

Research Article

Isolation and Characterization of Phenanthrene Degrading Bacteria from Soil in the District of Angul Near JSPL Industry

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) such as phenanthrene are persistent environmental pollutants commonly associated with industrial activities, posing a serious risk to soil ecosystems & human health due to their toxic & carcinogenic nature. The present study focuses on the isolation and characterization of phenanthrene-degrading bacteria from soil samples collected near Jindal Steel and Power Limited (JSPL) industrial area, where chronic hydrocarbon contamination is expected. To isolate effective degrading bacteria, soil samples were enriched in BHB supplemented with phenanthrene as the only carbon and energy source. By utilizing spectrophotometric analysis, bacterial isolates exhibiting notable growth were further tested for the ability to degrade phenanthrene. The strong isolates were characterized morphologically, physiologically, and biochemically using accepted microbiological procedures. The majority of the isolates belonged to genera like *Bacillus* and *Pseudomonas* that are known to degrade hydrocarbons. Degradation efficiency was found to be affected by temperature, phenanthrene content, and pH, with neutral pH exhibiting the best degradation. The findings show that industrially contaminated soils contain native bacteria that are metabolically adaptable and can use phenanthrene as a carbon source. This study emphasises the importance of native microbial populations in natural attenuation processes and their capacity for adaptation in contaminated environments. The identified isolates exhibit encouraging potential for use in bioremediation techniques meant to restore soils affected by PAHs near industrial areas.

Keywords

PAH, PAH – Degrading Bacteria, Biodegradation, Bioremediation, Phenanthrene

1. Introduction

1.1. The Global and Local Context of PAH Pollution

An inherent consequence of the industrial revolution is the

growth of persistent organic pollutants (POPs), of which polycyclic aromatic hydrocarbons (PAHs) are a ubiquitous class of environmental poisons. PAHs are hydrophobic, thermodynamically stable, and widely distributed in the atmosphere, hydrosphere, and pedosphere. They are structurally defined by

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two or more fused benzene rings [1]. The incomplete combustion of fossil fuels, waste incineration, and industrial activities, including coal gasification, coke manufacture, and petroleum refining, are the main human-caused sources of their presence in the environment [2]. Phenanthrene functions as a precursor for oxygenated derivatives (oxy-PAHs), which are frequently more cytotoxic and mobile in groundwater than the parent molecule, although not being categorised as a human carcinogen (Group 3 by the IARC) [3]. Phenanthrene buildup in soil also modifies the microbial ecology, lowers agricultural output, and increases the possibility of bioaccumulation in the terrestrial food chain [4].

1.2. The Angul-Talcher Industrial Belt

The trajectory of modern industrial development is often accompanied by an inevitable ecological footprint. The Angul-Talcher area in the eastern Indian state of Odisha serves

as evidence of this duality. A dense cluster of thermal power plants, aluminium smelters, coal mines, and integrated steel manufacturing units, notably the enormous facilities of Jindal Steel and Power Limited (JSPL), is located in this area, which is frequently referred to as the "energy capital" of Odisha. The centre of this industrial environment is the Jindal Steel & Power Limited (JSPL) complex, which includes captive power plants, a coal gasification unit, and an integrated steel production with a capacity of 6.0 MTPA (million tons per year) [5].

Terrestrial Accumulation: The processing of solid wastes (fly ash, blast furnace slag) and raw materials (coal, coke, iron ore) introduces a complex mixture of organic and inorganic contaminants into the soil. Since the JSPL facility's projected development to a 25.2 MTPA liquid steel capacity puts additional burden on land resources, strong environmental management strategies are necessary [6].

Table 1. Phenanthrene's Chemistry and Recalcitrance.

Property	Value	Implication for Biodegradation
Molecular Formula	C ₁₄ H ₁₀	Substrate for ring-hydroxylating dioxygenases [1]
Molecular Weight	178.23 g/mol	Classified as Low Molecular Weight (LMW) PAH; easier to degrade than 4+ ring PAHs [7]
Water Solubility	1.18 mg/L (at 25°C)	Extremely low; limits bioavailability to bacteria in aqueous soil pores [7]
Log Kow (Octanol-Water)	4.46	High lipophilicity; tends to adsorb strongly to soil organic matter [8]

1.3. The Study Area

1.3.1. Climate and Geographical Context

The industrial cluster around the JSPL facility in the Angul district of Odisha (20045/N-21010/) is the subject of the study. The area is part of the Brahmani River basin.

1.3.2. Climate

The area experiences a tropical climate with extreme summer temperatures reaching 45 °C and high humidity. Significant rainfall (~1421 mm) occurs during the monsoon season (June–September), which makes it easier for surface pollutants to seep into groundwater and for coal dust to spread over agricultural areas [9].

1.3.3. Features of the Soil

The natural soil has a sandy loam texture and is mostly lateritic (red). Because of its naturally occurring mild acidity (pH 5.5–6.5), cationic heavy metals like Zn and Cd are more soluble and mobile [8].

1.3.4. Process Emissions

Several high-emission units are involved in the steel-making process.

1.3.5. Coke Ovens

Pyrolysis of coal to produce coke releases coal tar, a primary source of Anthracene, Phenanthrene, and Naphthalene [10].

1.3.6. Sinter Plant

Dust, including metals and organics, is produced by the agglomeration of iron ore fines with the coke breeze [11].

1.3.7. CPP

The 810 MW Captive Power Plant (CPP) and its planned extension units burn coal, generating fly ash that contains trace metals [11].

2. Materials and Methods

2.1. Sample Collection

The soil sample was collected from a farming field close to a Shiva Temple of Jamunda Jungle village, Angul, within the month of Mid-August. The sample was taken in a very sterile polyethene bag & was taken to the laboratory for the isolation of the polyaromatic organic compound degrading bacterium. The pH of the soil sample collection was 6.8 [12, 13].

2.2. Isolation & Screening of the PAH Degrading Bacteria

BH broth medium was first autoclaved at 121 °C and 1.5 atmospheric pressure for twenty min, then this medium was kept inside the laminar flow several minutes for to cool down to 45 °C and supplemented with PAH as a unique carbon source 0.1gm of soil was inoculated into the 250 ml volume flask containing a 100 milliliter BH broth media supplemented with 1.0% poly aromatic hydrocarbon as initial concentration with 0.1% glucose. The medium was incubated at 37 °C under shaking conditions. (150 rpm) for two days. After the incubation period, this medium was serially diluted from 10^{-3} to 10^{-5} . Then 1ml of serial diluted sample of 10^{-3} & 10^{-5} was transferred to sterile Petri plates that contain BH broth agar medium supplemented with 1.0% of poly aromatic hydrocarbon to get separate colonies. 10^{-3} & 10^{-5} petriplates containing 122 & 73 distinct colonies [14]. Out of which five colony was streaking on BH agar media containing 1.0% poly aromatic hydrocarbon & 0.1% glucose, which gives a pure isolate, then that was preserved in BHB agar slant for further study purposes. Five purified isolates were screened on solid media supplemented with completely different PAH concentrations. of 1, 2, 3, & 4% to find the most potent poly aromatic hydrocarbon-degrading microorganism [15, 16]. From the screening profile, the microorganism isolate of PS-01 was chosen because the most potent microorganism isolate as a result of its ability to grow on poly aromatic hydrocarbon supplemented media till a PAH concentration of 4% [17, 18].

2.3. Identification and Characterization

The Phenanthrene degrading isolate of bacterium was examined morphologically for colonial characterization like color and form on agar media, followed by Gram's staining

another organic chemistry means biochemical tests were conducted [19, 20].

2.4. Optimization of Environmental Parameters & Nutritional Parameters to Enhance the Biodegradation of Phenanthrene

All experiments were dispensed in a 100ml Erlenmeyer flask containing 25ml NB media supplemented with an applicable amount of PAH compound. Then inoculated in NB media containing 1.0% of Phenanthrene and was incubated at 37°C under shaking conditions (150 rpm) till the growth reached 24h. About 1% of PS-01 after 24h was used as an inoculum in all the experiments [21, 22].

3. Results

3.1. Isolation of Phenanthrene Degrading Bacteria

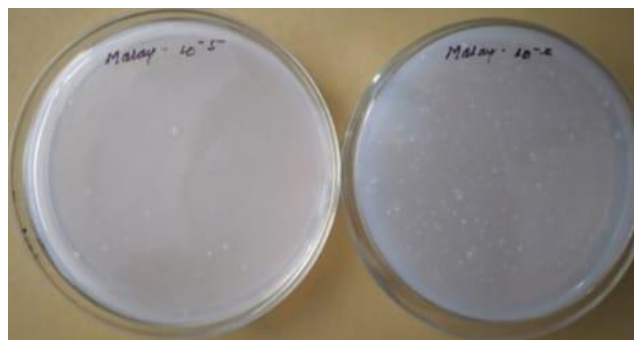


Figure 1. Isolation of bacteria by the spread plate method.



Figure 2. Isolation of bacteria by the streak plate method.

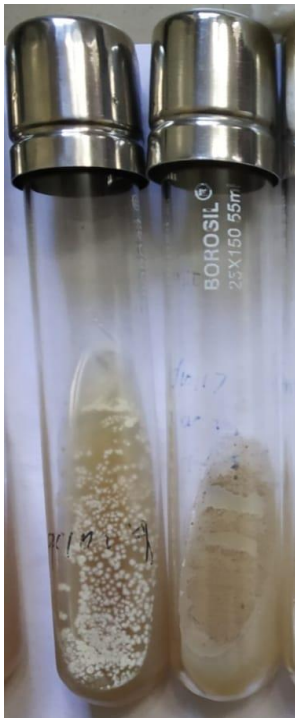


Figure 3. Slant culture.



Figure 6. Starch Agar Test.

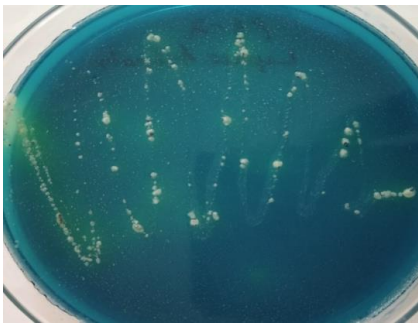


Figure 7. Lipase Test.

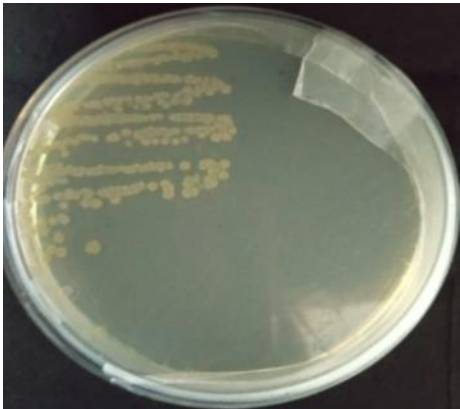


Figure 4. Bacteria growth from slant culture.

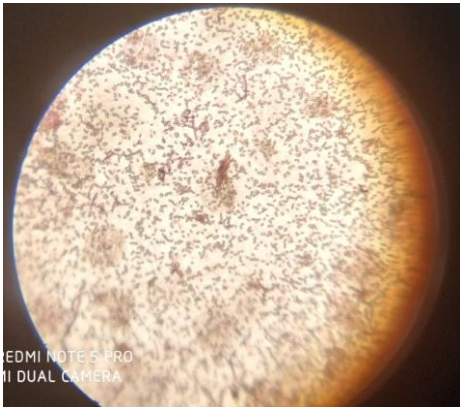


Figure 5. Identification of bacteria by Gram Staining.

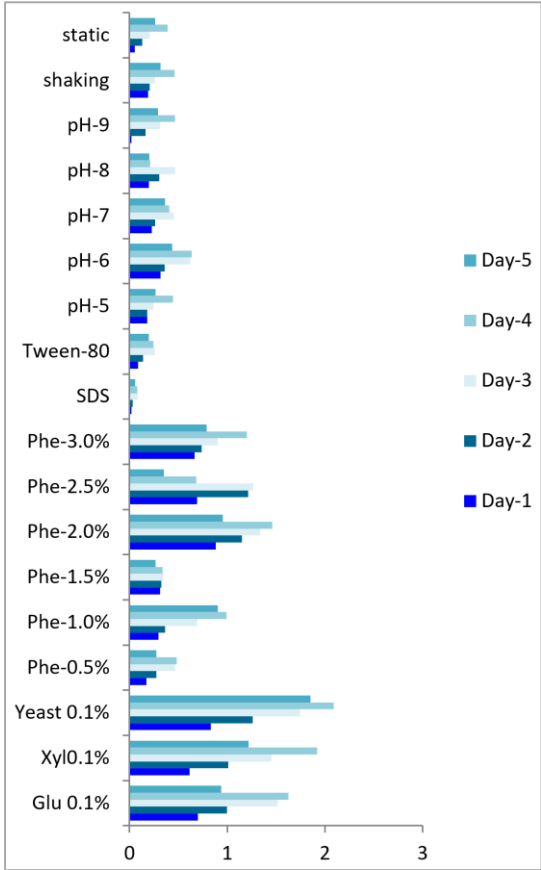


Figure 8. Bacteria growth on 600nm from Day-1 to Day-5 on environmental & nutritional parameters.

Table 2. Bacteria growth on different concentrations.

PAH Concentration						
Bacterial code	Gram Stain	0.5%	1%	2%	3%	4%
PS-01	+ve	+	+	+	+	+
PS-02	+ve	+	+	+	+	+
PS-03	+ve	+	+	-	-	-
PS-04	+ve	+	-	-	-	-
PS-05	+ve	+	+	+	-	-

Table 3. Colony characteristics of individual PS bacterial strains on BHB agar at 37°C within 24-36h.

Characteristics	PS001	PS-002	PS-003	PS-004	PS-005
Temperature	37°C	37°C	37°C	37°C	37°C
Media	BHB	BHB	BHB	BHB	BHB
Size	Small	Large	Small	Large	Small
Shape	Circular	Irregular	Circular	Irregular	Circular
Margin	Entire	Curved	Entire	Curved	Entire
Texture	Smooth	Smooth	Smooth	Smooth	Smooth
Elevation	Flat	Raised	Flat	Flat	Flat
Chromogenesis	Yellow	White	Slight-yellow	Chalky-white	Yellow

Table 4. Biochemical Characterization.

Test	PS-01 (Bacillus sp.)
Gram Stain	Positive (+) Rods
Motility	Motile
Catalase	Positive (+)
Oxidase	Variable
Starch Hydrolysis	Positive (+)
Lipase	Positive (+)
Pigmentation	Cream to brownish

Table 5. Bacteria growth at 600nm from Day-1 to Day-5 on environmental & nutritional parameters.

Different environment&nutritional Parameters	Day-1	Day-2	Day-3	Day-4	Day-5
Glu 0.1%	0.701	0.997	1.514	1.625	0.937
Xyl 0.1%	0.615	1.01	1.454	1.919	1.22

Different environment&nutritional Parameters	Day-1	Day-2	Day-3	Day-4	Day-5
Yeast 0.1%	0.831	1.263	1.745	2.088	1.851
Phe-0.5%	0.172	0.274	0.467	0.485	0.274
Phe-1.0%	0.297	0.362	0.692	0.994	0.902
Phe-1.5%	0.312	0.325	0.348	0.339	0.265
Phe-2.0%	0.882	1.15	1.337	1.459	0.956
Phe-2.5%	0.691	1.215	1.267	0.682	0.352
Phe-3.0%	0.667	0.738	0.904	1.201	0.791
SDS	0.017	0.031	0.083	0.08	0.058
Tween-80	0.087	0.137	0.259	0.247	0.2
pH-5	0.183	0.182	0.245	0.446	0.266
pH-6	0.319	0.36	0.621	0.638	0.438
pH-7	0.23	0.262	0.453	0.406	0.362
pH-8	0.2	0.306	0.468	0.213	0.203
pH-9	0.015	0.165	0.311	0.466	0.29
shaking	0.189	0.207	0.259	0.463	0.317
static	0.053	0.131	0.207	0.391	0.261

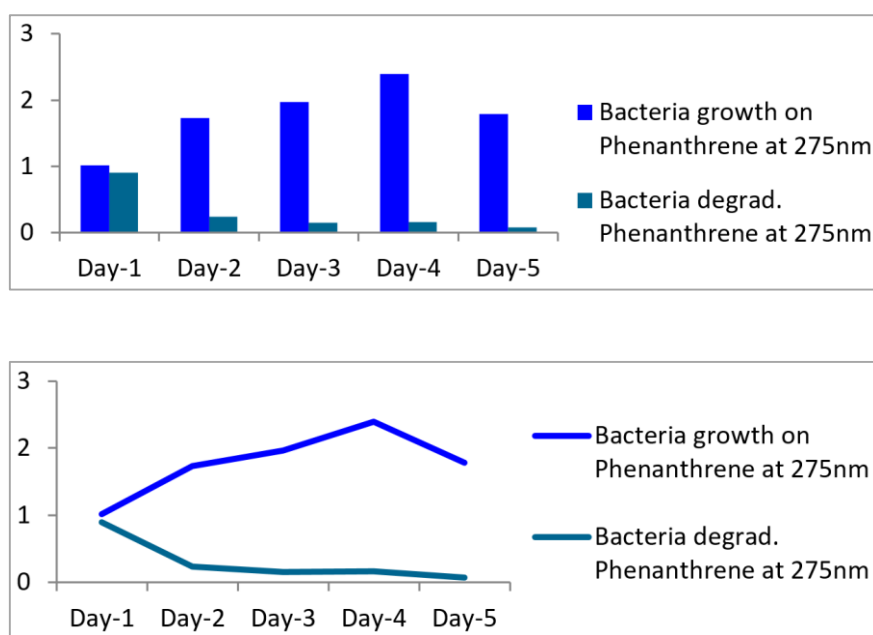


Figure 9. Bacteria Growth & Degradation analysis on Phenanthrene at 275nm.

Table 6. Bacteria Growth & Degradation analysis on Phenanthrene at 275nm.

Bacteria growth & degradation	Day-1	Day-2	Day-3	Day-4	Day-5
Bacteria growth on Phenanthrene at 275nm	1.018	1.732	1.969	2.397	1.786
Bacteria degrade. Phenanthrene at 275nm	0.898	0.238	0.151	.161	0.076

4. Discussion

4.1. Isolation & Screening of Phenanthrene Degrading Bacteria

During this study, five purified bacterial strains were isolated from the soil samples obtained from the farming field close to a Shiva Temple of Jamunda Jungle. These purified isolates had the power to tolerate high concentrations of PAH, so as to pick the most potent strain, isolates were subjected to completely different concentrations of PAH on solid media. All the isolates were Gram-positive. All five isolates had the power to grow on 1% PAH conc. (The concentration which is used for the microorganism isolation process). Some of the isolated bacterial strains do not have the ability to grow on 4% of PAH concentration. The most potent isolate had the ability to grow till 4% of PAH concentration. Therefore, all experiments were done at 1% PAH conc [17].

4.2. Identification and Characterization

PS-01 has the typical *Bacillus* profile, which includes catalase positivity, motility, and starch hydrolysis positivity. In Angul's dry, dust-filled soils, *Bacillus* species thrive [23].

4.3. Bacteria Growth & Degradation Analysis on Phenanthrene

Microorganisms' growth & degradation was analyzed on the Phenanthrene compounds from Day-1 (24hrs) to Day-5 (120 hrs). The growth & degradation were determined at 275nm. From the subsequent Figures, it is concluded that the growth of the bacteria on the phenanthrene compounds was significant, and it degraded the phenanthrene compound [24].

5. Conclusions

According to the results of the aforementioned experiment, the carbon sources, such as yeast, produced the greatest bacterial growth, while the surfactants, such as SDS, produced the least. Additionally, the best bacterial growth was found at pH 6, PAH conc-2%, and shaking conditions. A maximum growth of 2.397 (OD at 275 nm) was attained by Day 4, which coincided with a 91.5% decrease in phenanthrene. This demonstrates a significant inverse association between microbial biomass and substrate concentration.

Abbreviations

BHB	Bushnell Haas Broth
Mg/L	Milligram Per Liter
N.B.	Nutrient Broth
PAH	Polycyclic Aromatic Hydrocarbon

PAHDB	Polycyclic Aromatic Hydrocarbon Degrading
Phen	Phenanthrene
Ug/L	Microgram Per litre

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Author Contributions

Malaya Ranjan Pradhan: Conceptualizations, methodology, writing-original draft.

Namita Panigrahy: Supervision, Writing-review & editing, data curation, validation.

Abhimanyu Das: Resources, Visualization, formal analysis, investigation.

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Data Availability Statement

The data supporting the outcome of this research work have been reported in this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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<https://doi.org/10.46488/nept.2024.v23i03.021>

Biography



Malaya Ranjan Pradhan completed his M.Sc. in Botany from Central University of Jharkhand in 2019. Currently, he is pursuing his PhD at GIET University. He has also been working as a Guest Faculty in the Department of Botany at Government Autonomous College, Angul, for 5 years from 2019 to 2024. His academic interests include botany, environmental studies, rural development, and educational studies.



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Her research focuses on microbial bioremediation, Environmental Biotechnology and Industrial Microbiology for the treatment of industrial wastewater. She has authored over fifteen scientific articles and two book chapters from international publishers. She has presented and participated in numerous state, national, and international conferences, seminars, workshops, and symposiums. She also holds a published Indian patent

on algae-based nutritionally enriched fish feed. With extensive experience in environmental biotechnology and analytical techniques such as HPLC, GC–MS, FT-IR, and UV-Visible spectroscopy, she is dedicated to advancing environmental sustainability and mentoring students in life sciences research.



Abhimanyu Das is an accomplished academician, researcher, and youth development professional with extensive experience in higher education and community engagement. Holding multiple advanced qualifications, including M.Sc. in Botany, M.Ed., M.A. (Odia), Ph.D. in Education,

Ph.D. in Botany, PGDCA, PGDJMC, and PGDESD. He has established a distinguished career in the fields of Biological Sciences and Education. With 26 years of undergraduate teaching experience and 6 years of postgraduate teaching experience, Dr. Das has significantly contributed to academic excellence and student development. The primary area of research specialization is Biochemistry, with broader academic interests in Environmental Conservation and Youth Empowerment.

Research Field

Malaya Ranjan Pradhan: Botany, environmental studies, rural development

Namita Panigrahy: Microbial bioremediation, Environmental Biotechnology and Industrial Microbiology

Abhimanyu Das: Biochemistry, Environmental Conservation