CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBON (PAH)-DEGRADING SOIL BACTERIA IN URBAN PARKS: BIOREMEDIATION APPROACH FOR A CLEAN ENVIRONMENT

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Polycyclic aromatic hydrocarbons (PAHs) are organic compounds that contain two or more fused benzene rings pried with hydrogen and carbon atoms. They are classified as environmental pollutants due to their high carcinogenic, and mutagenetic nature towards to humans and other organisms. Among these PAHs pyrene and anthracene can commonly be found in the environment. PAHs can be neutralized in physical and chemical methods, but it causes adverse side effects on the environment. So, the best eco-friendly and cost-effective way is bioremediation. The aim of this study is to evaluate environmental PAH concentrations and to select soil bacteria which is capable of degrading PAHs such as pyrene and anthracene. First soil samples were collected from urban roadsides and parks in Akkaraipattu, Karainagar and Nawala in Sri Lanka. The optimum PAHs degrading bacterial strains were identified by using plate assay and their degradation percentages were evaluated by using spectrophotometric analysis. According to the spectrophotometric analysis results, bacterial strain RJ01, RJ00, RA00, RN00, and SH01 degrades anthracene more than 60% and strains RJ01, RJ00, RN00, SH04, SH06 and SH09 degrades pyrene more than 50%. The HPLC environmental analysis test results revealed that the soil samples were contain pyrene and anthracene in 50ppm concentration, in the range of 50-200ppm. In conclusion, the selected PAHs degrading soil bacteria can be identified as potential biological agents for degrading PAHs such as pyrene and anthracene.

Keywords: Bioremediation, Pyrene, anthracene, PAHs, Degradation percentage Bacteria

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INTRODUCTION

Polycyclic organic matter (POM) includes diverse compounds with three or more fused aromatic rings, typically composed of carbon, hydrogen, oxygen, nitrogen, and sulfur. While millions of POM variants are theoretically possible, only about 100 have been identified and studied, with polycyclic aromatic hydrocarbons (PAHs) being the most prevalent subset, containing only carbon and hydrogen. PAHs are ubiquitous environmental pollutants known for their carcinogenic, mutagenic, and genotoxic properties, posing significant health risks. They are produced by the combustion of carbonaceous materials and can be found in both outdoor and indoor environments, originating from activities such as smoking, cooking, and domestic heating (Organisation Mondiale De La Santé Bureau Régional De L'europe, 2000; Srogi, 2007).

PAHs with low molecular weights exist primarily in the vapor phase, while high molecular weight PAHs associate with particulates. Intermediate PAHs can partition between vapor and particulate phases depending on atmospheric conditions. PAHs can undergo degradation through photolysis, reactions with environmental pollutants, or metabolism by fungi and microorganisms. Due to their hazardous nature, effective bioremediation strategies are essential (Ifegwu & Anyakora, 2015).

Bioremediation employs microorganisms to degrade, confine, or alter pollutants, aiming to mitigate environmental contamination. Bacteria, as natural scavengers, have evolved to break down organic pollutants, making them valuable in biotechnological applications for environmental cleanup. Specifically, bacteria capable of degrading PAHs, such as naphthalene and phenanthrene, have been a focus of study for their potential to address PAH contamination (Aswin Thacharodi et al., 2023).The objective of the study is to isolate PAHs degrading bacteria from urban parks and evaluate their PAHs degradation percentages.

METHODOLOGY

Polluted soil samples were collected from urban roadsides and urbarn parks in Akkaraipattu (Ampara district), Karainagar (Jaffna district), and Nawala (Colombo district) in Sri Lanka since the earlier research was covered with other factors like water. There for we are conducting our research with soil sample. Additionally, there is a higher chance to obtain the PAHs in the soil sample because of industrial activities, petroleum waste inappropriate disposal. Approximately 100 1`g of each sample was placed in sterile zip-lock bags, labeled with location, date, and time, and stored at 4ºC until use. For the isolation of soil bacteria, the samples underwent serial dilution up to a 10^{-10} factor, with test tubes filled with 9ml of distilled water autoclaved at 121ºC for 2 hours. 5g of each soil sample was mixed with 50 ml of autoclaved water, and serial dilutions were performed. Using the spread plate technique, 0.1 ml of each $10^{-5}/10^{-10}$ diluted sample was spread on nutrient agar plates, which were sealed with parafilm and incubated at room temperature for 24 hours.
Morphologically distinct colonies from the spread plates were then selected, labeled, and streaked on nutrient agar plates using quadrant streaking; these plates were sealed with parafilm and incubated for 24 hours. For the primary screening, solidified BBH agar plates were divided into 25 squares, and selected bacterial isolates were transferred to each square, then sealed with parafilm and incubated for 3 days. A 100ppm solution of anthracene and pyrene in acetone was prepared and spread on BBH agar plates, and starved bacterial colonies were transferred to labeled squares on the plates, which were then sealed with parafilm and incubated for 24 hours.

For spectrophotometric analysis, test tubes with 2ml distilled water were autoclaved, filled with 2% methylene blue and bacterial colonies, and analyzed using a spectrophotometer with three readings taken per sample.

RESULTS AND DISCUSSION

Spread plate technique.

After the incubation of spread plates, the bacterial growth resulted as shown in figure 1 where morphologically different bacterial colonies were identified as given in table 1. And these identified colonies were named using a unique code such as RJ00, RJ01, RA01, RA00, RN00,SH01 and SH06.

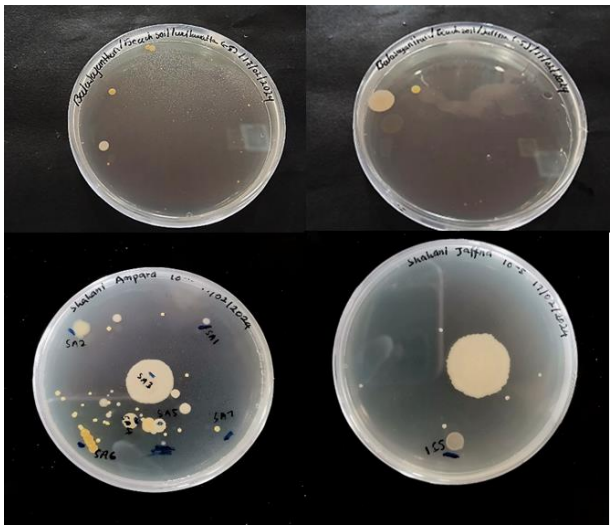


Figure 1*:* spread plate results obtained.

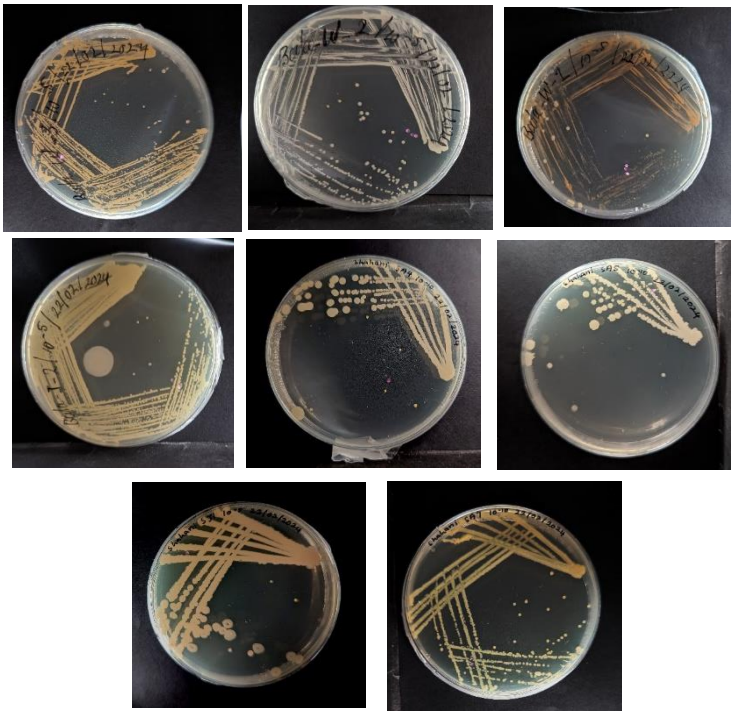
Table 1: Bacterial colony identification using its morphologically different features.

Colony	**Size**	**Colour**	**Texture**	**Elevation**	**Form**	**Margin**
RJ00	Moderate	creamy	Viscid	Flat	Round	Entire
RJ01	Small	Creamy orange	smooth	Convex	Round	Undulate
RA00	Large	White	Dry (powdery)	Flat	Irregular	Irregular
RN00	Large	White	Dry	Flat	Irregular	Serrate
RA01	Medium	Creamy	Smooth	Convex	Circular	Entire
SH01	Medium	Creamy	Smooth	Flat	Irregular	Erose
SH04	Small	Yellow	Mucoid	Convex	Punctiform	Entire
SH06	Medium	Creamy	Smooth	Flat	Concentric	Entire

Streak Plate technique.

After the incubation of streak plates single bacterial isolates of the morphologically different bacterial colony was obtained as shown in figure 2. This helped in clear identification of the bacterial colony as single isolates that can be further studied.

Figure 2: Streak plate results obtained.



Primary screening

Table 2: Primary screening results.

Bacteria	Pyrene	Anthracene
RJ01	0/25	25/25
RJ00	0/25	25/25
RJ02	0/25	0/25
RA02	0/25	0/25
RA00	0/25	0/25
RN00	0/25	25/25
SH01	23/25	25/25
SH04	24/25	24/25
SH06	24/25	23/25

The bacteria were starved for 2 days and then transferred to new BBH plates spiked with PAHs. The only carbon source in the new plate was PAHs, which allowed bacteria to identify their growth. Bacteria that could not degrade PAHs died. The starvation process was repeated every 2-3 days. 6 colonies successfully degraded anthracene and growth in all 25 squares, while only SH06, SH04, SH01 colonies showed pyrene degrading ability.

Spectrophotometric analysis

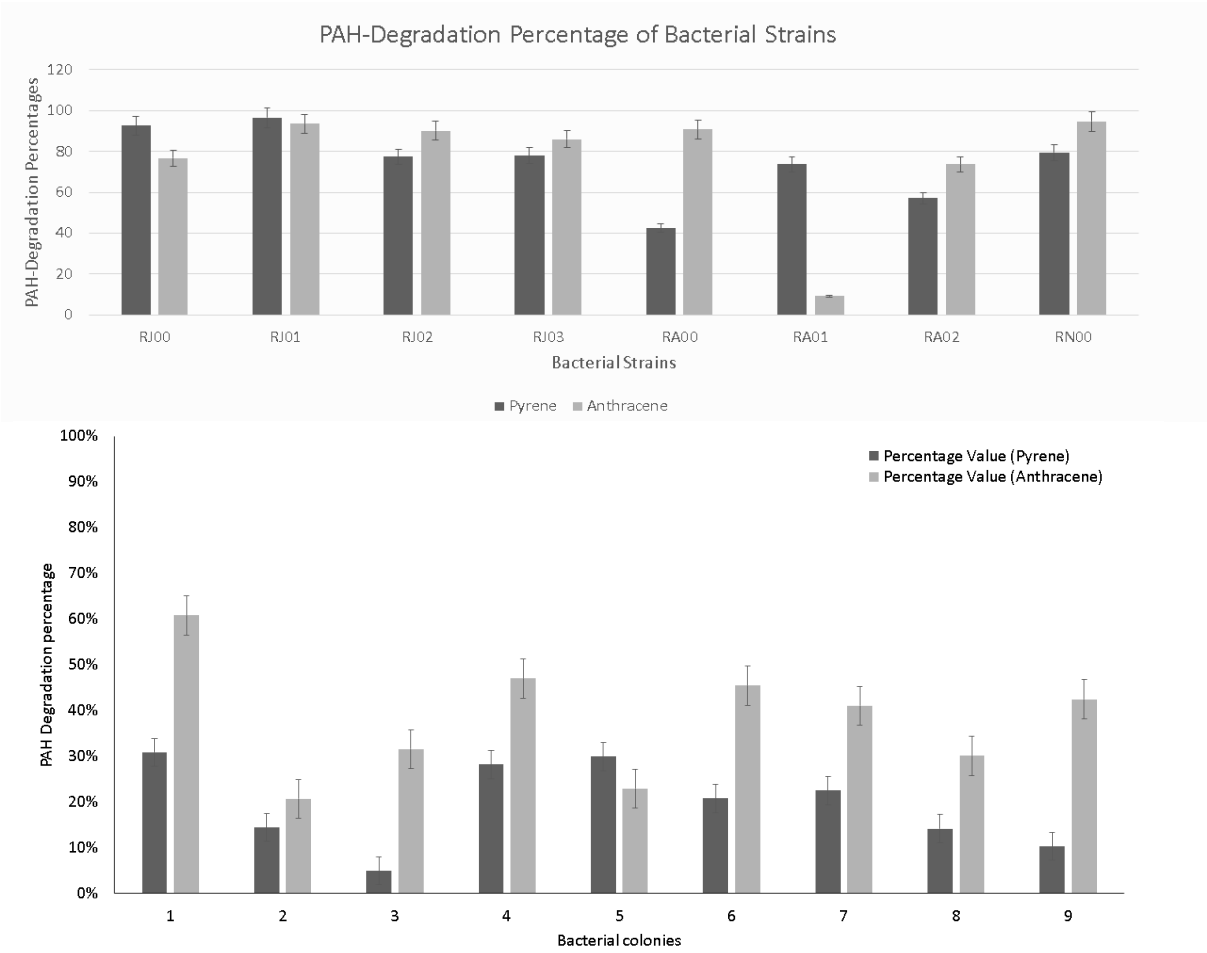


Figure 3: PAH degradation percentages

Secondary screening was done to calculate the degradation percentage by using following equation,

Equation 2 :

PAH degradation percentage=(Initial Absorbance-Final absorbance)/Initial absorbancex100

2% methylene blue is used in a study to identify the degradation percentages of PAHs in test tubes. The dye changes color based on redox state, and when reduced, it becomes colorless. Bacteria that degrade PAHs oxidize PAHs, reducing methylene blue. Absorbance measurements indicate the degradation percentage. PAH degradation percentages were calculated using absorbance data, shown in Figure 2 and 3.

HPLC

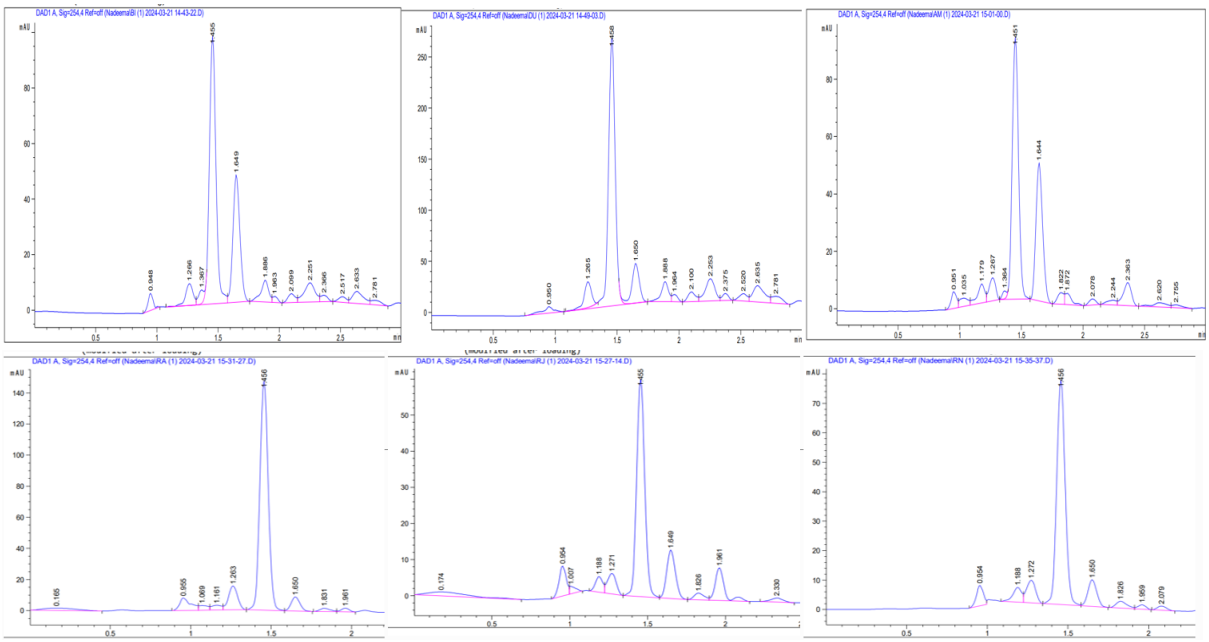


Figure 4: HPLC results

HPLC is a powerful analytical technique, due to its ability to separate, identify and quantify complex mixtures of compounds with high accuracy and sensitivity, this method was utilized for determine the concentration of PAHs in collected contaminated soil samples. HPLC capability to separate the compound based on their physical and chemical properties such as polarity and molecular size, allow for the selective detection and quantification of PAHs in the collected soil samples.

CONCLUSIONS

This study successfully isolated and identified various bacterial colonies capable of degrading polycyclic aromatic hydrocarbons (PAHs). The primary and secondary screenings demonstrated the efficiency of these isolates in degrading PAHs these bacterial strains can be incorporated into compost media for sustainable bioremediation of PAH-contaminated environments, providing a promising solution for addressing soil pollution. According to the spectrophotometric analysis results, bacterial strain RJ01, RJ00, RA00, RN00, and SH01 degrades anthracene more than 60% and strains RJ01, RJ00, RN00, SH04, SH06 and SH09 degrades pyrene more than 50%.

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