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RESEARCH ARTICLE

Enzyme Profiling of Petroleum Hydrocarbon-Degrading Bacteria Isolated from Oil Contaminated Soils

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Abstract

Objectives: The main objective of this study was to evaluate the activity of enzymes with hydrocarbon-degrading potential. **Methods:** Four petroleum hydrocarbon-degrading bacterial strains, S4P2, S14P1, S9D2, and S13D1, were isolated and used to screen the activity of catalase, lipases, and catechol 1, 2-dioxygenase. All the strains were characterized biochemically. The presence of catalase was studied using nutrient agar and absorbance was measured at 240 nm due to the disappearance of peroxide. Qualitatively, the presence of lipase was studied on tributyrin agar (TBA) plates. The lipase activity was quantified using the titrimetric method. The activity of catechol 1, 2-dioxygenase (C12O) was screened by the formation of the product cis, cis-muconic acid (CCMA) by measuring the absorbance at 260 nm. **Findings:** Bacterial strains S4P2, S14P1, S9D2, and S13D1 showed clear halo zones around TBA plates, indicating lipase activity. Similarly, quantitively lipase activity was highest in S4P2 (0.034$\pm$0.01 U/ml) and S13D1 (0.027$\pm$0.01 U/ml) followed by S9D2 (0.024$\pm$0.01 U/ml) and S14P1 (0.024$\pm$0.01 U/ml) respectively. The presence of catalase was confirmed by effervescence after the addition of H_2O_2 on cultured nutrient agar, quantitative screening was performed by checking the decrease in absorbance at 240 nm. The maximum activity was observed in isolates S9D2 and S14P1. The total catechol 1, 2-dioxygenase activity was found to be 18.48$\pm$0 U/ml and 8.96$\pm$0.56 U/ml in S9D2 and S13D1. In contrast, S14P1 and S4P2 showed 12.88$\pm$0.56 U/ml and 16. 8$\pm$0.U/ml. **Novelty:** Microbial enzymes play an important role in the degradation of hydrocarbons. The direct application of microbes offers many constraints, so the use of these enzymes as a cocktail may be a solution for bioremediation. In the present study, we aimed to elucidate the enzymes responsible for hydrocarbon degradation in previously unreported strains, *Pseudomonas donghuensis* and *Azospirillum zeae*. Therefore, this enzyme can be used as a bioremediation agent for petroleum-contaminated sites.

Keywords: Petroleum; Catalase; Lipases; Catechol 1,2-dioxygenase; Hydrocarbons

1 Introduction

Petroleum and its products are widely used as sources of energy, but they can be very harmful to the environment. Studies have shown that diesel and gasoline hydrocarbons have catastrophic and ecotoxicological effects. To address this problem, various clean-up strategies have been applied, including expensive conventional methods, which require large machinery and consume a considerable amount of power [1]. Alternatively, bioremediation using enzymes is eco-friendly, facile, and sustainable solution. According to the reports of the U.S Environmental Protection Agency (EPA) enzymes are reported as bioremediation agents that accelerate hydrocarbon biodegradation [2].

A recent study explored the potential of cellulase, protease, lipase, and amylase in crude oil degradation using a *Bacillus licheniformis* strain. Only 2% of microorganisms have been found to produce enzymes with bioremediation capacity that are used in industries [3]. Therefore, further research is needed to identify new strains with catabolic enzymes that can aid in bioremediation. A comprehensive understanding of novel microbial enzymes is essential for improving eco-sustainable bioremediation strategies for petroleum hydrocarbon degradation [4]. Most autochthonous hydrocarbonoclastic species are reported to produce extracellular and intracellular lipases. Microbial lipases play a crucial role in breaking down lipids and hydrolyzing the ester bonds of triglycerides into glycerol and fatty acids and are a unique feature in the degradation of organic pollutants [Figure 1]. Many published studies have highlighted that lipases are involved in lipolytic reactions and hydrocarbon uptake. Hence, this enzyme was the ideal choice for the current study.

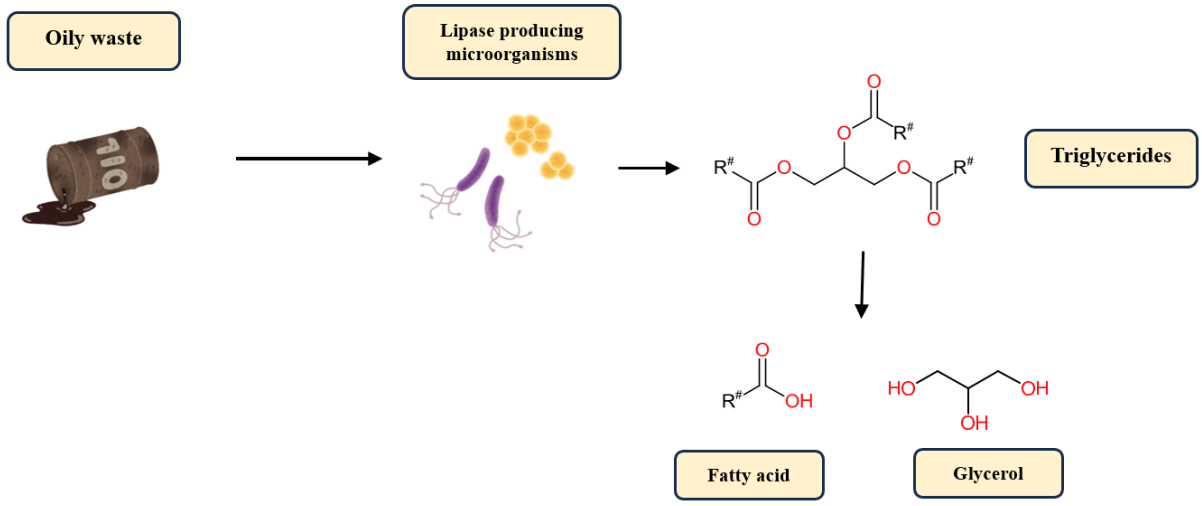

Fig 1. Mechanism of lipase enzyme on oily waste

According to a recent report by Bekele et al. [5], two indigenous species, *Bacillus subtilis* and *Pseudomonas aeruginosa*, are commonly found in hydrocarbon-contaminated sites. The report also screened the catalase and urease enzyme activity of these species qualitatively, but further quantitative screening of catalase activity is necessary to obtain crucial information about hydrocarbon degradation. Hence, in the current studies an attempt has been made to perform qualitative and quantitative assay of catalase from novel strains. Catalase is considered as one of the important indicator enzymes in hydrocarbon biodegradation.

The fractions of aromatic hydrocarbons include monoaromatic and polyaromatic hydrocarbons, such as benzene, toluene, xylene, ethyl benzene, fluorene, naphthalene, and pyrene, which are often observed in gasoline fuel. A recent study examined the environmental fate and detrimental effects of aromatic hydrocarbons on the flora and fauna. Microorganisms exhibit a remarkable ability to biodegrade such aromatic hydrocarbons through the aerobic degradation pathway, which involves the incorporation of oxygen and opening of the aromatic ring with the aid of dioxygenase, as illustrated in [Figure 2]. This study highlights the microbial biodegradation of aromatic hydrocarbons through the utilization of monooxygenases and dioxygenases [6]. Most recent metagenomic studies have explored dioxygenase genes involved in hydrocarbon degradation [7]. However, considering the cost value, a simple spectrophotometric method was used in the current study using catechol as a substrate, which can be further measured at 260 nm.

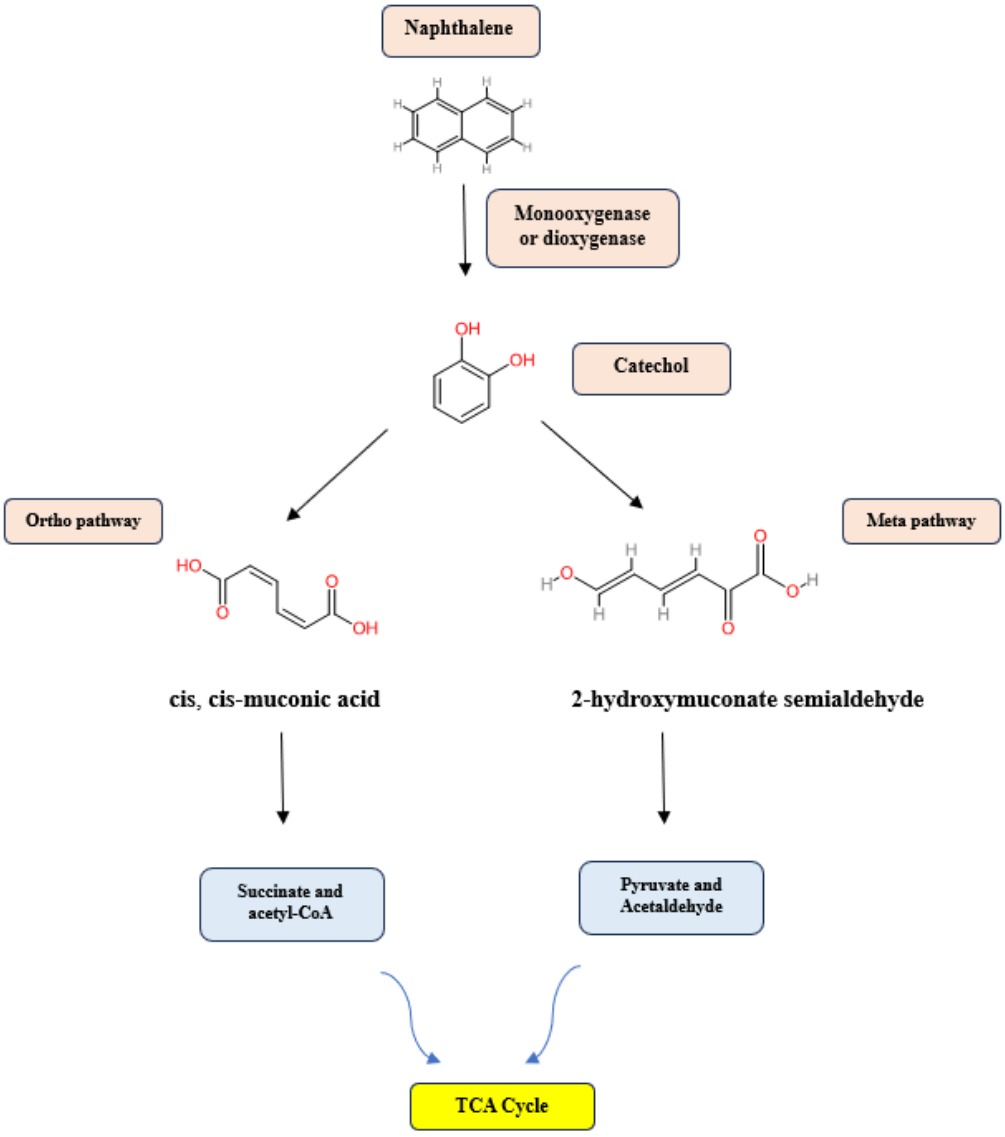

Fig 2. Representation of aromatic hydrocarbon degradation pathway

Although various microbial strains have been identified for petrol and diesel hydrocarbon biodegradation, there is still a need to identify novel strains that possess enzymatic activities capable of combating hydrocarbon pollution under extreme conditions. Bioprospecting for enzymatic profiling of lipase, catalase, monooxygenase, dioxygenase, laccase, dehydrogenase, and alkane dehydrogenase in new isolates can reveal unique activities that may have biotechnological potential. The present study aimed to screen the catalase, lipase, and catechol 1,2-dioxygenase activities of hydrocarbon-degrading bacteria isolated from contaminated oil regions in the Raigad District, Maharashtra, India. The current research is the initial investigation to assess the enzymatic properties of the newly discovered *Pseudomonas donghuensis* and *Azospirillum zeae* strains in the biodegradation of petrol and diesel hydrocarbons. An understanding of enzymatic profiling of newly reported strains of petroleum hydrocarbons can pave the way for enhanced eco-sustainable bioremediation technology.

2 Methodology

2.1 Source of isolates

Four isolates (S9D2) *Rhodococcus ruber*, (S13D1) *Azospirillum zeae*, (S4P2) *Pseudomonas chengduensis*, and, (S14P1) *Pseudomonas donghuensis* were previously characterized by 16S rRNA sequencing and isolated from oil-contaminated sites of Raigad region, India, using diesel and petrol as sole source of carbon[8]. All isolates were biochemically characterized according to Bergey's Manual of Determinative Bacteriology. The isolates were tested for different biochemical test such as:

Carbohydrate test: The utilization of sugar and production of gas formation was confirmed by inoculating a loopful of culture in sterile peptone water containing 1% glucose, 1 % lactose, 1 % mannitol, and 1 % sucrose separately with Andrade's indicator and inverted Durham's tube.

Oxidase test: Oxidase activity was determined on filter paper by spotting the isolates and freshly prepared oxidase reagent on the culture. Violet coloration in the colonies confirmed the presence of activity.

Urease test: The enzyme urease was studied by inoculating the culture in Sterile Christensen's urea broth at 37 °C for 48 h. The colour change of broth into magenta pink is indication of presence of urease.

Indole test: This test was performed using sterile tryptone water. The culture suspension was inoculated into tryptone water and incubated at 37 °C for 24-48 h. The formation of a red ring at the top of the media indicates the production of tryptophanase.

Methyl red: The test was carried out using sterile glucose phosphate broth with a methyl red indicator. Development of red colour in the broth indicating positive test.

Voges Proskauer: The biochemical test is done by inoculating loopful of culture suspension in Sterile glucose phosphate broth. The broth was then incubated at 37 °C for 48 h. A red colour development after addition of $\propto$- naphthol and strong alkali (40% KOH) indicates positive test.

Starch hydrolysis: Sterile nutrient agar plates containing 1% starch were spot inoculated with the culture. A clear halo zone around the colony was recorded after the addition of iodine reagent.

Triple sugar ion test: This test was performed using sterile triple sugar iron agar slants. Changes in the colour of slant/acid/alkaline/H_2S production were analyzed.

Citrate utilization: The isolates were screened for sodium citrate utilization by streaking on Sterile Simmon's citrate agar. The colour change of the slant from green to blue was observed after 24 h of incubation[9].

2.2 Screening for catalase activity

To study catalase activity, strains were grown overnight in sterile nutrient broth to prepare the enzyme extract. The cells were centrifuged at 10000 rpm (REMI) at 4 °C for 15 min. Crude enzyme extract was used for the assay. The reaction mixture consisted of the enzyme (0.1 ml), 1.2 ml phosphate buffer (pH 7.0), and 0.2 ml hydrogen peroxide (15 mM). The catalase activity was recorded by a decrease in the absorbance at 240 nm which was measured using a UV-Visible spectrophotometer (JASCO V-630)[10].

2.3 Screening for lipase activity

To check for the presence of extracellular lipase enzyme activity in the isolate's qualitative screening method, tributyrin agar (TBA) plates (Himedia) supplemented with oil were used. Individual isolates were spotted on TBA plates and incubated at 37°C for 48 h. The appearance of a clear zone around the inoculated colony indicated the presence of extracellular lipase[11].

Lipase activity was quantitatively studied using the titrimetric method. The reaction mixture consisted of 1 ml of culture supernatant, 3 ml of olive oil as the substrate, and 1 ml of 0.1 M tris HCL buffer (pH 7.0). The reaction mixture was incubated for 24 h. A control was maintained using a pre-boiled enzyme extract. The reaction was stopped using 3 ml of an acetone–ethanol mixture. The fatty acid liberated was measured by titrating against 0.1 N NaOH and 0.1 % phenolphthalein indicator[12].

2.4 Preparation of cell extract and screening for catechol 1,2-dioxygenase activity

Isolates were grown individually in 100 ml of sterile nutrient medium and incubated at 37°C. Cells were harvested after 24 h of the incubation period and centrifuged at 10000 rpm at 4 °C for 15 min. The supernatant was decanted, and the pellets were resuspended and washed in 50 mM phosphate buffer (pH 7.2). The activity of catechol 1, 2 dioxygenase enzyme was measured using a UV-visible spectrophotometer (JASCO V-630). The reaction mixture of a total of 1 ml contained 20 μL of 50 mM catechol, 960 μL of 50 mM sodium phosphate buffer, and 20 μL of crude enzyme. The formation of cis, cis-muconic acid was measured at 260 nm.

One unit of enzyme activity is represented as the amount of catechol 1, 2 dioxygenases required to generate 1 μmol of product cis, cis-muconic acid per minute. The activity is calculated with the formula: {($\varepsilon \times$ L/V) (ΔOD/min)}, where a change in optical density (ΔOD), molar extinction coefficient (ε), the volume of the reaction mixture (V), and path length (L) [13].

3 Results and Discussion

3.1 Biochemical identification of isolates

The bacterial strains were characterized according to Bergey's Manual of Determinative Bacteriology. Isolate S9D2 was distinguished from other isolates with a gram-positive nature and cocci morphology. [Table 1] illustrates the biochemical identification results of individual isolates.

Table 1. Biochemical test of hydrocarbon degrading bacteria

Isolates / Biochemical Test	S9D2	S13D1	S4P2	S14P1
Gram's Nature	Gram Positive	Gram Negative	Gram Negative	Gram Negative
Glucose utilization	G	A/G	A	A
Lactose utilization	A	A/G	A	A/G
Mannitol utilization	A	A/G	–	A
Sucrose utilization	–	A/G	A/G	A
Catalase test	+	+	+	+
Urease test	–	+	+	–
Indole test	–	–	–	–
Methyl Red test	–	–	–	+
Voges Proskauer test	–	–	–	–
Starch Hydrolysis	+	+	+	–
Triple sugar iron test	–	–	–	+
Citrate Utilization	+	+	+	+

+ Positive, – Negative, A- Acid formation, G- Gas formation

3.2 Screening for catalase activity

Qualitatively, catalase activity in the bacterial isolates was measured based on the appearance of effervescence. All four isolates showed effervescence after the addition of hydrogen peroxide. Catalase activity was quantified by measuring the reduction in the absorbance at 240 nm. The highest activity was recorded by S9D2 (22.00$\pm$0.01 μmol H_2O_2/min), S14P1 (13.74$\pm$0.03 μmol H_2O_2/min), S13D1 (10.32$\pm$0.01 μmol H_2O_2/min) and S4P2 (9.97$\pm$0.01 μmol H_2O_2/min) respectively as shown in [Figure 3]. A study on crude oil degradation by a consortium with predominant genera of *Acinetobacter* and *Alcaligenes* showed the presence of catalase activity with a range of 10-20 U during a 60-day incubation period [14]. However, the catalase activity of *Rhodococcus ruber* was higher than that reported previously. The most common methodology for the quantitative measurement of catalase activity is either potassium permanganate titration or the oxidization of potassium dichromate. Catalase activity was monitored spectrophotometrically by measuring the decrease in the absorbance at 240 nm. A recent study reported that crude oil degradation by immobilized *Pseudomonas* sp. Biochemically, a study of catalase activity showed that all three strains, *Pseudomonas oryzahabitans, Pseudomonas luteola*, and *Pseudomonas stutzeri*, were catalase negative [15]. Alternatively, the current study stands out by the above-reported work showing catalase activity in both *P. chengduensis* and *P. donghuensis* strains.

Some reports have highlighted the importance of enzyme ureases, catalase, dehydrogenases, peroxidases, and lipases in the decomposition and biodegradation of pollutants in oil-contaminated soil. These studies reported a correlation between enzyme activity and petroleum degradation. This emphasizes the need to study enzyme activity in microbes to monitor the bioremediation process [16]. The presence of catalase activity in all isolates revealed that the microorganisms could protect against oxidative stress. Catalase activity can enhance the aerobic pathway of petroleum biodegradation by providing additional oxygen, which can add to the functionality of the bacterial strains.

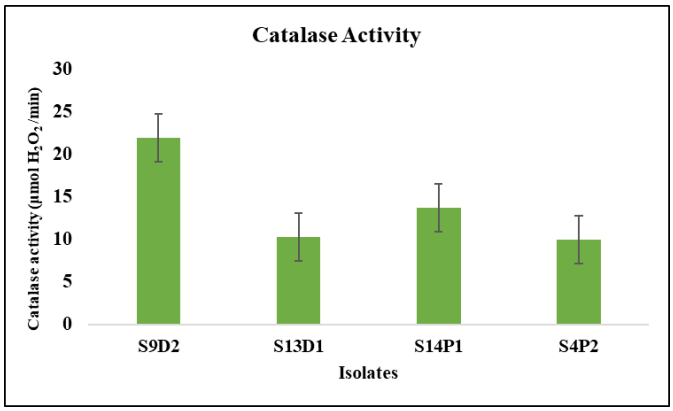

Fig 3. Catalase activity of petroleum hydrocarbon degrading bacteria

3.3 Screening for Lipase activity

In the last few decades, the industrial application of lipase has attracted significant interest. The role of lipase enzymes in the utilization of petroleum hydrocarbons, emulsification, and biodegradation has been reported in various studies. All four strains in the current study, S4P2, S14P1, S9D2, and S13D1, showed a zone of clearance on Tributyrin Agar (TBA) plates after 48 h of incubation. S4P2 and S13D1 showed an elevated clearance zone compared with S14P1 and S9D2, as shown in [Figure 4]. These results are supported by similar work done on *Pseudomonas* sp. screened from petroleum-contaminated areas in Kerela, India, which showed the highest extracellular lipase activity [17].

Fig 4. Lipase activity on Tributyrin Agar plate after 48 h of incubation

An investigation of the screening of lipase-producing bacteria using tributyrin (TBA) agar media was performed using *Chryseobacterium gleum* and *Bacillus velezensis* isolated from oily sludge sewage [18]. The zone of clearance of *Pseudomonas chengduensis* and *Azospirillum zeae* when compared to the above species showed a greater zone of clearance around TBA plates, indicating higher lipase activity. The presence of lipase activity signifies the ability of the isolates to break down hydrophobic hydrocarbons into simple by-products. Lipase is a versatile enzyme that usually works with other enzymes, such as dioxygenase, leading to accelerated degradation of hydrocarbons. This synergistic action played a crucial role in bioremediation. Lipolytic activity study of *Burkholderia cepacia* was performed on a tributyrin agar plate. This strain resulted in a clearance zone after incubation [19].

Compared with the lipolytic results of the zone of clearance observed for *Burkholderia cepacian* isolates, *Pseudomonas chengduensis* and *Azospirillum zeae* reported in the current study showed better results. The titrimetric assay, which is a reliable method, was used to measure lipase activity in the current study and has an advantage over other methods in eliminating turbidity. The quantitatively isolated S4P2 showed a maximum 0.034±0.01 U/ml activity while S13D1 showed 0.027±0.01 U/ml activity. The activity showed by isolates S9D2 and S14P1 was 0.024±0.01 U/ml and 0.024±0.01 U/ml as illustrated in [Figure 5]. Studies on *Pseudomonas aeruginosa* and *Bacillus subtilis* screened from hydrocarbon-contaminated soil showed maximum lipase activity at pH 8 and a temperature range of 40°C-50°C [20]. The current report documented maximum lipase activity at a neutral pH and temperature of 37°C. Exploring the activities of degradative enzymes in potential new strains for hydrocarbon studies can gauge the extent of petroleum pollution in the ecosystem and can help in the future for the effective development of consortiums for bioremediation of polluted sites.

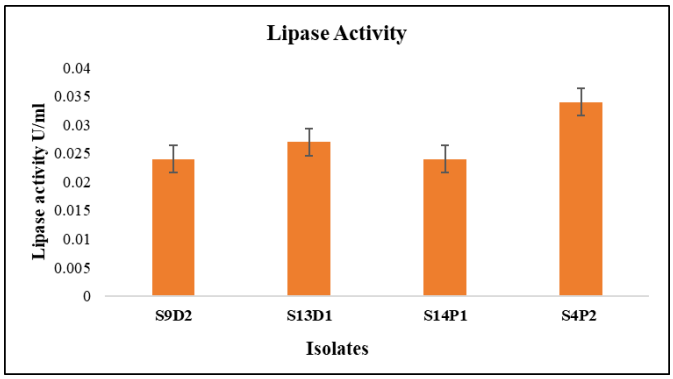

Fig 5. Lipase activity of petroleum hydrocarbon degrading bacteria by titrimetric method

3.4 Screening of catechol 1,2-dioxygenase activity

The total C12O activity of S9D2 and S13D1 was found to be 18.48±0 U/ml and 8.96±0.56 U/ml respectively. Isolates S14P1 and S4P2 showed 12.88±0.56 U/ml and 16.8±0 U/ml activity, respectively [Figure 6]. A previous study on a *Pseudomonas putida* strain for naphthalene catabolism was performed, emphasizing the degradative enzyme activity of catechol 1,2 dioxygenase, naphthalene 1,2-dioxygenase, and catechol 2,3 dioxygenase. The total C120 activity in *Pseudomonas putida* was found to be between 0.0186 ± 0.0018 and 0.0064±0.0016 μmol/min*mg of protein [21]. Compared to the C120 activity of *Pseudomonas putida*, the activity reported in *Pseudomonas chengduensis* and *Pseudomonas donghuensis* crude extracts was relatively higher, indicating the potential of current isolates to work in aromatic hydrocarbon biodegradation in petroleum-contaminated sites.

Similarly, phenol degradation in a *Rhodococcus* sp. isolate reported catechol 1,2-dioxygenase activity and the absence of the catechol 2,3-dioxygenase enzyme. The highest specific activity of catechol 1,2-dioxygenase was 23.603 U/mg after incubation for 10 min [22]. The isolation and optimization of catechol 1,2-dioxygenase production from the bacterial strain *M. hydrothermale* has been reported. The isolate showed maximum activity of 0.140 ± 0.002 U/ml at 35°C, pH 8.0 [23]. In comparison to catechol 1,2-dioxygenase activity in isolate *M. hydrothermale*, the current study isolates *Rhodococcus ruber*, (S13D1) *Azospirillum zeae*, (S4P2) *Pseudomonas chengduensis* and (S14P1) *Pseudomonas donghuensis* exhibited maximum activity of 18.48±0 U/ml, 8.96±0.56 U/ml 12.88±0.56 U/ml and 16.8±0 U/ml at a pH 7.2 respectively.

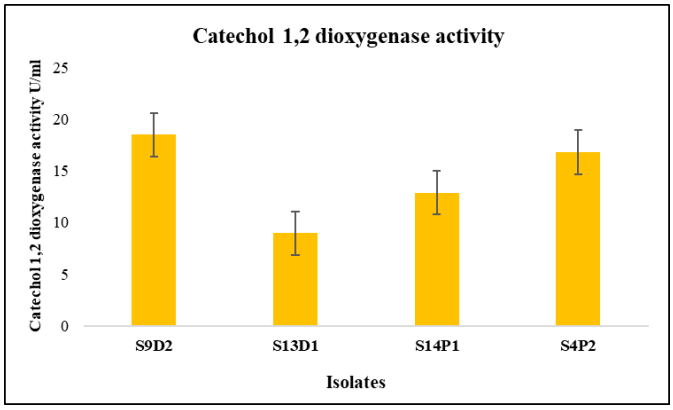

Fig 6. Catechol 1,2 dioxygenase activity of petroleum hydrocarbon degrading bacteria

Hydrocarbonoclastic species mostly reported in petroleum bioremediation have been explored, including *Alcaligenes, Sphingomonas, Burkholderia, Bacillus subtilis, Pseudomonas aeruginosa, Pseudomonas stutzer, Pseudomonas putida, Gordonia sihwensis, Bacillus licheniformis*, and *Rhodococcus equi* [24]. However, the novelty of this study is that the previously unexplored species of *Pseudomonas* and *Azospirillum* were found to have an enzymatic activity that can aid in breaking down petroleum complex hydrocarbons and could be proven to have useful applications in clean-up oil spills and environmentally polluted sites.

4 Conclusion

The current study covered the biochemical characterization and enzyme activity of hydrocarbon-degrading bacteria isolated from oil-contaminated soils in the Raigad region. Interestingly, isolates S4P2, S14P1, S9D2, and S13D1 exhibited enzymatic activity. The different enzymatic activities of the strains reflect the tolerance of the isolates to the substrate. The highest lipase activity was observed in *Pseudomonas chengduensis* and *Azospirillum zeae*. A zone of clearance was observed for all isolates. The maximum catalase activity of (22.00 ± 0.01 μmol H_2O_2/min, 13.74 ± 0.03 μmol H_2O_2/min) was observed in *Rhodococcus ruber* and *Pseudomonas donghuensis*. The presence of effervescence in the inoculated slants further confirmed catalase activity. Moreover, catechol 1,2-dioxygenase activity (18.48 ± 0 U/ml, 16.8 ± 0 U/ml) was higher in *P. chengduensis* and *R. ruber*. The current study tailored the significance of enzyme profiling to hydrocarbon biodegradation. Enzyme profiling in unexplored new strains *Pseudomonas donghuensis* and *Azospirillum zeae* in bioremediation of hydrocarbons can enhance robustness and provide better insights into petroleum hydrocarbon bioremediation. These unique indigenous isolates could be used together in the future as a consortium or coculture for the prevailing issues in petrol and diesel degradation. A cocktail of enzymes can be developed for the remediation of contaminated soil. In the future, metagenomics studies of the strains can reveal the pollutant degrading genes involved in the bioremediation.

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References

1) Trejos-Delgado C, Cadavid-Restrepo GE, Hormaza-Anaguano A, Agudelo EA, Barrios-Ziolo L, Loaiza-Usuga JC, et al. Oil Bioremediation in a Tropical Contaminated Soil Using a Reactor. *Anais da Academia Brasileira de Ciências*. 2020;92(2):1–18. Available from: https://dx.doi.org/10.1590/0001-3765202020181396.
2) Kanwal M, Ullah H, Gulzar A, Sadiq T, Gul Z, Ullah M, et al. Biodegradation of petroleum hydrocarbons and the factors effecting rate of biodegradation. *American Journal of Biomedical Science and Research*. 2022;16(1):6–15. Available from: http://dx.doi.org/10.34297/AJBSR.2022.16.002182.
3) Srimathi M, Suganthi M, Sugitha S, Kumar KA, Ramprasath C, Abirami G. Characterization of Crude Oil Degrading Marine Bacterium Bacillus licheniformis. *Indian Journal of Microbiology*. 2024;p. 1–3. Available from: https://dx.doi.org/10.1007/s12088-024-01222-9.
4) Radhakrishnan A, Balaganesh P, Vasudevan M, Natarajan N, Chauhan A, Arora J, et al. Bioremediation of Hydrocarbon Pollutants: Recent Promising Sustainable Approaches, Scope, and Challenges. *Sustainability*. 2023;15(7):1–18. Available from: https://dx.doi.org/10.3390/su15075847.
5) Bekele GK, Gebrie SA, Abda EM, Sinshaw G, Haregu S, Negie ZW, et al. Kerosene Biodegradation by Highly Efficient Indigenous Bacteria Isolated From Hydrocarbon-Contaminated Sites. *Microbiology Insights*. 2023;16:1–9. Available from: https://dx.doi.org/10.1177/11786361221150759.
6) Pandolfo E, Caracciolo AB, Rolando L. Recent Advances in Bacterial Degradation of Hydrocarbons. *Water*. 2023;15(2):1–18. Available from: https://dx.doi.org/10.3390/w15020375.
7) Eze MO. Metagenome Analysis of a Hydrocarbon-Degrading Bacterial Consortium Reveals the Specific Roles of BTEX Biodegraders. *Genes*. 2021;12(1):1–14. Available from: https://doi.org/10.3390/genes12010098.
8) Nanekar RD, Kokitkar SS. Isolation, genetic identification and optimization of hydrocarbon degrading bacteria from petroleum contaminated soil in Raigad region. *Ecology, Environment and Conservation*. 2023;29(4):1554–1561. Available from: https://dx.doi.org/10.53550/eec.2023.v29i04.014.
9) Hossain MF, Akter A, Sohan MSR, Sultana N, Reza MA, Hoque KMF. Bioremediation potential of hydrocarbon degrading bacteria: isolation, characterization, and assessment. *Saudi Journal of Biological Sciences*. 2022;29(1):211–216. Available from: https://dx.doi.org/10.1016/j.sjbs.2021.08.069.
10) Tondo ML, de Pedro-Jové R, Vandecaveye A, Piskulic L, Orellano EG, Valls M. KatE From the Bacterial Plant Pathogen Ralstonia solanacearum Is a Monofunctional Catalase Controlled by HrpG That Plays a Major Role in Bacterial Survival to Hydrogen Peroxide. *Frontiers in Plant Science*. 2020;11:1–12. Available from: https://dx.doi.org/10.3389/fpls.2020.01156.
11) Faiz S, Nasreen Z, Sha A, Naz S. Isolation, screening and characterization of lipase from bacterial isolates and its application in detergents and oily waste water degradation. *Pure and Applied Biology*. 2021;10(1):209–224. Available from: http://dx.doi.org/10.19045/bspab.2021.100022.
12) Bamitale OM, Ayomikun AM. Biodegradation potential of tropical hydrocarbon degrading Providencia stuartii. *Trends in Applied Sciences Research*. 2020;15(3):253–259. Available from: https://scialert.net/abstract/?doi=tasr.2020.253.259.
13) Sarwade V, Funde S. Biotransformation of nitro aromatic amines in artificial alkaline habitat by pseudomonas DL17. *Environmental Analysis Health and Toxicology*. 2022;37(1):1–16. Available from: https://dx.doi.org/10.5620/eaht.2022001.
14) Pi YR, Bao MT. Investigation of kinetics in bioaugmentation of crude oil via high-throughput sequencing: Enzymatic activities, bacterial community composition and functions. *Petroleum Science*. 2022;19(4):1905–1914. Available from: https://dx.doi.org/10.1016/j.petsci.2022.01.022.
15) Gayathri V, Krishnaprema K. Bioremediation of Crude Oil using Hydrocarbon degrading Pseudomonas luteola by Immobilization methods. *Ecology, Environment and Conservation*. 2023;29(January Suppl. Issue):S109–S116. Available from: https://dx.doi.org/10.53550/eec.2023.v29i01s.017.
16) Xiao F, Zhou BB, Duan ML, Chen XP. Effect of Different Remediation Methods on the Degradation Rate of Petroleum Hydrocarbon and Enzyme Activity in Petroleum Contaminated Soil. *Applied Ecology and Environmental Research*. 2023;21(4):2819–2832. Available from: https://dx.doi.org/10.15666/aeer/2104_28192832.
17) Jacob EL, Mohan AP, Joseph AV. Bioremediation of Petroleum-Polluted Soil Using Biosurfactant Producing Bacteria, Pseudomonas sp. *Journal of Scientific Research*. 2022;66(1):224–231. Available from: https://dx.doi.org/10.37398/jsr.2022.660124.
18) Layly IR, Meryandini A, Helianti I, Astuti RI. Identification of lipase producing bacteria from palm oil sewage sludge processing plant at Malimping, Banten, Indonesia. *Biodiversitas Journal of Biological Diversity*. 2021;22(10):4512–4524. Available from: https://dx.doi.org/10.13057/biodiv/d221045.

19) Szymczak T, Cybulska J, Podleśny M, Frąc M. Various Perspectives on Microbial Lipase Production Using Agri-Food Waste and Renewable Products. *Agriculture*. 2021;11(6):1–22. Available from: https://dx.doi.org/10.3390/agriculture11060540.
20) Abubakar A, Abioye OP, Aransiola SA, Maddela NR, Prasad R. Crude oil biodegradation potential of lipase produced by Bacillus subtilis and Pseudomonas aeruginosa isolated from hydrocarbon contaminated soil. *Environmental Chemistry and Ecotoxicology*. 2024;6:26–32. Available from: https://dx.doi.org/10.1016/j.enceco.2023.12.001.
21) Pozdnyakova-Filatova I, Petrikov K, Vetrova A, Frolova A, Streletskii R, Zakharova M. The Naphthalene Catabolic Genes of Pseudomonas putida BS3701: Additional Regulatory Control. *Frontiers in Microbiology*. 2020;11:1–12. Available from: https://dx.doi.org/10.3389/fmicb.2020.01217.
22) Lee GLY, Zakaria NN, Convey P, Futamata H, Zulkharnain A, Suzuki K, et al. Statistical Optimisation of Phenol Degradation and Pathway Identification through Whole Genome Sequencing of the Cold-Adapted Antarctic Bacterium, Rhodococcus sp. Strain AQ5-07. *International Journal of Molecular Sciences*. 2020;21(24):1–20. Available from: https://doi.org/10.3390/ijms21249363.
23) Olukunle OF. Characterization of polycyclic aromatic hydrocarbon (PAH) degrading bacteria and optimization of physicochemical conditions for the production of catechol 1, 2-dioxygenase. *Nigerian Journal of Pure and Applied Sciences*. 2023;36(1):4547–4558. Available from: https://njpas.com.ng/wp-content/uploads/2023/05/10-NJPAS-22-B16.pdf.
24) Amran R, Jamal MT, Pugazhendi A, Al-Harbi M, Ghandourah M, Al-Otaibi A, et al. Biodegradation and Bioremediation of Petroleum Hydrocarbons in Marine Ecosystems by Microorganisms: A Review. *Nature Environment and Pollution Technology*. 2022;21(3):1149–1157. Available from: https://dx.doi.org/10.46488/nept.2022.v21i03.019.