagenic, carcinogenic, and teratogenic, as determined by this study. The concentrations of PAH contaminants in estuary water and sediments, marine water and sediments, freshwater lakes, polar ponds, freshwater sediments, ground water, and urban terrestrial soil have all increased. Therefore, bacterial species gain the ability to utilise refractory polycyclic aromatic hydrocarbons as a source of carbon and energy through adaptation and the acquisition of genes critical for PAH consumption from the bacterial population.

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