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Investigations Into the Potential for Anthracene Decomposition of Novel Soil Bacterium, *Pseudomonas putida* P7

Kushalatha M.[1], Umesh H.R.[1,*]

Abstract

Bioremediation of hazardous contaminants holds immense importance for the preservation of a clean and healthy environment. Conversely, the accumulation of toxicants can be mitigated with the assistance of microorganisms capable of breaking down these harmful substances into benign molecules. Anthracene is frequently used as a model molecule for studies on PAH pollution because it is found in many carcinogenic PAHs. Therefore, the hunt for new microorganisms with the ability to break down anthracene is necessary. This work intended to the soil of a petroleum refinery near Bangalore was used in this study to isolate twenty different bacterial strains. These strains were cultured on solid nutrient agar and then purified by cultivation on mineral media supplemented with anthracene. Successful bacterial growth and anthracene degradation were confirmed through optical density measurements. The growth of bacterial isolate was observed in media at a pH of 7.0 and temperature of 28°C. However, the presence of certain nitrogen and carbon sources inhibited bacterial growth during anthracene breakdown. The efficacy of anthracene degradation by the isolate)1.8x1022 colony forming units/ml and optical density of 0.92) was determined to be 85.36% after one hundred twenty hours of incubation. Gas chromatography-Mass spectrometer analysis revealed the conversion of anthracene to anthraquinone and 9, 10-dihydroxy-anthracene. Through the biochemical tests and 16S rRNA analysis, the highly effective bacterial isolate was identified as P_7, a novel strain of Pseudomonas putida, with 98% homology. High-performance liquid chromatography analysis demonstrated the substantial reduction in anthracene levels (up to 83.25%) and the formation of reaction products by the bacterial strain P_7 sourced from the soil. The newly identified bacterial strain P_7 shows promise as an effective tool for bio-remediating hydrocarbon pollutants to protect the environment from polyaromatic hydrocarbon contaminants.

Keywords: Anthracene decomposition, strain, *Pseudomonas*, 16S rRNA, phylogenetic analysis

***Author for Correspondence**
Umesh H.R.
E-mail: umeshhr_76@ymail.c

[1]Research Scholar, Department of Biochemistry, Maharani Lakshmi Ammanni College for Women, Bangalore, Karnataka, India
[1]Assistant Professor, Department of Biochemistry, Maharani Lakshmi Ammanni College for Women, Bengaluru, Karnataka India

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INTRODUCTION

A considerable proportion of petroleum derivatives consists of polycyclic aromatic hydrocarbons (PAHs), which are fused-ring aromatic compounds. PAHs have been identified as significant atmospheric pollutants by the Environmental Protection Agency in the United States [1, 2]. These pollutants are predominantly present in industrial areas and released through vehicle emissions, posing a consistent threat to human health [3]. The health risks associated with PAHs and their derivatives include hypersensitivity, anemia, spleen enlargement, and various types of tumors [4]. Certain lower-mass PAHs have been linked to abnormalities in potency and notably high mortality rates in aquatic organisms [5, 6].

Anthracene is frequently used as a representative molecule for studying PAH pollution because of its presence in several carcinogenic PAHs. Its hydrophobic nature and tendency to accumulate in biological systems make it an ideal model molecule for investigating the absorption characteristics and degradation rates of PAHs in the environment [4]. Despite similarities in the structures of phenanthrene and anthracene in terms of the number of aromatic rings, anthracene exhibits distinct degradation patterns owing to its hydrophobic properties [7–9]. The presence of molecules such as anthracene in the environment must be rigorously addressed because of their significant adverse effects on animal life [3, 10]. Contaminated soils containing PAHs must be managed to prevent any detrimental impacts on the environment and human health. Microbial organisms may play a crucial role in converting pollutants into nonharmful components [4].

In recent years, bioconversion has gained importance as a cost-effective, viable, and safe method for remediating polluted sites [6, 11]. PAHs found in soil or sediment can be entirely converted by specific microbial strains. [11]. Studies have shown that specific bacteria, algae, and fungi can thrive on toxic PAHs and produce harmless byproducts [12]. Microbes demonstrating catechol 1,2-dioxygenase enzyme activity can utilize PAHs and break down catechol [13–15]. The capability of numerous microbes to degrade hydrocarbons has been documented over the years through metabolic and secondary-metabolic research [5, 16–18]. For the biotransformation of PAHs, more effective microbial strains need to be identified. Current research efforts have focused on identifying new strains of beneficial microbes that can decompose pollutants, leading to cleaner environments. To achieve this goal, investigations have been conducted to isolate soil bacteria capable of decomposing anthracene, and to identify strains that degrade PAHs in polluted soil environments.

METHODS

The techniques employed in the present study consist of the subsequent procedures.

Soil Sample Collection

Soil specimens tainted with oil were procured from a petroleum refinery located near Bangalore. The soil was extracted in its entirety and stored in a plastic zip-lock bag. Subsequently, the specimens were promptly transported to the facility and preserved at a temperature of 4°C until the procedures were performed.

Separation of Bacteria from Soil Sample

The soil sample, containing bacteria, underwent a filtration process utilizing a 2 mm filter to ensure uniformity in size. Following this, one gram of the filtrate was serially diluted in double-distilled water and cultured using the spread plate method after the filtration step [19]. Incubation of the cultured plates was performed at 28°C for 4 days until bacterial colonies became visible. Subsequently, the newly formed colonies were meticulously harvested and cultivated on culture plates containing nutrient agar. The inoculation process was repeated several times to obtain a pure bacterial culture. Finally, pure cultures of soil bacteria were preserved in slants containing anthracene at a temperature of 4°C.

Evaluation of the Separated Bacterial Strains in Solid and Liquid Media Having Anthracene

The separated bacterial strains were assessed in solid and liquid media containing anthracene. Bacterial isolates were examined for their ability to utilize anthracene as the sole source of energy and carbon necessary for degradation. A validated spread plate assay was employed to assess the efficiency of the isolated bacterial strains grown on a medium supplemented with anthracene [20, 21]. Anthracene, initially dissolved in acetone, was sprayed onto the culture plates. Following the vaporization of acetone, the bacteria were permitted to degrade anthracene, which remained in the medium. The evaluation in liquid medium involved the use of 250 ml conical flasks containing 10% bacterial inoculum in 100 ml of PNR medium and 1000 mg/L anthracene [22, 23]. The media were then incubated at 28°C, and bacterial cultures were observed for 120 hours at 24-hour intervals. The decomposition of anthracene and bacterial growth were measured at 600 and 540 nm, respectively, using a spectrophotometer.

Determination of Optimum Culture Conditions for the Purified Strains

The determination of optimal culture conditions for the purified strains involved standardization of various criteria, such as anthracene concentration, incubation pH, and temperature of the bacterial culture media. A range of parameters was explored, including anthracene concentrations from 200 to 1000 ppm, pH levels from 4 to 9, and temperatures from 25 to 50°C to achieve optimization. The effect of inoculum concentration on bacterial growth was assessed by varying the percentage of inoculum from 0 to 18% of the pure culture. In addition to anthracene, different carbon sources, such as sucrose, glucose, and fructose, have been investigated to evaluate their effects on bacterial culture development with respect to designated PAHs [24, 25]. Bacterial isolate P_7 was exposed to UV light for 15 min before being introduced into media containing varying concentrations of anthracene [26]. Throughout the experiment, samples of sterile liquid culture were collected daily to measure the optical density, with additional samples stored at 4°C to assess the decomposition potential. Colony forming units were counted every 24 h for 120 h to evaluate the sustainability of the isolated bacterial culture. To guarantee the precision and coherence of the findings, each experiment was performed three times.

Anthracene Decomposition Study

Decomposition experiments were conducted by utilizing 250 ml conical flasks containing 10% bacterial inoculum, 100 ml of PNR media, and 1000 mg/L anthracene mixed in acetone. After acetone was vaporized, 100 ml culture was transferred to conical flasks containing various concentrations of anthracene, 10% bacterial inoculum was added, and the conical flasks were kept at 30°C [27].

GC–MS Probing of Anthracene Extract

A Shimadzu fused silica capillary column was used for GC–MS probing of the anthracene extract. The column temperature was adjusted to 100°C for one minute, 15°C/min to 160°C, and 5°C/min to 300°C for eight minutes. With a split less duration of three minutes, the GC injector was maintained isothermally at 280°C. The flow rate of helium used as the carrier gas was regulated to one milliliter per minute by electronic pressure control. The GC–MS interface temperature was set at 280°C [27].

HPLC Analysis of Residual Anthracene

High-performance liquid chromatography was used to measure the sum of leftover anthracene and the decomposition of PAHs [14]. A dual-piston reciprocal pump system and reverse phase column [Waters] comprised a High-performance liquid chromatography (HPLC) with a diode-array detector. The injector had a 6-port binary valve system; the injection volume was set to 10 µL, and the isocratic eluent was pumped at a rate of one milliliter per minute. To calculate the PAH concentrations, the absorbance of anthracene at 254 nm was measured. The isocratic eluent had a pH of 3.6 and was composed of 70% acetonitrile and 30% water. After vacuum filtration, the mobile phase was ultrasonicated for 15 min. The standard was filtered through a membrane filter after dissolution in 10 mg/25 ml of the mobile phase. The sample was taken straight, passed through an injector into the system, and then filtered using a membrane filter [28, 29].

Molecular Characterization of the Bacterial Isolate, P_7

Bacterial isolate P_7, which is capable of decomposing anthracene, was subjected to molecular characterization using 16s rRNA sequencing. This method involves a series of well-established procedures, including separation of genomic DNA, electrophoresis using agarose gel, amplification using a thermocycler, DNA sequencing, and phylogenetic analysis. To separate the genomic DNA, the culture of the bacterial isolate was blended in lysis buffer, and the cellular remains were then treated with various solvent extractions and centrifugation steps. Genomic DNA was then condensed by mixing it with a solution of 0.1 volume of 3M sodium acetate at pH 7.0 and 0.7 ml of isopropanol.

Seventy percent ethanol was used to wash the resulting DNA precipitate. After diffusing the genomic DNA in TE buffer, RNAse enzyme was added to remove any remaining RNA. After preliminary evaluation, the 16S rRNA gene was the target of the forward and reverse primers used for PCR amplification of the genomic DNA.

A thermocycler was used for PCR amplification, with 30 cycles at 94°C for 1 min, 50°C for 1 min, and 70°C for 2 min, followed by a final extension of 7 min at 70°C. The amplified genomic DNA bands were analyzed by gel electrophoresis. The PCR product sequence was analyzed using the ABI3130xl platform [30–35]. The obtained nucleotide sequences were further analyzed for sequence homology and phylogenetic relationships using bioinformatics tools such as the BLAST program of NCBI and Clustal Omega of Mega11 online software [28, 29].

RESULTS

The study findings were documented, analyzed, and presented as outlined below.

Bacterial Isolation from Soil Sample

Twenty different bacterial strains were identified in soil samples. These bacterial strains were cultivated in both liquid and solid media, supplemented with anthracene.

Assessment of Distinct Bacterial Strains on Solid Medium Supplemented with Anthracene

Among the 20 bacterial strains grown on nutrient agar (NA) media supplemented with anthracene, 11 were identified as utilizing anthracene as their main energy source in PNR media containing anthracene (Table 1).

Table 1. Evaluation of bacteria isolated from anthracene amended media.

No.	Isolate	Anthracene concentration in ppm						
		0	***200***	***400***	***600***	***800***	***1000***	***1200***
1.	P_1	+++	+++	+++	+++	+++	+++	+++
2.	P_2	+++	+++	+++	+++	+++	+++	+++
3.	P_3	+++	+++	+++	+++	+++	+++	++
4.	P_4	+++	+++	+++	+++	+++	+++	++
5.	P_5	+++	+++	+	+	-	-	-
6.	P_6	+++	+++	+	+	-	-	-
7.	P_7	+++	++	+++	+++	+++	+++	+++
8.	P_8	+++	+++	++	+	+	-	-
9.	P_9	+++	+++	++	++	++	+	+
10.	P_{10}	+++	+++	++	++	++	+	+
11.	P_{11}	+++	+++	++	++	++	++	+
12.	P_{12}	+++	+++	++	++	++	++	++
13.	P_{13}	+++	+++	++	+	+	+	-
14.	P_{14}	+++	++	+	+	+	-	-
15.	P_{15}	+++	+++	++	+	+	-	-
16.	P_{16}	+++	+++	+++	+++	++	+	-
17.	P_{17}	+++	+++	+++	++	++	+	-
18.	P_{18}	+++	+++	++	++	+	+	+
19.	P_{19}	+++	+++	++	++	-	-	-
20.	P_{20}	+++	+++	+++	++	+	+	+

+++ = Rich growth
++ = Medium growth
+ = Poor growth
[-] = No growth

Evaluation of the Separated Bacterial Strains in Liquid Media

The bacterial strains that displayed the most promising outcomes on PNR-anthracene (solid) media were systematically assessed in liquid media. Of the 20 isolates, 11 demonstrated the highest optical density and were selected for further investigation (Table 2). The growth of bacteria in liquid media in

the presence of anthracene was monitored spectrophotometrically at 600 nm and 540 nm over a period of 120 h, with samples taken every 24 h to track bacterial growth and the degradation of anthracene in PNR media (Figure 1). The isolate that exhibited the highest OD at 600 nm was chosen for subsequent analysis.

Table 2. Bacterial culture growth on anthracene after 3 days incubation in a large test tube (500 ppm) PNR.

No.	Strain	OD_{600nm}
1.	P_1	0.199
2.	P_2	0.321
3.	P_3	0.211
4.	P_4	0.159
5.	P_7	0.425
6.	P_9	0.269
7.	P_{10}	0.223
8.	P_{11}	0.265
9.	P_{12}	0.169
10.	P_{18}	0.243
11.	P_{20}	0.231

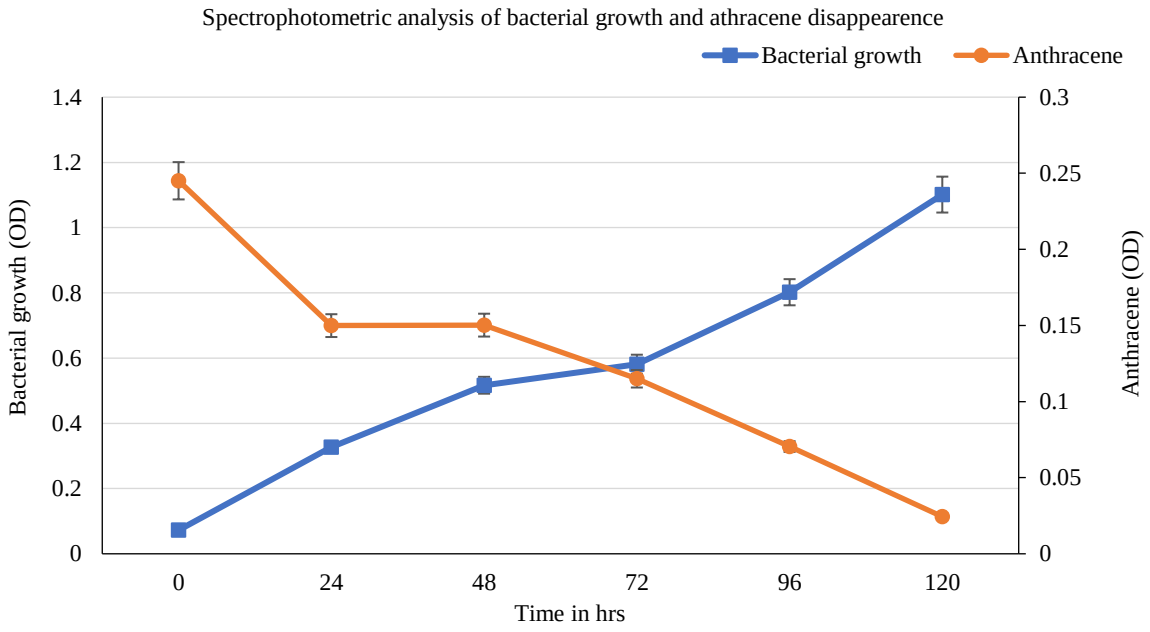
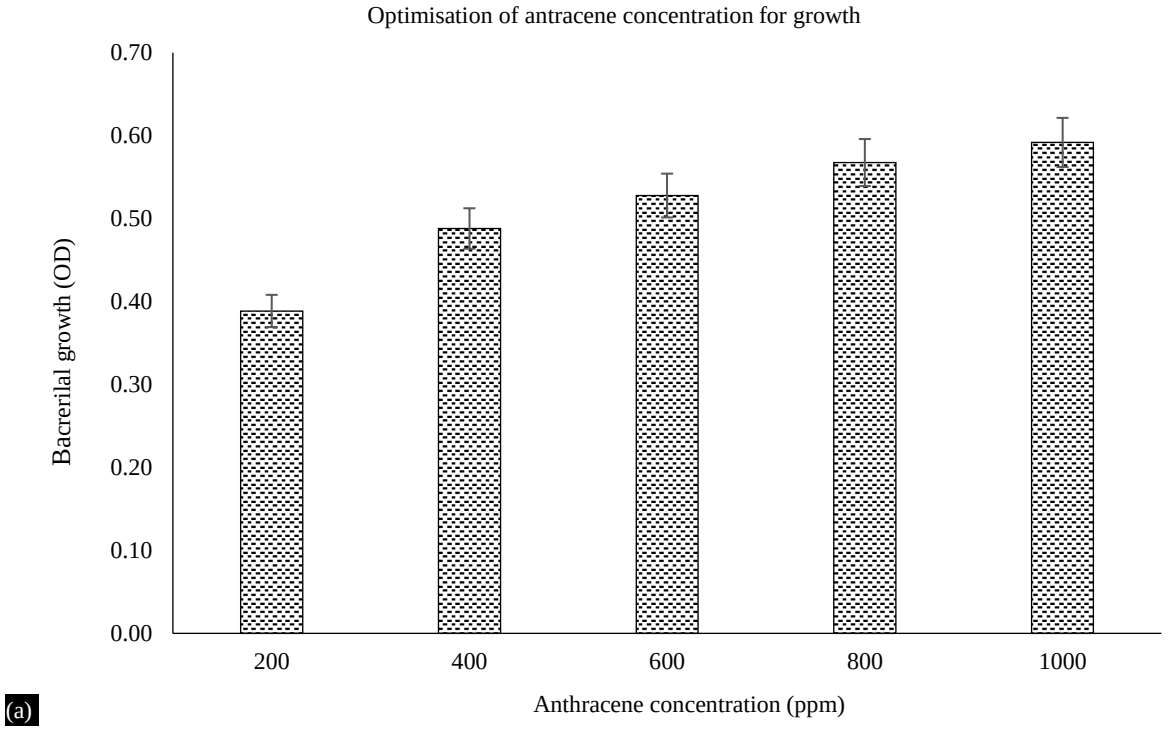


Figure 1. Analysis of anthracene disappearance and bacterial growth using UV spectrophotometry. The optical density of bacterial growth was observed at 600 nm; the Optical density of anthracene concentration was observed at 540 nm. Each data point represents the mean triplicated data with ± S.E. The data points with similar letters are not significantly different at $P<0.05$.

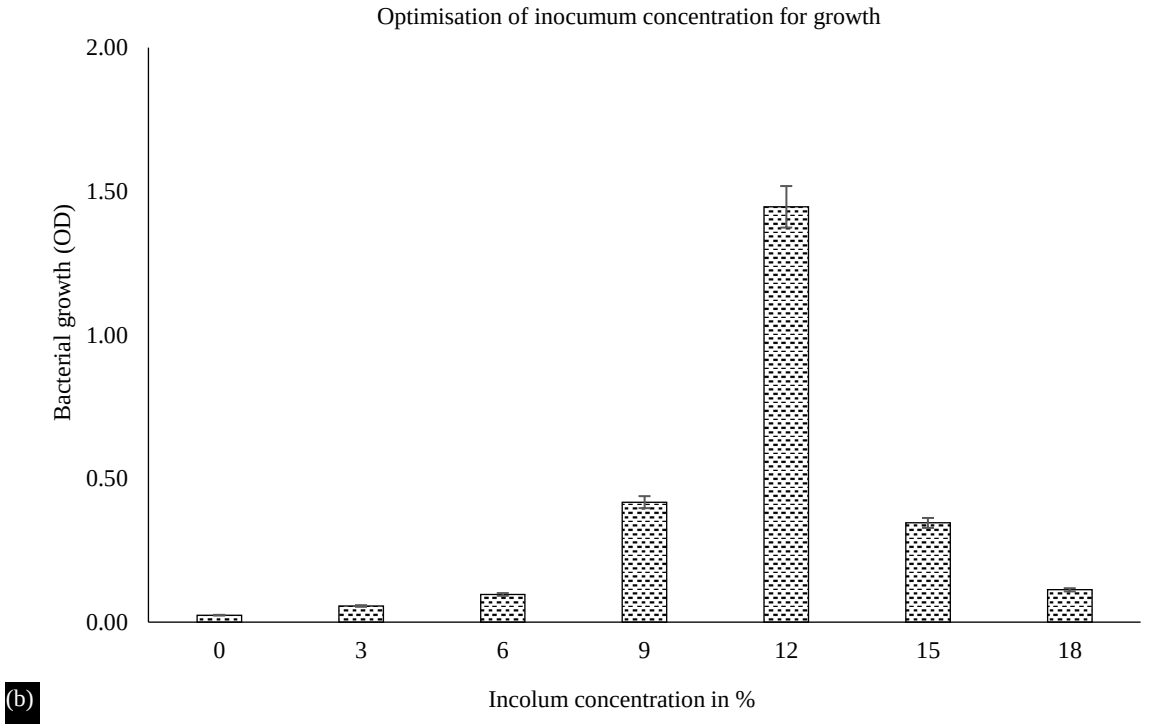
The information provided below presents the results obtained from the standardization of various specific frameworks, including anthracene concentration, pH level, media temperature, alternate nitrogen and carbon sources, inoculum size, and UV light.

Effect of Anthracene Concentration and Bacterial Inoculum on Growth Optimization

In this study, we determined that the optimal concentration of anthracene for promoting growth was 1000 ppm for strain P_7 (Figure 2(a)). Additionally, the highest rate of decomposition was achieved at a bacterial inoculum concentration of 12% vv^{-1}, as illustrated in Figure 2(b).



(a)

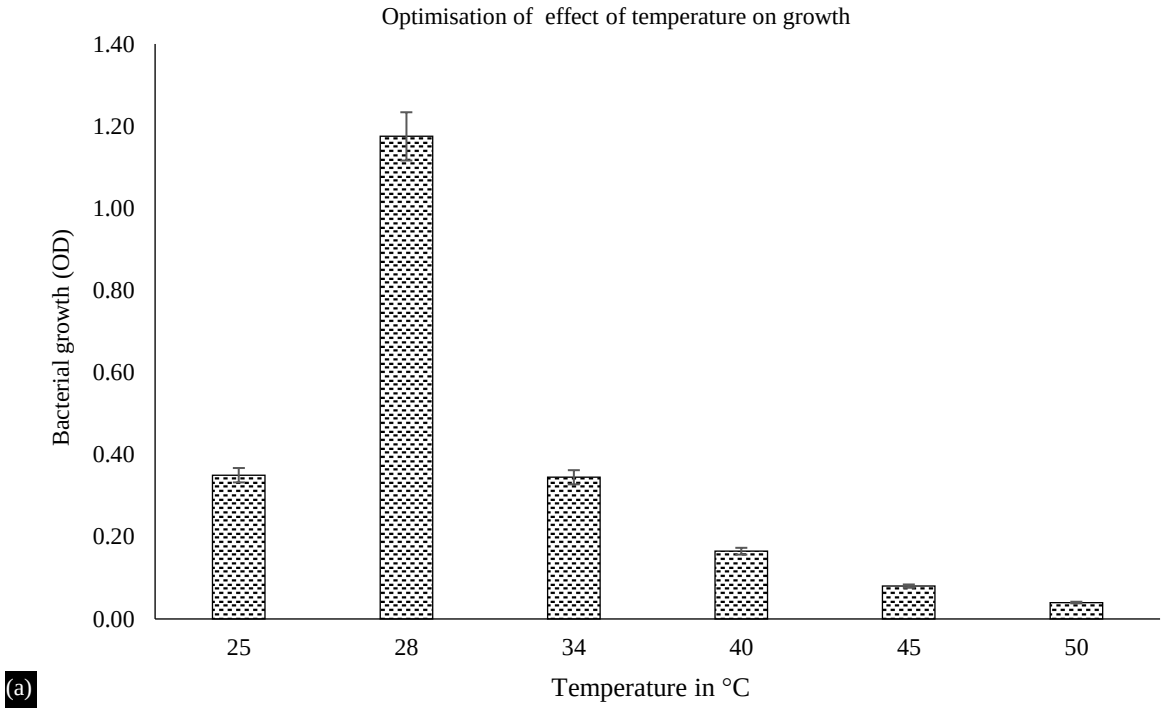


(b)

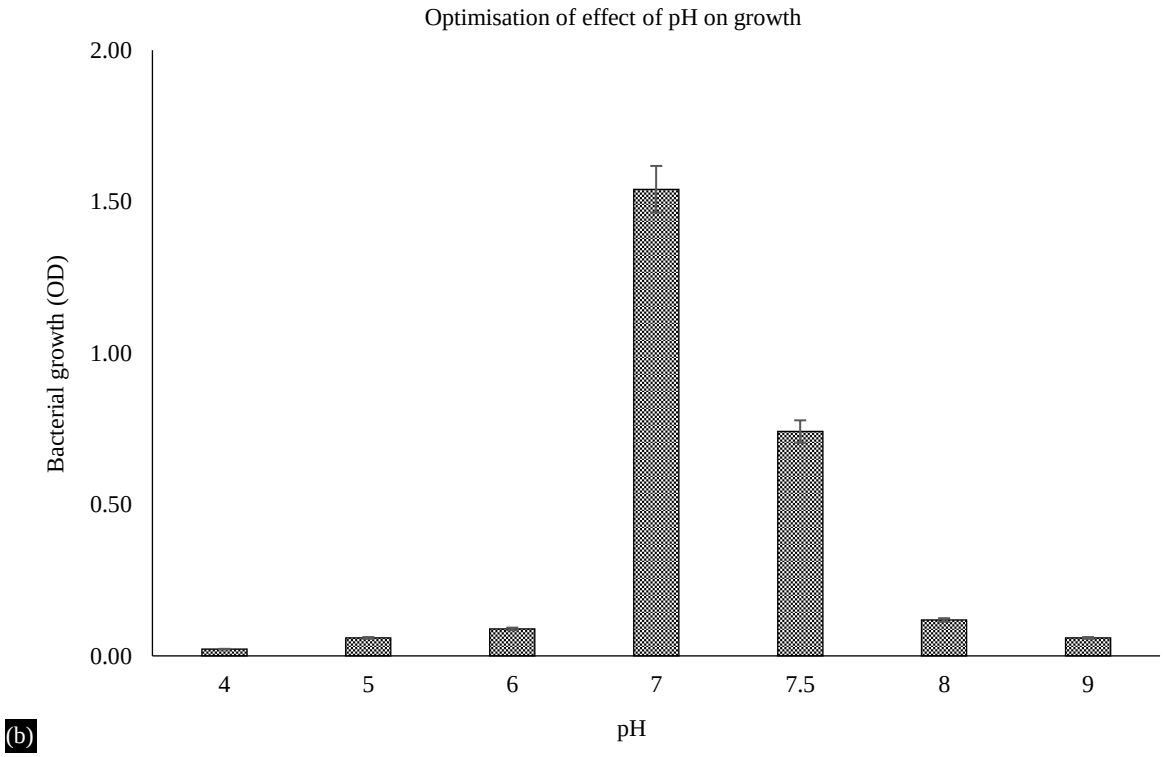
Figure 2. Enhancing the environment to support the development of bacterial isolate P7. (a) Optimization of anthracene concentration v/s growth of isolates P_7. (b) Represents optimization of inoculum % v/s growth of isolate P_7.

Effect of Temperature and pH for Optimization of Anthracene Decomposition

In this study, the effects of pH and temperature on the increase in anthracene degradation were examined. Bacterial isolate P7 exhibited the highest growth rate at 28°C, as illustrated in Figure 3(a), and at a pH of 7, as shown in Figure 3(b).



(a)

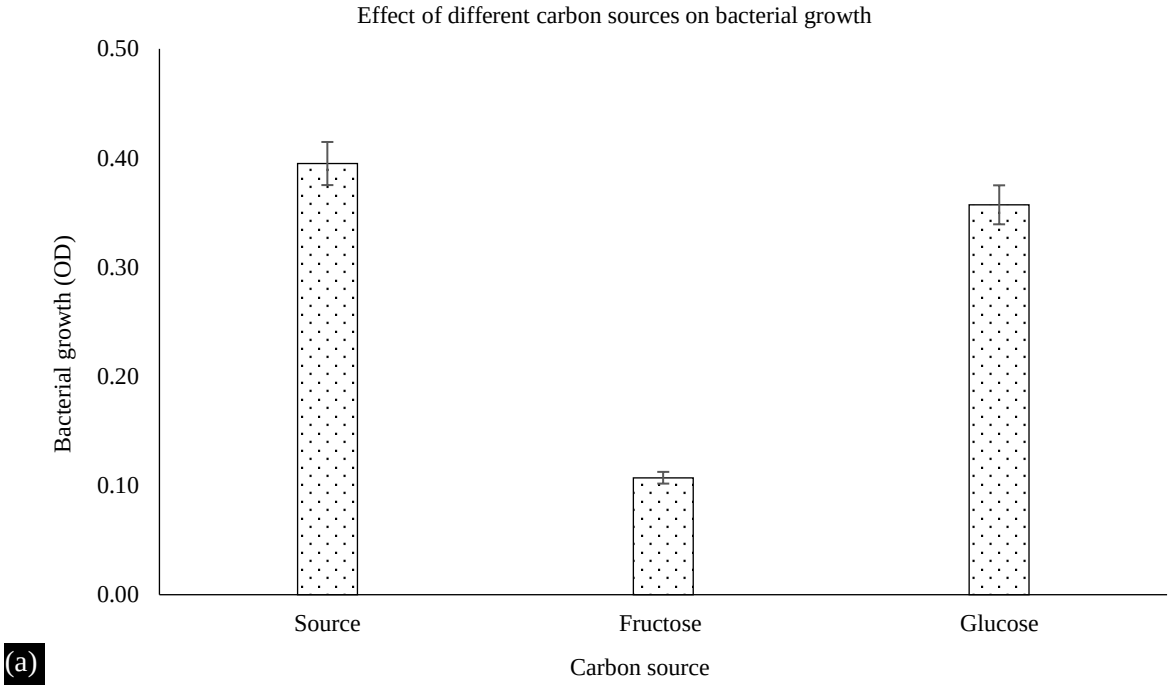


(b)

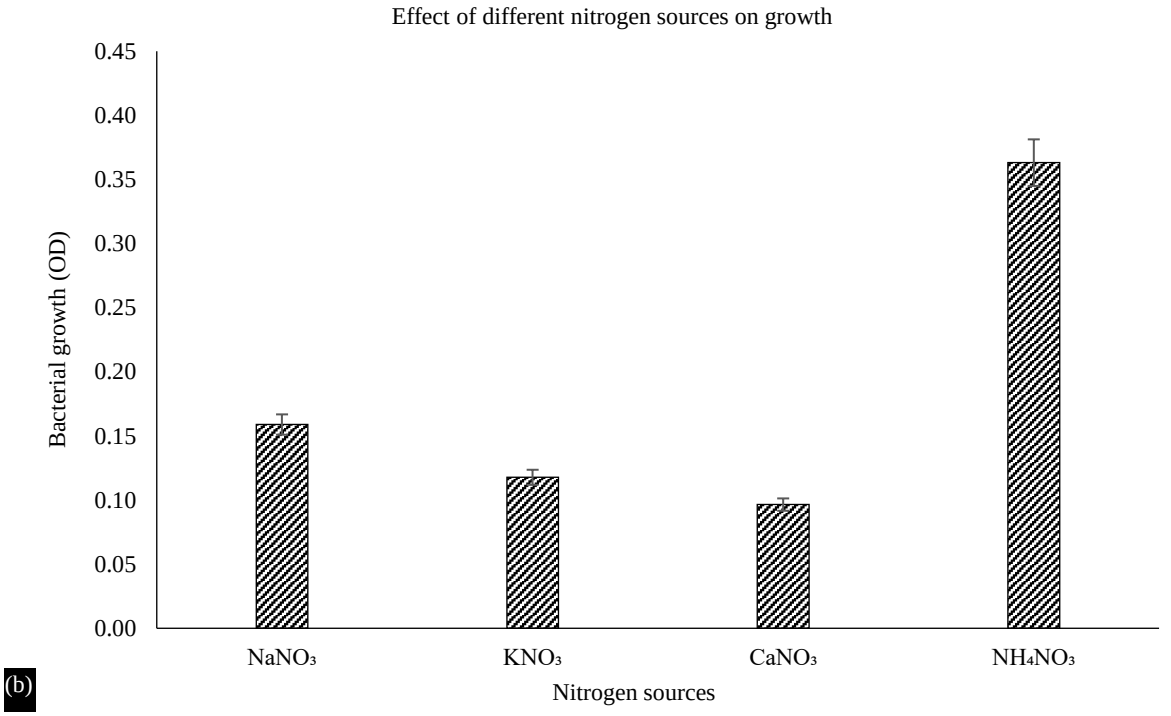
Figure 3. Conditions for P7 bacterial isolate growth are optimized. Figure 3(a) depicts the growth of isolate P7 as a function of optimization temperature. The optimization of media pH in relation to isolate growth is shown in Figure 3(b). With ± standard error, each bar displays the mean of three sets of data. At $P<0.05$, there is no significant difference between the bars with comparable letters.

Effect of Various Nitrogen and Carbon Sources on Culturing of Strain P_7

The growth of strain P_7 was hindered by the nitrogen and carbon sources tested, except for ammonium nitrate and sucrose, which resulted in the enhanced growth of the isolated strain (Figure 4(a) and (b)).



(a)



(b)

Figure 4. The growth of bacterial isolate P7 in response to various carbon and nitrogen sources. Figure 4(a) illustrates how various carbon sources affect isolate P7's growth, while Figure 4(b) shows how various nitrogen sources affect isolate P7's growth. The mean of the triplicated data is shown by each bar with ±S.E. When $P<0.05$, there is no significant difference between the bars with comparable lettering.

Effect of UV Light on Anthracene Utility

The growth outcome of the P7 isolates exposed to UV light showed significant improvement with increasing concentrations of anthracene when compared to the control group. This observation is illustrated in Figure 5.

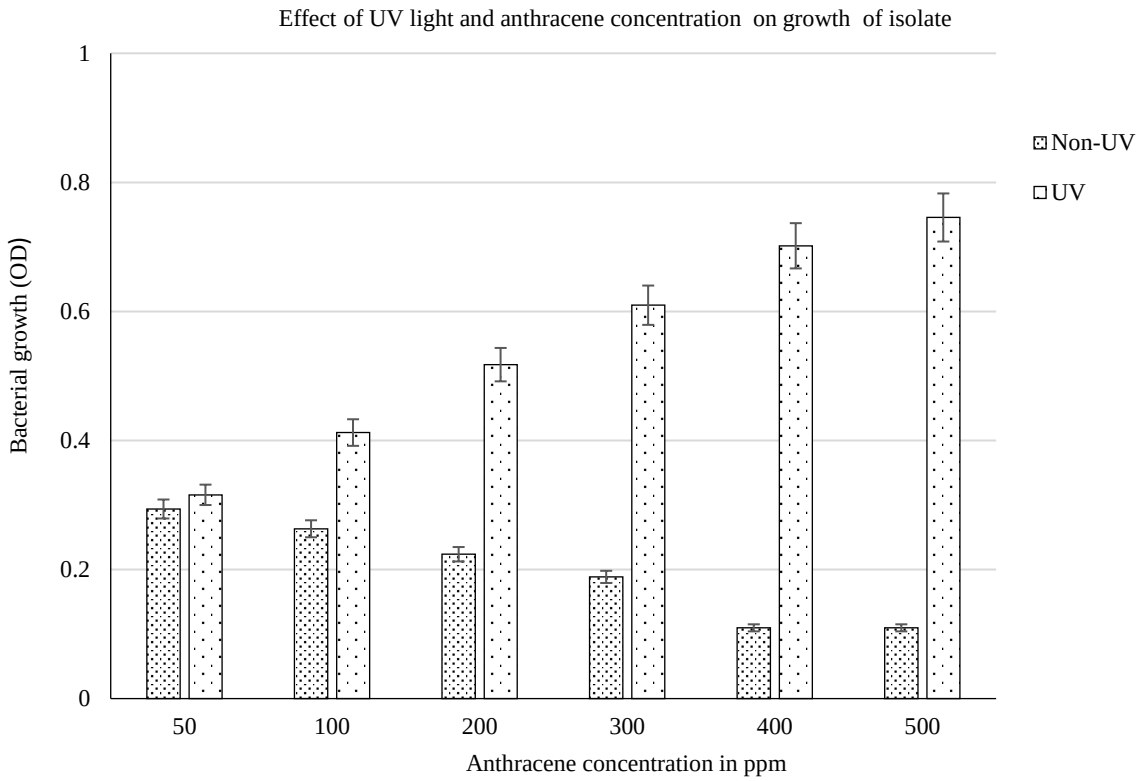


Figure 5. shows how the concentration of anthracene and UV light affect the growth of isolate P7. Each bar shows the triplicate data mean ±S.E. At $P<0.05$, there was no significant difference between the bars with similar letters.

CFU Per Ml of Isolated Bacteria

To assess the viability of the bacteria, samples were collected at 24-hour intervals for 120 h. The results, presented in Table 3, indicate that the bacterial cells exhibited significant growth, with the initial concentration of 1.3×10^5 per ml increasing to 1.8×10^{22} after 120 h.

Table 3. $CFUmL^{-1}$ of the bacterium isolates P_7.

Time (h)	0	24	48	72	96	120
$CFUmL^{-1}$	1.3×10^5	3.1×10^{10}	4.5×10^{13}	1.2×10^{15}	2.6×10^{19}	1.8×10^{22}

Bacterial Decomposition of Anthracene

The breakdown of anthracene by the bacteria and the optical density of the bacterial culture is illustrated in Figure 6. Within 120 h, strain P7 decomposed 85.36% of anthracene. The bacterial strain P_7 effectively decomposed anthracene during the incubation period from 24 to 96 h, with the bacterial culture reaching its peak growth (OD 0.92) at the 120-hour mark. An increase in the growth of strain P_7 was observed through an optical density range of 0.5–0.92 between 96 and 120 hours, with only 52.59% of anthracene being utilized. The confirmation of anthracene biodegradation by strain P_7 was carried out through GC–MS analysis, which revealed the presence of 9,10-dihydroxy anthracene and anthraquinone as the identified products.

HPLC Analysis

HPLC analysis was used to track the degradation of anthracene and the production of reaction byproducts while treating a soil sample with the bacterial isolate P_7. The retention time profiles exhibited a consistent and rapid decline in the anthracene concentration (Figure 7). Upon evaluating degradation efficiency, it was determined that the isolate achieved an 83.25% degradation rate.

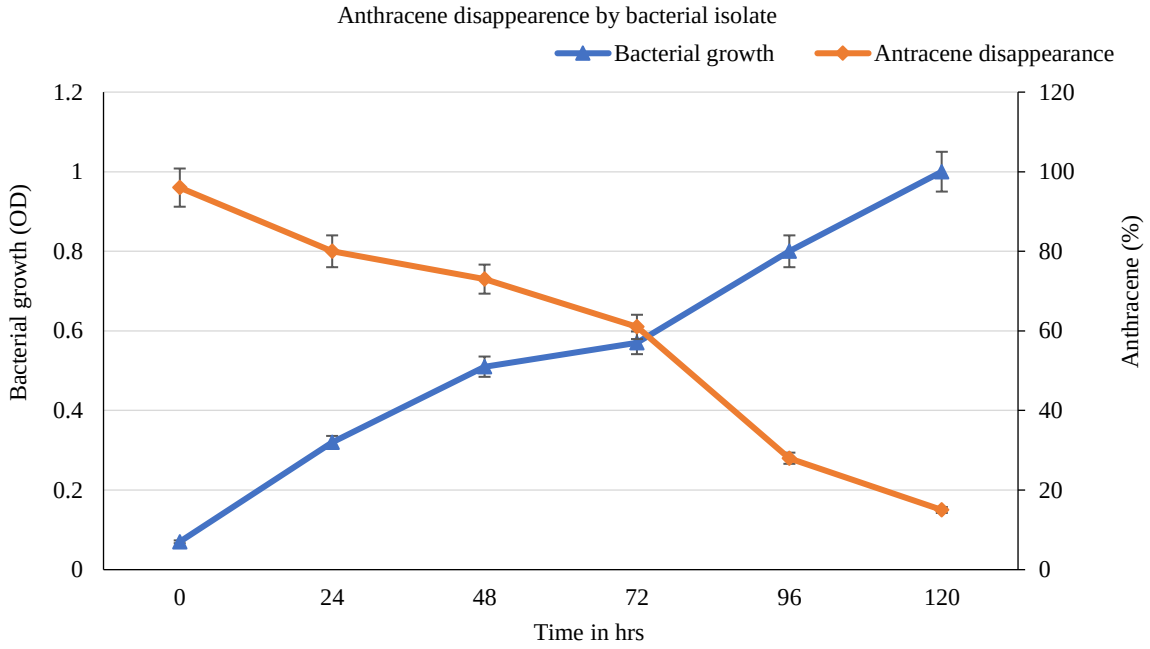
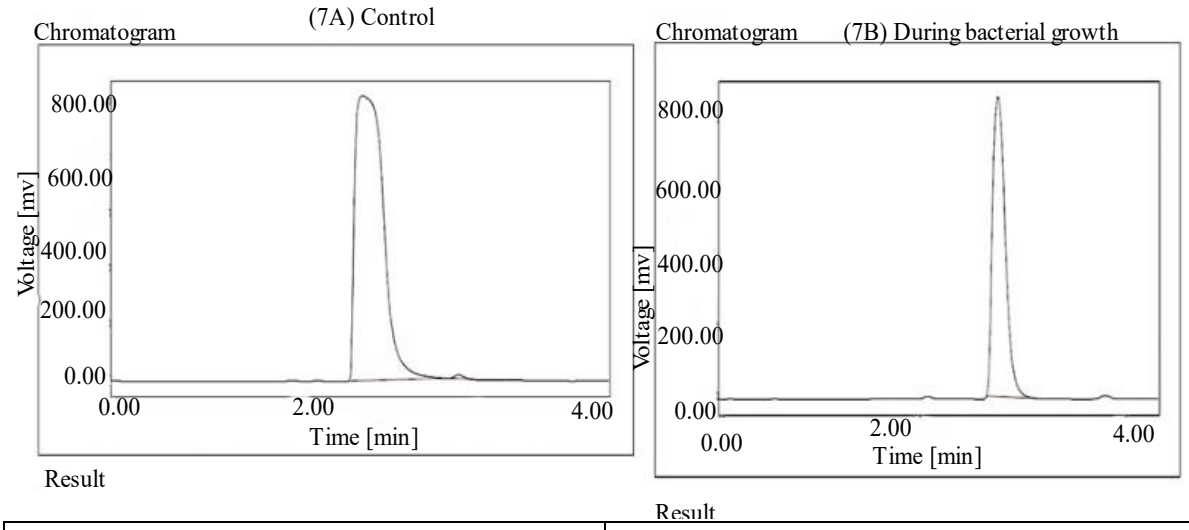


Figure 6. Bacterial isolate P7 disappears along with anthracene. With ±S.E., each bar shows the mean of three sets of data. If P is less than 0.05, there is no significant difference between the bars with similar letters.



No.	Name	RT (Min)	Area (mV*s)	Height (mV)	Amount
1		2.7833	22260.9746	1024.8849	0.0000
2		3.8333	92.2491	14.3552	0.0000
Sum			22353.2246	1039.2401	0.0000

No.	Name	RT (Min)	Area (mV*s)	Height (mV)	Amount
1		2.7833	7640.3364	865.2044	0.0000
Sum			7640.3364	865.2044	0.0000

Figure 7. HPLC analysis of anthracene degradation: (a) control, (b) during growth of the bacterium. A significant amount of anthracene degradation was observed and reported.

Identification of Bacterial Isolate P_7

Bacterial isolate P_7 was identified at the genus level using biochemical tests. The findings indicated that the isolate was gram-negative, motile, rod-shaped, catalase-positive, and negative for urease, starch hydrolysis, and citrate (Table 4). The colony morphology on the agar plates displayed smooth white colonies with entire edges, leading to the identification of the isolate as *Pseudomonas* sp.

Table 4. Features of the isolated anthracene-decomposing bacteria's morphology and metabolism.

Microscopy	**Biochemical tests**				
Rods	Gram's Test	Catalase	Starch hydrolysis	Citrate	Urease
	-ve	+ve	-ve	-ve	-ve

Characterization of the Anthracene Utilizing Bacterial Isolate P_7 with Phylogenetic Analysis

Bacterial strain P_7, which is capable of decomposing anthracene, was characterized by molecular identification. This involved amplifying the 16S rRNA gene sequence of the strain by PCR. The amplified genes were visualized using horizontal gel electrophoresis with agarose, as shown in Figure 8. Gel electrophoresis results indicated that the size of the PCR-amplified gene products of the strain was approximately 1380 bp. After the PCR product sequence was established, it was formatted into the FASTA format. Next, the NCBI nucleotide database was examined for this sequence using the BLAST-N software. The comparison revealed a 97.8% match between isolate P7 and *Pseudomonas putida* strain ICMP 2758. Finally, the nucleotide sequence was submitted to NCBI for accession numbers. Furthermore, a phylogenetic tree analysis was conducted to study the relationship between the isolated anthracene-decomposing bacteria, P_7, and the results are presented in Figure 8.

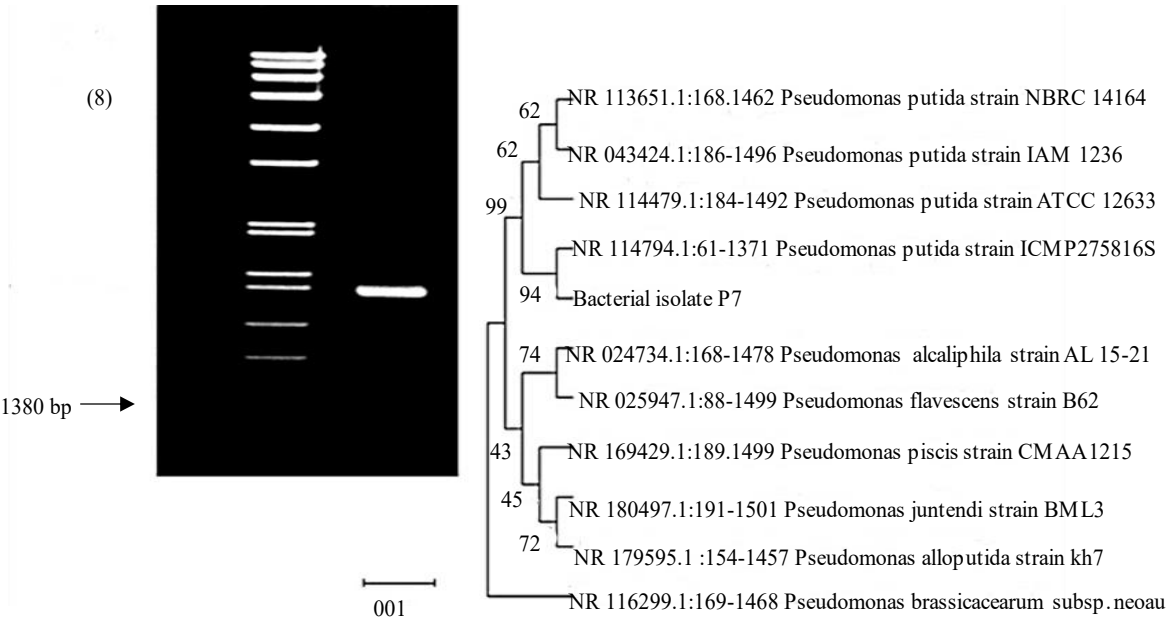


Figure 8. Study of strain P7's phylogenetic relationship. By first selecting the topology with the superior log likelihood value, the evolutionary history was inferred using the Tamura-Nei model Maximum Composite Likelihood (MCL) technique. Using the number of substitutions per site as a measure for branch lengths, the tree is drawn to scale. In MEGA11, analyses of evolution were performed.

DISCUSSION

Polycyclic aromatic hydrocarbons (PAHs) are major pollutants that pose a health hazard to the biosphere. In this study, a bacterial isolate named P_7 was identified in a soil sample collected from a petroleum refinery area near Bangalore.

Anthracene is a PAH that can be used with this isolate. It was also noted that when the anthracene concentration in the medium rose from 0-1000 ppm, microbial isolate growth was enhanced. Previous research carried out in a medium enriched with anthracene [31] revealed comparable results.

The growth and activity of bacteria in PAH-enriched media can be significantly affected by several variables, such as pH, temperature, and nutrient availability. The temperature of the medium can affect

both the solubility [36] and the decomposition of anthracene-like compounds by bacteria. Higher temperatures can increase the bioavailability of PAHs but can also reduce the solubility of oxygen, which is detrimental to aerobic bacteria. Therefore, previous studies have primarily focused on optimizing the temperature of the medium. Similarly, in the current study, elevated anthracene decomposition occurred at 28°C, which is the most favorable temperature for the growth of the newly discovered isolate. Optimal culture conditions can stimulate bacteria to produce and release enzymes into the surrounding environment, which can effectively decompose pollutants. Inappropriate temperatures can hinder enzyme function by impeding access to the substrate due to low solubility and low temperature or by affecting the three-dimensional structure of the enzymes at higher temperatures. Most bacteria exhibit optimal growth and activity within a specific pH range, and deviations from this range negatively affect cellular composition, metabolism, and culturing [3]. The results of this study indicated that the bacterial isolate P_7 demonstrated heightened activity and culturing efficiency at pH 7 due to the balanced movement of ions across cell membranes. Conversely, fluctuations in pH levels can disrupt ionic equilibrium, leading to disturbances in the culture and cellular metabolism of isolate P7, resulting in reduced decomposition of anthracene-like compounds.

Although certain bacteria can utilize anthracene as an energy and carbon source [37], the presence of carbohydrates in the medium can hinder this capability [38]. Our research supports these findings by demonstrating that bacterial isolate P_7 was able to utilize anthracene as the sole carbon source in the presence of carbohydrates. The assimilation of glucose and other carbohydrates as carbon sources can suppress the activity of anthracene-degrading enzymes in bacteria [39]. In contrast, the growth and anthracene decomposition potential of the bacterial isolate P_7 were not compromised when cultured with various nitrogen sources such as KNO_3, $NaNO_3$, $CaNO_3$, and NH_4NO_3. This suggests that the presence of different carbon and energy sources in this study acted as competitors to anthracene, resulting in lower bioavailability of the compound.

The water-repelling properties of PAHs are responsible for their reduced bacterial biodecomposition. The rate of removal of toxic substances depends on the type of decomposing strain, both internal and external factors, and the chemical properties of the decomposed molecule. Various microorganisms, including algae, fungi, and bacteria, can decompose PAHs via unique metabolic reactions [16]. These microbes must be acclimatized to optimal growth conditions to facilitate rapid culture [40, 41]. Additionally, adaptations in bioavailability are crucial for microbes to establish connections with pollutants and increase their accessibility [42].

The presence of different pollutants can negatively affect the decomposition capabilities of bacteria, which is a cause for concern. Despite extensive research, there is limited knowledge available regarding the fundamental aspects of microbe-plant associations in relation to the phytochemical remediation of anthracene-like molecules [43, 44]. In the current study, 82.29% of anthracene was decomposed within 120 h in the PNR culture media, surpassing previous studies where only 74.8% decomposition was achieved after 10 days of incubation in the BSM medium. Furthermore, the comprehensive decomposition of anthracene into autoclaved soil by *Burkholderia* sp. took approximately 20 days [10. 45]. The rate of breakdown and bio-enhancement are greatly influenced by the chemical makeup of the molecule and the specific type of bacteria involved.

The results of this study confirmed the hypothesis that strain P_7 efficiently decomposes anthracene within 7 days, surpassing previous research findings [42, 46]. Through 16S rRNA analysis, bacterial isolate P_7 was identified as a highly promising new strain of *Pseudomonas putida*, exhibiting 98% homology [47]. HPLC analysis revealed a significant decrease in anthracene levels (up to 83.25%) and the generation of decomposed compounds in soil samples treated with strain P_7.

CONCLUSION

Pseudomonas putida P_7 has shown great potential for the bioremediation of polycyclic aromatic hydrocarbons (PAHs), such as anthracene, which is a major contaminant in the partial burning processes

carried out by petroleum factories. Through our investigation, we discovered a new bacterial strain capable of decomposing anthracene. This novel isolate utilized anthracene as its sole source of carbon and energy, making it a promising candidate for the biodegradation of other PAHs. Specifically, *Pseudomonas putida* P7 has demonstrated effectiveness in breaking down harmful aromatic hydrocarbons and helping restore the biosphere by removing PAH contaminants. Despite the progress made, there is still a pressing need for accurate and innovative methods to address the challenges posed by complex polyaromatic hydrocarbon blends.

Abbreviations

GC–MS: Gas chromatography-Mass spectrometer
CFU: Colony forming units
OD: Optical density
PAH: Poly aromatic hydrocarbons
nm: nanometer
HPLC: High-performance liquid chromatography
PCR: Polymerase chain reaction
RNase: Ribonuclease enzyme
BLAST: Basic local alignment search tool
NCBI: National center for biotechnology information
ppm: Parts per million
UV: Ultraviolet
KNO_3: Potassium nitrate
$NaNO_3$: Sodium nitrate Ammonium nitrate calcium nitrate
NH_4NO_3: Ammonium nitrate calcium nitrate
$CaNO_3$: Ammonium nitrate calcium nitrate
FASTA: Fast adaptive shrinkage threshold algorithm

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Author's Contributions

HR carried out sample collection, separation, and evaluation of novel anthracene-degrading bacterial strains and molecular characterization of the bacterial isolate P7. KL participated in the anthracene decomposition study, GC–MS probing, and HPLC analysis of anthracene decomposition by bacterial isolate P7. Both HR and KL participated in the design of the study and drafting of the manuscript. All the authors have read and approved the manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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