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Article: Investigations Into The Potential for Anthracene Decomposition of Novel Soil Bacterium, *Pseudomonas putida* P7

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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 antracene 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.

Key words: Anthracene decomposition, strain, Pseudomonas, 16S rRNA, Phylogenetic analysis.

1. Introduction

A considerable proportion of petroleum derivatives consist of polycyclic aromatic hydrocarbons [PAHs], which are fused ring aromatic compounds. PAHs have been identified by the Environmental Protection Agency in the United States as significant pollutants in the atmosphere [1,2]. These pollutants are predominantly present in industrial areas and are released through vehicle emissions, posing a consistent threat to human health [3]. The health risks associated with PAHs and their derivatives include conditions such as 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 due to its presence in many 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 rate of PAHs in the environment [4]. Despite similarities in structure between phenanthrene and anthracene in terms of the number of aromatic rings, anthracene exhibits distinct degradation patterns due to its hydrophobic properties [7-9]. The presence of molecules like anthracene in the environment must be addressed rigorously due to their significant adverse effects on animal life [3, 10]. Contaminated soils containing PAHs need to be managed to prevent

any detrimental impact on the environment and human health. Microbial organisms may play a crucial role in converting pollutant PAHs into non-harmful components [4].

In recent years, bioconversion has gained importance as a cost-effective, viable, and safe method for remediation of polluted sites [6, 11]. PAHs found in soil or sediments 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 by-products [12]. Microbes demonstrating catechol 1,2-dioxygenase enzyme activity are capable of utilizing PAHs and breaking 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 effecient microbial strains still need to be found. Current research efforts are 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.

2. Methods

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

2.1. Soil sample collection

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

2.2. 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 subjected to serial dilution in double distilled water and then cultured using the spread plate method post the filtration step [21]. Incubation of the cultured plates took place at a temperature of 28˚C for duration of 4 days until bacterial colonies became visible. Subsequently, the newly formed colonies were meticulously harvested and cultivated in culture plates containing nutrient agar. It was necessary to repeat the inoculation process several times in order to achieve a pure bacterial culture. Finally, the pure cultures of soil bacteria were preserved in slants with anthracene at a temperature of 4˚C.

2.3. Evaluation of the separated bacterial strains in solid and liquid media having anthracene

The assessment of the separated bacterial strains on solid and liquid media containing anthracene was conducted. The 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 in growing on a medium supplemented with anthracene [22, 23]. Anthracene, initially dissolved in acetone, was sprayed onto the culture plates. Following the vaporization of acetone, bacteria were permitted to degrade the anthracene, which remained on the medium. The evaluation in liquid medium involved the use of 250 ml conical flasks containing 10% bacterial inoculum in 100 ml of PNR media and 1000 mg/L anthracene [19, 20]. The media were then incubated at 28˚C, and the bacterial cultures were observed for 120 hours with 24-hour intervals. The decomposition of anthracene and bacterial growth were measured at 600 nm and 540 nm, respectively, using a spectrophotometer instrument.

2.4. Determination of optimum culture conditions for the purified strains

The determination of optimal culture conditions for the purified strains involved the 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 spanning from 4 to 9, and temperatures ranging from 25 to 50˚C to achieve optimization. The impact 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 were investigated to evaluate their effects on bacterial culture development with respect to designated PAHs [24, 25]. The

bacterial isolate P_7 was exposed to UV light for fifteen minutes before being introduced into media containing varying anthracene concentrations [26]. Throughout the experiment, samples of sterile liquid culture were collected daily to measure optical density, with additional samples stored at 4˚C to assess decomposition potential. Colony-forming units were counted every 24 hours for a period of 120 hours to evaluate the sustainability of the isolated bacterial culture. To guarantee precision and coherence of findings, every experiment was carried out three times.

2.5. Anthracene decomposition study

Decomposition experiment was conducted by utilizing 250 ml conical flasks having 10% of bacterial inoculum, 100 ml of PNR media and 1000 mg/L antrhracene mixed in acetone. After acetone vaporized, 100ml culture was transferred to the conical flasks having various concentrations of anthracene and 10% inoculums of bacteria was included and conical flasks were kept at 30^{o}C [27].

2.6. GC-MS probing of anthracene extract

Shimadzu fused silica capillary column was used for GC-MS probing of anthracene extract. The temperature of the column 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 carrier gas was regulated to one milliliter per minute by electronic pressure control. The GC–MS interface temperature was set at 280˚C [27].

2.7. HPLC analysis of residual anthracene

High performance liquid chromatography investigation was utilized to measure the sum of leftover anthracene and decomposition of PAHs [14]. A dual piston reciprocal pump system and a reverse phase column [Waters] make up an HPLC with a diode-array detector. The injector has 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 concentrations of PAHs, anthracene's absorbance at 254 nm was utilized. 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 subjected to 15 minutes of ultra sonication. The standard was filtered through a membrane filter after being dissolved in 10 mg/25 ml of mobile phase. The sample was taken straight, put through an injector into the system, and then filtered using a membrane filter [28, 29].

2.7. Molecular characterization of the bacterial isolate, P_7

The bacterial isolate P_7, which is capable of decomposing anthracene, was subjected to molecular characterization using 16s rRNA sequencing. This method involved a series of well-established procedures, including the 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 a lysis buffer, and the cellular remains were then treated with various solvent extraction and centrifugation steps. The 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 resultant DNA precipitate. After diffusing the genomic DNA in TE buffer, the RNAse enzyme was added to remove any remaining RNA. After a preliminary evaluation, the 16S rRNA gene was the target of particular forward primers and reverse primers used for PCR amplification of the genomic DNA.

The thermocycler was used for PCR amplification, with 30 cycles at 94°C for 1 minute, 50°C for 1 minute, and 70°C for 2 minutes, followed by a final extension of 7 minutes at 70°C. The amplified genomic DNA band was then checked using 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 the clustal omega of Mega11 online software [28, 29].

3. Results

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

3.1. Bacterial isolation from soil sample

Twenty different bacterial strains were identified in the soil sample. These bacterial strains were cultivated on both liquid and solid media that were supplemented with anthracene.

3.2. 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 also 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

3.3. 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. Out of the twenty isolates, eleven 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 600nm and 540nm over a period of 120 hours, with samples taken every 24 hours to track bacterial growth and the degradation of anthracene in PNR media [Fig.1]. The isolate that exhibited the highest OD at 600nm was chosen for subsequent analysis.

No	Strain	OD_{600nm}
1.	**P_1**	**0.199**
2.	**P_2**	**0.321**
3.	**P_3**	**0.211**
4.	**P4**	**0.159**
5.	**P7**	**0.425**
6.	**P9**	**0.269**
7.	**P10**	**0.223**
8.	**P11**	**0.265**
9.	**P12**	**0.169**

10.	**P18**	**0.243**
11.	**P20**	**0.231**

Table 2 Bacterial culture growth on anthracene after 3 days incubation in large test tube [500ppm] PNR

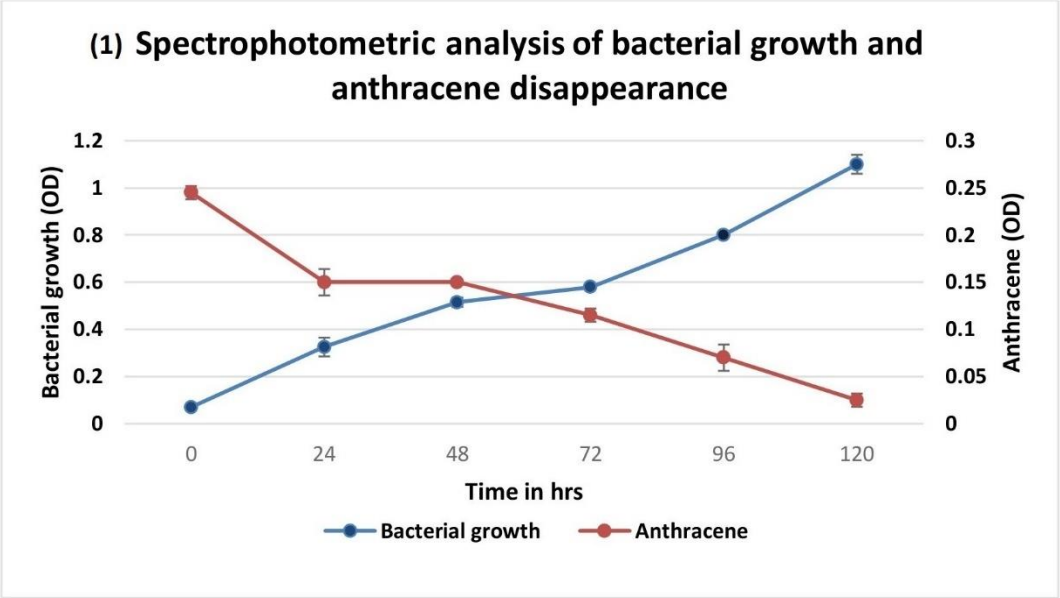


Fig 1 Analysis of anthracene disapparance and bacterial growth using UV spectrophotometry. The optical density of bacterial growth was observed at 600nm; Optical density of anthracene concentration was observed at 540nm. Each data point represents the mean triplicated data with ± S.E. The datapoint with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

The below provided information presents the results obtained from the standardization process of various specific frameworks, including anthracene

concentration, pH level, media temperature, alternate nitrogen and carbon sources, inoculum size, and UV light.

3.3.1. Effect of anthracene concentration and bacterial inoculum on growth optimization

In this study, it was determined that the optimal concentration of anthracene for promoting growth was 1000 ppm for strain P_7 [Fig.2A]. Additionally, the highest rate of decomposition was achieved with a bacterial inoculum concentration of 12% vv^{-1}, as illustrated in Fig.2B.

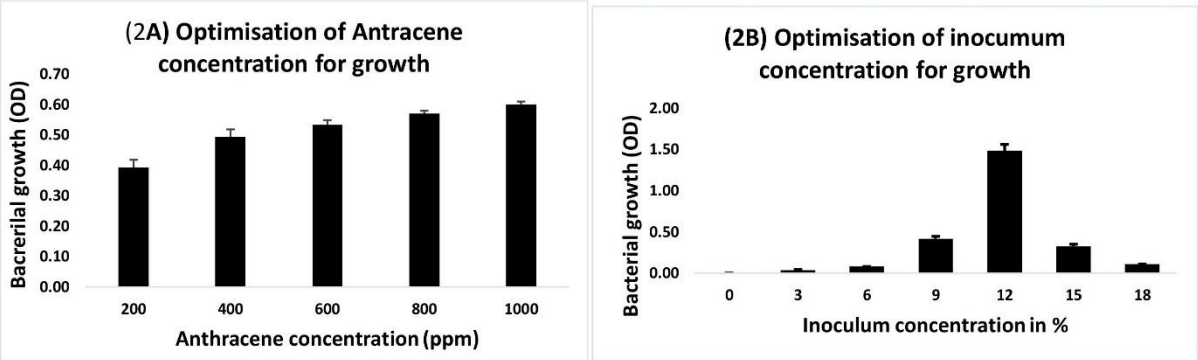


Fig.2 Enhancing the environment to support the development of bacterial isolate P7. 2A optimization of anthracene concentration v/s growth of isolates P_7. 2B represents optimization of inoculum % v/s growth of isolate P_7.

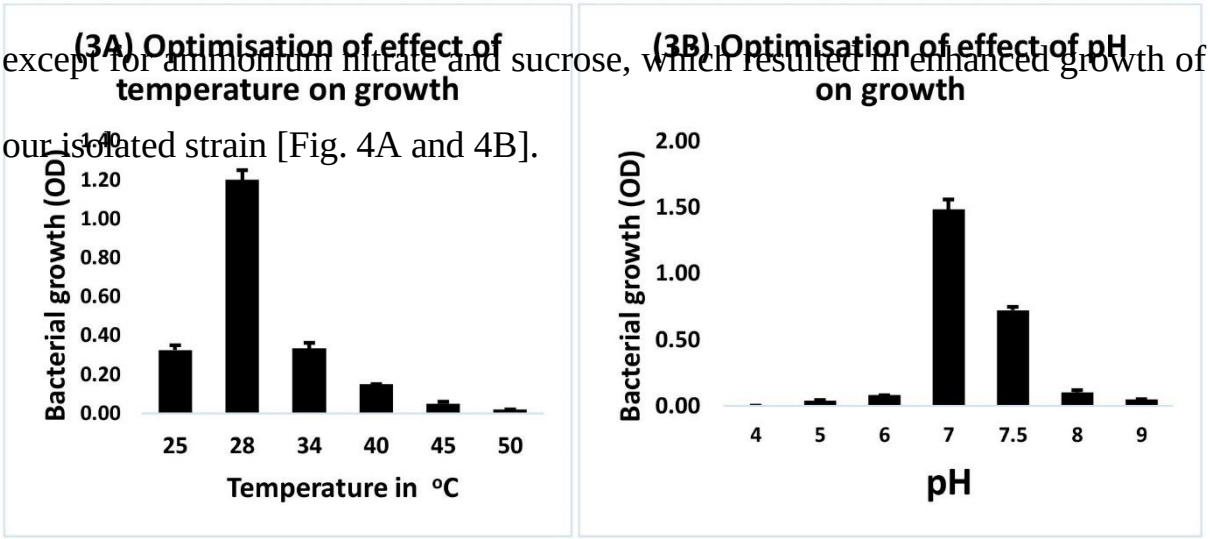
3.3.2. Effect of temperature and pH for optimization of anthracene decomposition.

In this work, the effects of pH and temperature on the increase of anthracene degradation were examined. The bacterial isolate P7 exhibited the highest growth rate at 28˚C, as illustrated in Figure 3A, and at a pH of 7, as shown in Figure 3B

Fig. 3: Conditions for P7 bacterial isolate growth are optimized. Figure 3A 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 3B. 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.

3.3.3. 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 enhanced growth of our isolated strain [Fig. 4A and 4B].



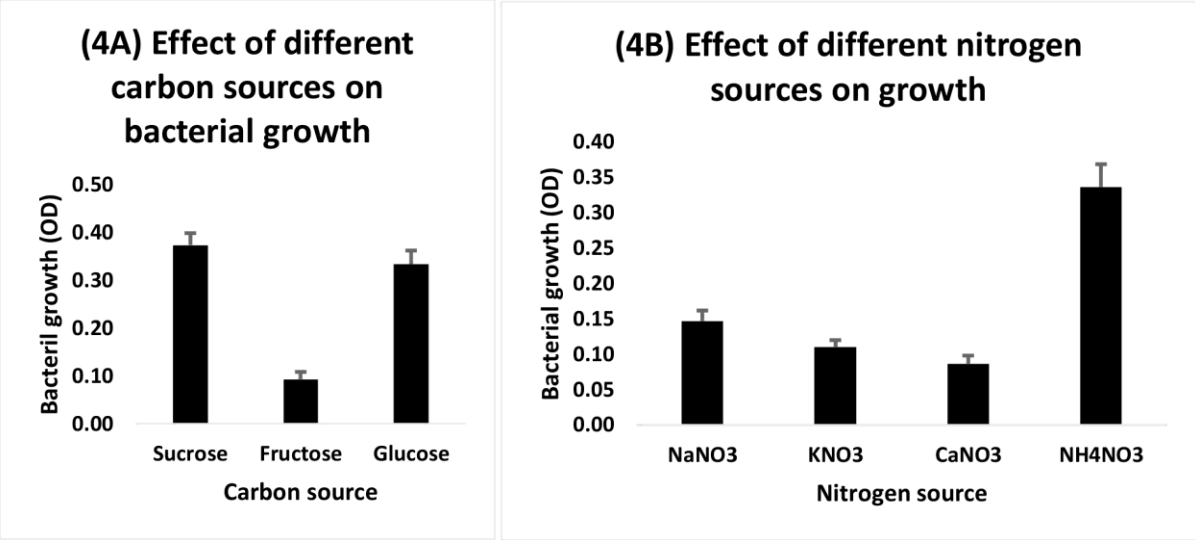


Fig. 4: The growth of bacterial isolate P7 in response to various carbon and nitrogen sources. Figure 4A illustrates how various carbon sources affect isolate P7's growth, while Figure 4B shows how various nitrogen sources affect isolate P7's growth. The mean of the triplicated data is shown by each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

3.3.4. Effect of UV light on anthracene utility

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

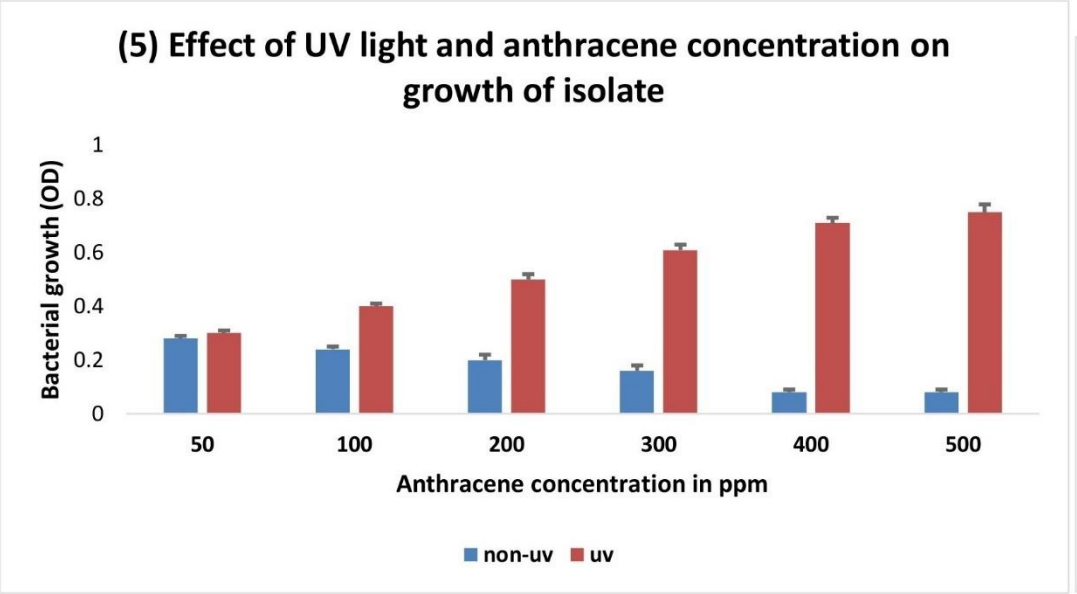


Fig 5 shows how the concentration of anthracene and UV light affect isolate P7's growth. Every bar shows the triplicated data mean with ±S.E. At P<0.05, there is no significant difference between the bars with similar lettering.

3.4. CFU per ml of isolated bacteria

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

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}

3.5. Bacterial decomposition of anthracene

The breakdown of anthracene by bacteria and the optical density of the bacterial

culture are illustrated in Figure 6. Within a span of 120 hours, the strain P7 managed to decompose 85.36% of the anthracene. The bacterial strain P_7 effectively decomposed anthracene during the incubation period from 24 to 96 hours, with the bacterial culture reaching its peak growth [OD-0.92] at the 120-hour mark. The increase in the growth of strain P_7 was observed through an optical density range of 0.5-0.92 between 96-120 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, revealing the presence of 9,10-dihydroxy anthracene and anthraquinone as the identified products.

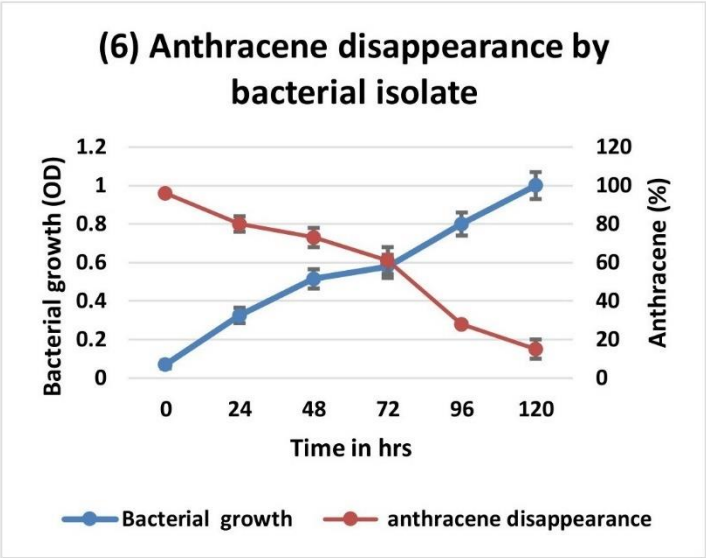
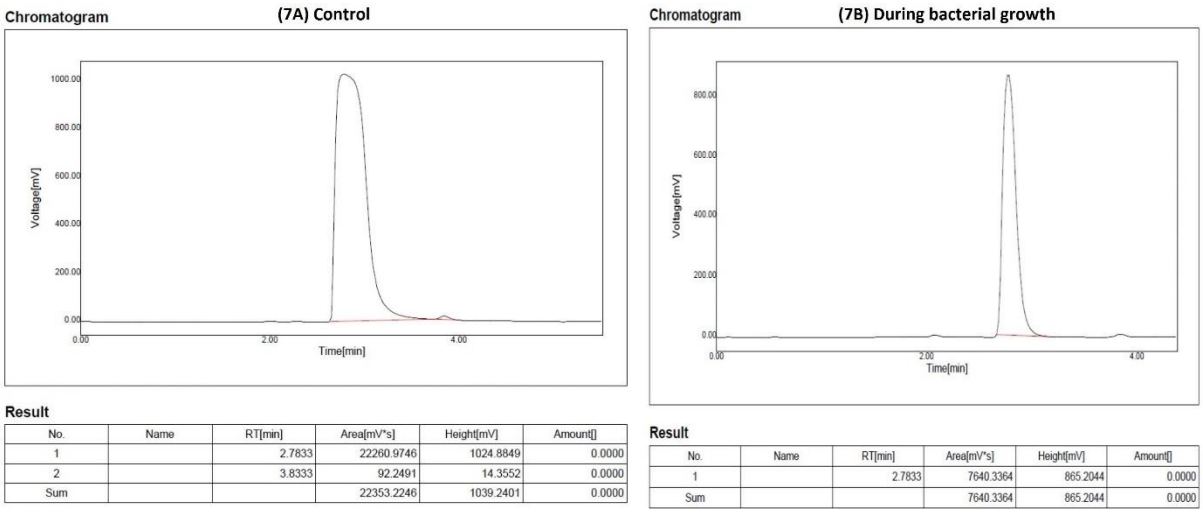


Fig. 6: Bacterial isolate P7 disappears along with anthracene. With ±S.E., each bar shows the mean of three sets of data. If P is less than 0.05, there is no significant difference between the bars with similar letters.

3.6. HPLC analysis

HPLC analysis was utilized 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 swift decline in anthracene concentration [Fig.7]. Upon evaluating the degradation efficiency, it was determined that the isolate achieved an 83.25% degradation rate.



Result

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

Result

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

Fig 7 HPLC analysis of anthracene degradation: a] Control b] During growth of the bacterium. Significant amount of anthracene degradation was observed and reported

3.7. Identification of bacterial isolate P_7

The bacterial isolate P_7 was identified at the genus level through 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 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

3.8. Characterization of the anthracene utilising Bacterial isolate P_7 with phylogenetic analysis

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

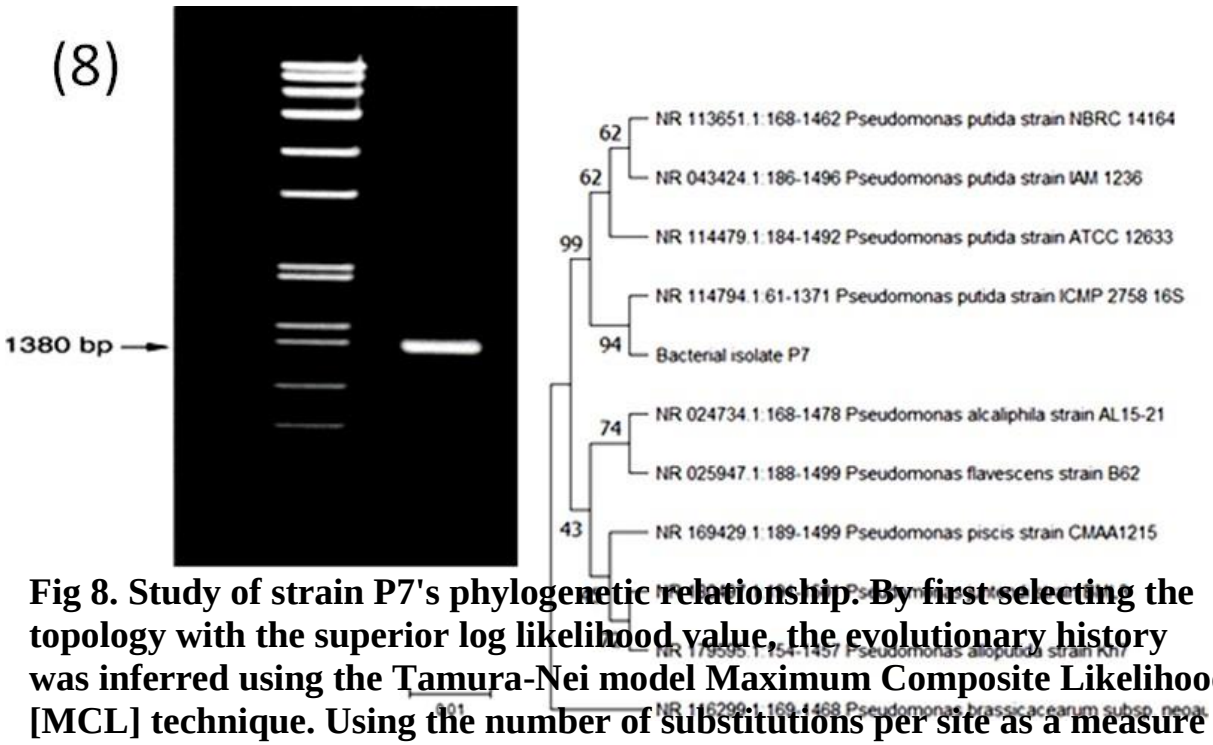


Fig 8. Study of strain P7's phylogenetic relationship. By first selecting the topology with the superior log likelihood value, the evolutionary history was inferred using the Tamura-Nei model Maximum Composite Likelihood [MCL] technique. Using the number of substitutions per site as a measure for branch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

4. Discussion

Polycyclic aromatic hydrocarbons [PAHs] are major pollutants that pose health hazards to the biosphere. In this research study, a bacterial isolate named P_7 was identified from a soil sample taken from a petroleum refinery area near Bangalore.

Anthracene is one kind of PAH that this isolate may be able to use. It was also noted that when the anthracene concentration in the medium rose from 0-1000

ppm, the microbial isolate's growth enhanced. Previous research carried out in a medium enriched with anthracene [31] has revealed comparable results.

The growth and activity of bacteria in a PAH-enriched media can be significantly impacted by a number of variables, such as pH, temperature, and the availability of nutrients. The temperature of the medium can have an impact on both the solubility [36] and 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, it was observed that elevated anthracene decomposition occurred at 28°C, which is the most favorable temperature for the growth of the newly discovered isolate. The optimal culture conditions can stimulate the bacteria to produce and release a set of enzymes into the surrounding environment, which can effectively decompose the pollutant. Inappropriate temperatures can hinder enzyme function by impeding its 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, with deviations from this range negatively impacting cellular composition, metabolism, and culturing [3]. The results of the study indicate 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 culturing and cellular metabolism of the isolate P7, resulting in reduced decomposition of anthracene-like compounds.

While 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 the 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]. On the contrary, the growth and anthracene decomposition potential of bacterial isolate P_7 were not compromised when cultured with various nitrogen sources such as KNO3, NaNO3, CaNO3, and NH4NO3. This suggests that the presence of different carbon and energy sources in this study acted as competitors to anthracene, resulting in a lower bioavailability of the compound.

The water repelling properties of PAHs are responsible for their reduced biodecomposition by bacteria. 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 molecule being decomposed. Various microorganisms, including algae, fungi, and bacteria, have the ability to decompose PAHs through unique metabolic reactions [16]. These microbes

must acclimatize to optimal growth conditions in order to facilitate rapid culturing [40, 41]. Additionally, adaptations in bioavailability are crucial for microbes to establish a connection with pollutants and increase their accessibility [45].

The presence of different pollutants can negatively impact 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 [42, 43]. In the current study, it was observed that 82.29% of anthracene was decomposed within 120 hours in the PNR culture media, surpassing previous studies where only 74.8% decomposition was achieved after 10 days of incubation in BSM medium. Furthermore, the comprehensive decomposition of anthracene into autoclaved soil by *Burkholderia* sp. took approximately 20 days [10. 44]. The rate of breakdown and bio enhancement are greatly influenced by the chemical makeup of the molecule and the specific kind of bacteria involved.

The results of this study have confirmed the hypothesis that strain P_7 efficiently decomposed anthracene within a 7-day period, surpassing previous research findings [45, 46]. Through 16S rRNA analysis, the bacterial isolate P_7 was identified as a highly promising new strain of *Pseudomonas putida*, exhibiting 98% homology [47]. High Performance Liquid Chromatography [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.

5. 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 have discovered a new strain of bacteria that is capable of decomposing anthracene. This novel isolate utilizes anthracene as its sole source of carbon and energy, making it a promising candidate for the biodegradation of other PAHs as well. Specifically, *Pseudomonas putida* P7 has demonstrated effectiveness in breaking down harmful aromatic hydrocarbons and helping to restore the biosphere by removing PAH contaminants. However, 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 : Ultra violet
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 strain, and molecular characterisation of 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 work and drafting of the manuscript. All authors have read and approved the manuscript.

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