Sahel Journal of Life Sciences FUDMA 4(1): 417-424, 2026

Sahel Journal of Life Sciences FUDMA (SAJOLS)
March 2026 Vol. 4(1): 417-424
ISSN: 3027-0456 (Print)
ISSN: 1595-5915 (Online)
DOI: https://doi.org/10.33003/sajols-2026-0401-49

Research Article

Preliminary Screening of Pyrene-Degrading Bacteria from the Semi-Anoxic Bovine Gut

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ABSTRACT
Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants whose remediation remains a global challenge in oxygen-limited and resource-constrained environments. While microbial degradation of PAHs has been studied in soils and aquatic systems, the gastrointestinal microbiota of ruminants remains largely unexplored as a potential reservoir of hydrocarbon-degrading bacteria. This study evaluates the PAH-degrading potential of bacterial isolates from the semi-anoxic intestinal lining of cows inhabiting crude oil-impacted environments. Samples were aseptically collected from the intestinal gut lining of *Bos tarus* at the point of slaughter and cultured with a Pyrene supplemented Bushnell Haas medium. A total of sixteen distinct bacterial isolates were obtained and characterized using morphological and biochemical techniques. Their capacity to metabolize pyrene, a model high-molecular-weight PAH, was assessed using a 2,6-dichlorophenol indophenol (DCPIP) redox assay in combination with growth kinetics monitoring (OD_{600nm}). Approximately 70% of the isolates demonstrated DCPIP reduction, indicating active redox metabolism associated with hydrocarbon utilization. Notably, *Citrobacter freundii* and *Pseudomonas alcaligenes* exhibited pronounced and sustained DCPIP reduction alongside measurable shifts in growth dynamics, suggesting enhanced metabolic adaptation to pyrene. The applied methods provide preliminary evidence of PAH-associated metabolic activity, the observed diversity among isolates highlights the functional versatility of gut-associated microbiota under semi-anoxic conditions. These findings position the ruminant intestinal microbiome as an underutilized source of bacteria with potential relevance for bioremediation in oxygen-limited environments and presents a low-cost screening framework suitable for resource-limited laboratories and provides a foundation for further investigations involving molecular identification, enzymatic pathway analysis, and analytical validation of hydrocarbon degradation.

Keywords: Bioremediation; DCPIP assay; PAH biodegradation; Pyrene; Ruminant microbiome; Semi-anoxic environment

Citation: Urhibo, V.O., & Oshoma, C.E. (2026). Preliminary Screening of Pyrene-Degrading Bacteria from the Semi-Anoxic Bovine Gut. *Sahel Journal of Life Sciences FUDMA*, 4(1): 417-424. DOI: https://doi.org/10.33003/sajols-2026-0401-49

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a prominent class of persistent organic pollutants widely recognized for their carcinogenic, mutagenic, and toxic properties (Nwankwegu *et al*., 2022; Varjani *et al*., 2023). These hydrophobic compounds are primarily generated through incomplete combustion of organic materials such as fossil fuels, biomass, and industrial wastes, leading to their accumulation in soils, sediments, and aquatic systems (Kuppusamy *et al*., 2022). In oil-producing regions of sub-Saharan Africa, particularly Nigeria, chronic PAH contamination is exacerbated by crude oil exploration, pipeline failures, artisanal refining activities, and weak remediation enforcement, posing serious threats to ecological integrity and public health (Alotaibi *et al*., 2024).

Bioremediation using microorganisms has emerged as an environmentally sustainable and cost-effective approach for mitigating PAH contamination compared with conventional physicochemical methods (Varjani *et al*., 2023). Numerous bacterial genera, including *Pseudomonas*, *Mycobacterium*, *Rhodococcus*, and *Sphingomonas*, have been reported to degrade both low- and high-molecular-weight PAHs through enzymatic pathways involving monooxygenases and dioxygenases that facilitate aromatic ring cleavage and mineralization (Sazonova *et al*., 2022; Davletgildeeva *et al*., 2024). These microbial processes are particularly attractive for developing countries because they can be implemented using locally sourced organisms and relatively simple laboratory infrastructure.

Despite extensive studies on PAH-degrading bacteria from contaminated soils and aquatic environments, the gastrointestinal tracts of ruminant animals remain an underexplored ecological niche with significant biotechnological potential (Hussain *et al*., 2022) because of the exposure levels of the ruminant animal to pollutants. The semi-anoxic intestinal environment of cows supports diverse microbial communities adapted to metabolize complex and recalcitrant organic substrates. Ruminant gut microbiota is well known for its exceptional enzymatic capacity to degrade lignocellulosic plant materials, suggesting that they may also possess metabolic versatility for transforming structurally similar xenobiotic compounds such as PAHs (Oduro-Mensah *et al*., 2021).

Recent studies suggest that gut-associated bacteria harbour redox-active and hydrolytic enzymes capable of mediating hydrocarbon degradation under oxygen-limited conditions (Li *et al*., 2021; Zhou *et al*., 2024). Such traits are particularly relevant for the remediation of PAH-contaminated soils and sediments, which are often characterized by limited oxygen availability. Harnessing bacteria from semi-anoxic intestinal environments may therefore enhance the effectiveness of bioaugmentation strategies in similar environmental settings.

In resource-limited regions, identification of effective PAH degraders requires rapid and low-cost screening approaches. The use of 2,6-dichlorophenol indophenol (DCPIP) as a redox indicator provides a simple and sensitive method for qualitatively assessing microbial hydrocarbon degradation potential prior to more expensive analytical techniques (Adebusoye, 2023). When combined with growth kinetics and basic biochemical characterization, this approach enables efficient selection of promising bacterial candidates for further molecular and field-scale evaluation.

Against this background, the present study aimed to isolate and characterize bacterial strains from the semi-anoxic intestinal lining of cows and to evaluate their ability to degrade pyrene, a representative high-molecular-weight PAH, under controlled laboratory conditions. By integrating qualitative DCPIP-based screening with quantitative growth assessment, this study seeks to identify locally adapted bacterial isolates with potential application in low-cost, sustainable bioremediation strategies suitable for oil-impacted environments common in Nigeria and other African countries.

Isolation and Characterization of Bacterial Isolates

Bacterial isolates were obtained from the semi-anoxic intestinal lining of cows sourced from Gariki Uti Effurun, Delta State abattoir at the point of slaughter. Isolation was performed using standard microbiological techniques, followed by morphological and biochemical characterization, including catalase, oxidase, urease, citrate utilization, and sugar fermentation assays (Ajiboye *et al*., 2020).

DCPIP Redox Assay for Hydrocarbon Metabolism

The PAH-associated metabolic activity of the isolates was assessed using a modified DCPIP redox assay. Cultures were grown in Bushnell-Haas medium supplemented with pyrene (1,000 mg/L) as the sole carbon source. DCPIP served as an electron acceptor, with reduction indicated by a colour change from blue to colorless. All assays were conducted in triplicate, with appropriate positive and negative controls.

Growth Kinetics Measurement

Bacterial growth was monitored spectrophotometrically at OD_{600nm} over a 96-hour period. Changes in optical density were interpreted as indicators of substrate utilization dynamics.

Data Analysis

Data were analyzed using one-way ANOVA followed by Tukey’s post-hoc test. Statistical significance was set at $p \leq 0.05$.

Data Integration

The results obtained from the screening assays, including the DCPIP assay, biochemical tests, and growth measurements, were integrated to identify bacterial isolates with the most promising PAH-degrading abilities. The isolates were further characterized based on their morphological and biochemical profiles, as well as their sugar fermentation capabilities, to determine the correlation between specific traits and PAH degradation potential. The best isolates were identified and documented.

RESULTS AND DISCUSSION

Isolation and Identification of PAH-Degrading Bacteria

A total of sixteen (16) distinct bacterial isolates were successfully recovered from the semi-anoxic intestinal lining of cows. The isolates were identified based on their morphological and biochemical tests. The isolates exhibited diverse morphological forms, ranging from rods, cocci, and diplococci, with varying biochemical profiles, including catalase, urease, oxidase, and coagulase activities (Table 1). These results are consistent with findings from previous studies which highlight the presence of diverse bacterial communities in the gut microbiota of ruminants, capable of utilizing a wide array of substrates, including polycyclic aromatic hydrocarbons (PAHs) (Mishra and Das, 2019).

Screening for PAH-Degrading Potential

The capacity of bacterial isolates to degrade polycyclic aromatic hydrocarbons (PAHs), specifically pyrene, was evaluated using a 2,6-dchlorophenol indophenol (DCPIP) redox indicator assay. The results (Table 2) revealed significant variation in the PAH-degrading capabilities of the isolates, demonstrating the diverse metabolic potential of bacteria sourced from the semi-anoxic intestinal lining of cows. This variation underscores the complex nature of PAH degradation, which can depend on a range of factors including the bacterial species, environmental conditions, and the specific type of PAH being targeted.

After 96 hours of incubation, several isolates showed a distinct discoloration in the medium, shifting from blue to colourless, which was indicative of successful pyrene degradation. Notably, *Citrobacter freundii* and *Pseudomonas alcaligenes* exhibited the most prominent colour change, transitioning from blue to colourless, signifying efficient pyrene degradation. These isolates demonstrated superior PAH-degrading potential, supporting their candidacy for future bioremediation applications aimed at PAH contamination. The presence of discoloration in these isolates is consistent with the results reported by Сазонова *et al.* (2022), who identified *Pseudomonas* species as capable of degrading PAHs through enzymatic reduction of DCPIP.

The positive control (MSM + Pyrene + DCPIP) exhibited minimal colour change, validating the experimental setup and confirming the assay's effectiveness in detecting microbial degradation of PAH. Conversely, the negative control (MSM + Pyrene without bacteria) showed no discoloration, confirming that the absence of bacteria would prevent any degradation. These results align with the findings of Roy *et al.* (2002) and Koma *et al.* (2003), who also utilized the DCPIP assay to measure bacterial PAH degrading capabilities, highlighting the effectiveness of this method in distinguishing PAH degrading bacteria.

The ability of *Pseudomonas alcaligenes* and *Citrobacter freundii* to degrade pyrene is consistent with previous reports identifying *Pseudomonas* as a highly efficient PAH-degrading genus. For example, *Mishra and Das* (2019) demonstrated that *Pseudomonas* strains possess the enzymatic machinery required for the breakdown of both low and high molecular weight PAHs, making them valuable candidates for bioremediation applications.

The identification of *Citrobacter freundii* as an effective pyrene degrader is particularly noteworthy, as this species has not been as widely studied for PAH degradation as other genera like *Pseudomonas*. However, *Citrobacter* species have been shown to possess a variety of catabolic pathways capable of breaking down environmental pollutants, including aromatic compounds like PAHs. The findings of Hennessee and Li (2016) support this, as they emphasized the significance of *Citrobacter* species in biodegradation processes. The capacity of *Citrobacter freundii* to degrade pyrene suggests that this species could play an important role in future bioremediation efforts, particularly in agricultural and industrial environments where PAH contamination is prevalent. Therefore, the screening assay confirmed the PAHs degrading potential of several bacterial isolates, with *Citrobacter freundii* and *Pseudomonas alcaligenes* standing out as the most effective pyrene degraders. These results support the growing body of literature on the bioremediation potential of bacterial strains in degrading persistent environmental pollutants such as PAHs, and provide valuable insights into the bacterial diversity found in the semi-anoxic intestinal environment of cows. Future research should focus on elucidating the specific enzymatic pathways responsible for PAH degradation in these strains, as well as optimizing the environmental conditions to maximize their bioremediation potential.

Table 1: Morphological, Biochemical and sugar fermentation assessment of isolates on MSM enriched with PAH (Pyrene)

Morphology	Microscopy	Biochemical								Sugar Fermentation						
Shape	Grams reaction	Urease	Oxidase	Catalase	Coagulase	Indole	VP	MR	Citrate	Glucose	Lactose	Sucrose	H_2S	Acid	Gas	Probable Organism
Rod	-	-	-	+	-	-	+	-	-	+	-	-	+	+	+	*Salmonella pullorum*
Cocci	+	+	-	+	-	+	+	+	-	+	+	+	+	+	+	*Citrobacter freundii*
Diplo-cocci	+	+	-	+	-	+	-	+	-	+	+	+	+	+	+	*Staphylococcus warneri*
Rod	-	+	-	+	-	+	-	+	-	+	+	+	-	+	-	*Leclerciaade carboxylata*
Cocci		+	-/+	+	-	-	+	-	+	+	+	+	-	+	+	*Micrococcus varians*
Rod	-	-	+	+		-	+	-	+	-	-	-	-	-	-	*Pseudomonas alcaligenes*
Diplo-cocci	-	+	-	-	-	+	-	+	+	+	-	+	-	+	+	*Klebsiella oxytoca*
Diplo-cocci	-	+	-	+	-	-	+	-	+	+	-	+	-	+	+	*Raoultella ormithinolytica*
Diplo-cocci	-	+	-	+	-	+	+	-	+	+	+	+	-	+	+	*Raoutellasp*
Rod	-	-	-	+	-	+	+	-	+	+	+	+	-	+	+	*Kluyvera ascorbate*
Cocci	-	+	+	-	-	-	+	-	-	+	+	+	+	+	-	*Actinobacillus minor*
Cocci	-	-	-	-	-	-	+	-	+	+	+	+	-	+	+	*Enterobacter aerogenes*
Rod	-	+	-	-	-	-	-	+	-	+	-	+	-	+	-	*Proteus penneri*
Diplo-cocci	-	+	-	+	-	+	-	+	-	+	-	-	-	+	+	*Morganella morganii*
Diplo-cocci	+	-	-	+	-	-	+	-	-	+	+	+	+	+	+	*Micrococcus roseus*
Rod	+	+	-	+	-	-	+	-	+	+	+	+	-	+	+	*Cellobiosococcus sp*

Key: - = negative, + = positive

Table 2: Qualitative analysis PAH Degradation Potential of Bacterial Isolates Using DCPIP Redox Indicator

Sample	**Time (hours)**				
Isolate	**4**	**24**	**48**	**72**	**96**
Salmonella pullorum	NC	NC	SCC	MCC	CD
Citrobacter freundii	SCC	SCC	MCC	CD	CD
Staphylococcus warneri	NC	NC	NC	SCC	SCC
Leclercia adecarboxylata	NC	NC	SCC	MCC	MCC
Micrococcus varians	NC	SCC	SCC	MCC	MCC
Pseudomonas alcaligenes	NC	SCC	CD	CD	CD
Klebsiella oxytoca	NC	NC	SCC	SCC	SCC
Raoultella ornithinolytica	NC	SCC	SCC	MCC	CD
Raoultella sp.	NC	NC	SCC	MCC	CD
Kluyvera ascorbate	NC	NC	NC	NC	SCC
Actinobacillus minor	NC	NC	NC	NC	NC
Enterobacter aerogenes	NC	SCC	SCC	MCC	MCC
Proteus penneri	NC	NC	NC	NC	NC
Morganella morganii	SCC	SCC	SCC	MCC	CD
Micrococcus roseus	NC	NC	NC	NC	NC
Enterobacter cloacae	NC	NC	NC	NC	NC
Enterococcus faecalis	NC	SCC	MCC	MCC	MCC
Pseudomonas putida	SCC	SCC	SCC	SCC	CD
Rhodococcus erythropolis	NC	SCC	MCC	MCC	MCC

Keys: NC = No change, SCC = Slight colour change, MCC = Moderate colour change, CD = Complete discolouration

The positive control (MSM + Pyrene + DCPIP) showed the expected colour change, confirming the validity of the assay, while the negative control (MSM + Pyrene without bacteria) showed no discoloration, indicating no degradation. These results agree with the findings of Сазонова *et al*. (2022), who reported that bacteria such as *Pseudomonas* are capable of degrading PAHs through enzymatic pathways that involve the reduction of DCPIP.

Quantitative Assessment of PAH Degradation

To gain deeper insights into the correlation between bacterial growth and polycyclic aromatic hydrocarbon (PAH) degradation, the growth kinetics of the bacterial isolates were quantitatively assessed by monitoring optical density ($OD_{600\ nm}$) over 96 hours, reflecting the utilization of pyrene as a carbon source (Table 3). The results confirmed a significant relationship between bacterial growth and pyrene degradation, as the reduction in OD_{600nm} readings correlated with the consumption of pyrene.

Among the isolates, *Citrobacter freundii* and *Pseudomonas alcaligenes* exhibited the highest initial growth rates, with OD_{600nm} readings of 1.45 ± 0.01 and 1.25 ± 0.02 at 0 hours, respectively. By the 96-hour mark, a marked decrease in OD_{600nm} was observed, suggesting that these bacteria efficiently utilized pyrene, leading to its degradation. This pattern of growth reduction is consistent with the findings of Sazonova *et al.* (2022), who reported that bacteria from genera such as *Pseudomonas* demonstrate high PAH-degrading potential through the utilization of PAHs as carbon sources, which subsequently results in decreased bacterial growth when the PAHs are consumed.

The reduction in OD_{600nm} also suggests that *Citrobacter freundii* and *Pseudomonas alcaligenes* are likely employing enzymatic pathways to degrade pyrene, an approach commonly observed in PAH-degrading bacteria (Mishra and Das*,* 2019; Sazonova *et al.,* 2022). These findings align with studies by Liang *et al.* (2014), who emphasized the role of enzymatic systems in bacterial PAH degradation, particularly in Gram-negative bacteria such as *Pseudomonas*, which possess specific dioxygenases capable of breaking down complex PAHs like pyrene.

Interestingly, isolates such as *Raoultella ornithinolytica* and *Pseudomonas alcaligenes* showed slower growth rates but still exhibited considerable PAH degradation, as evidenced by the reduction in OD_{600nm} readings. This observation suggests that even bacteria with slower growth kinetics can effectively degrade PAHs, possibly due to specific metabolic pathways that enable the degradation of high-molecular-weight PAHs. This is consistent with findings from Yu *et al.* (2020), who demonstrated that the ability to degrade PAHs does not necessarily correlate with rapid growth, as some bacterial strains can inhabit metabolic pathways that allow them to utilize PAHs efficiently, even in the absence of significant growth.

The observed variability in bacterial growth and PAH degradation capabilities further emphasizes the complexity of the microbial processes involved in PAH biodegradation. As Hennessee and Li (2016) indicated, the degradation potential of bacteria can be influenced by various factors, including the presence of specific PAH-degrading enzymes, the availability of co-substrates, and environmental conditions. Moreover, as suggested by Zhao *et al.* (2011), the addition of surfactants or biostimulants could enhance bacterial surface interactions with hydrophobic PAHs, potentially increasing degradation rates in certain bacterial strains. Therefore, the quantitative assessment of bacterial growth and PAH degradation clearly demonstrated a correlation between the two, particularly for *Citrobacter freundii* and *Pseudomonas alcaligenes*, which exhibited efficient pyrene degradation. Additionally, the slower-growing isolates such as *Raoultella ornithinolytica* highlight the importance of considering metabolic capabilities and enzyme production in evaluating the overall potential of bacteria for PAH biodegradation. These findings are in line with Yu *et al.* (2020) and Mishra and Das (2019), who stressed the importance of understanding bacterial growth dynamics and enzymatic pathways in optimizing bioremediation strategies.

Table 3: Growth Kinetics and PAH Degradation by Bacterial Isolates

Probable organisms Isolate	**Time in hours ± Standard deviation**				
	4	**24**	**48**	**72**	**96**
Salmonella pullorum	1.50 ± 0.01	1.45 ± 0.02	1.21 ± 0.02	1.12 ± 0.03	1.05 ± 0.01
Citrobacter freundii	1.25 ± 0.02	1.45 ± 0.02	1.30 ± 0.01	1.25 ± 0.01	1.05 ± 0.01
Staphylococcus warneri	1.50 ± 0.01	1.47 ± 0.01	1.42 ± 0.01	1.18 ± 0.02	1.10 ± 0.01
Leclercia adecarboxylata	1.50 ± 0.03	1.45 ± 0.02	1.30 ± 0.02	1.15 ± 0.02	1.10 ± 0.01
Micrococcus varians	1.60 ± 0.02	1.55 ± 0.02	1.45 ± 0.03	1.30 ± 0.02	1.10 ± 0.01
Pseudomonas alcaligenes	1.50 ± 0.02	1.40 ± 0.03	1.25 ± 0.02	1.30 ± 0.02	1.05 ± 0.01
Klebsiella oxytoca	1.50 ± 0.01	1.40 ± 0.02	1.25 ± 0.01	1.00 ± 0.01	0.95 ± 0.01
Raoultella ornithinolytica	1.45 ± 0.02	1.20 ± 0.02	1.10 ± 0.03	1.00 ± 0.01	0.95 ± 0.02
Kluyvera ascorbate	1.45 ± 0.03	1.45 ± 0.02	1.43 ± 0.01	1.20 ± 0.01	1.20 ± 0.02
Actinobacillus minor	1.50 ± 0.01	1.45 ± 0.02	1.40 ± 0.01	1.35 ± 0.02	1.30 ± 0.02
Enterobacter aerogenes	1.50 ± 0.02	1.35 ± 0.02	1.25 ± 0.01	1.20 ± 0.01	1.15 ± 0.01
Proteus penneri	1.60 ± 0.01	1.60 ± 0.01	1.54 ± 0.01	1.53 ± 0.01	1.45 ± 0.02
Morganella morganii	1.25 ± 0.01	1.20 ± 0.01	1.10 ± 0.02	1.00 ± 0.02	0.95 ± 0.02
Micrococcus roseus	1.50 ± 0.02	1.46 ± 0.01	1.46 ± 0.01	1.40 ± 0.01	1.40 ± 0.02
Enterobacter cloacae	1.40 ± 0.01	1.40 ± 0.02	1.38 ± 0.01	1.35 ± 0.01	1.35 ± 0.02
Enterococcus faecalis	1.45 ± 0.01	1.40 ± 0.02	1.35 ± 0.01	1.35 ± 0.02	1.35 ± 0.01
Pseudomonas putida	1.35 ± 0.02	1.25 ± 0.01	1.15 ± 0.01	1.10 ± 0.01	0.97 ± 0.01
Rhodococcus erythropolis	1.40 ± 0.02	1.30 ± 0.01	1.20 ± 0.01	1.15 ± 0.02	1.05 ± 0.02

Initial concentration @ OD 600nm: 1.60

Morphological, Biochemical, and Sugar Fermentation Characterization

A comprehensive morphological, biochemical, and sugar fermentation assessment was conducted to further characterize the PAH-degrading bacteria. The results from these tests are summarized in Table 4. The bacterial isolates displayed diverse phenotypic characteristics, with Gram-negative bacteria such as *Pseudomonas alcaligenes*, *Citrobacter freundii*, and *Raoultella ornithinolytica* demonstrating robust enzymatic activities such as catalase and oxidase production. Moreover, these isolates were positive for fermentation of glucose and sucrose, which is consistent with their ability to metabolize PAHs efficiently. These findings corroborate previous studies which highlight the role of such metabolic pathways in the bioremediation of environmental contaminants (Liang *et al*., 2014; Zhao *et al*., 2011).

Interestingly, *Pseudomonas alcaligenes* and *Citrobacter freundii* demonstrated a strong ability to degrade pyrene, as well as robust enzymatic activity and sugar fermentation profiles, suggesting that these isolates may possess specialized pathways for PAH degradation. This observation is consistent with the work of Mishra and Das (2019), who reported that *Pseudomonas* strains are commonly associated with high PAH degradation capabilities due to their ability to utilize aromatic compounds as sole carbon sources.

Growth Kinetics and Quantitative Evidence of Pyrene Utilization

Quantitative assessment using optical density (OD_{600nm}) measurements provided further insight into the relationship between bacterial growth dynamics and pyrene degradation. Isolates that exhibited strong DCPIP discolouration also showed notable reductions in OD_{600nm} values over time, suggesting depletion of pyrene as a carbon source rather than simple biomass accumulation. *Citrobacter freundii* and *Pseudomonas alcaligenes* displayed the clearest coupling between growth patterns and substrate utilization, reinforcing their roles as efficient pyrene degraders.

Interestingly, some isolates exhibited slower growth rates yet still demonstrated measurable PAH degradation. This observation indicates that high degradation efficiency does not necessarily correlate with rapid biomass proliferation. Similar patterns have been reported in recent studies where slow-growing bacteria possess specialized enzymatic systems that enable effective transformation of recalcitrant hydrocarbons under energy-limited or oxygen-restricted conditions (Li *et al*., 2021; Zhou *et al*., 2024).

Several key correlations can be drawn between the results of the screening assay (Table 2) and the optical density measurements (Table 3):

There is a general correlation between bacterial growth and PAH degradation. Isolates such as *Citrobacter freundii* and *Pseudomonas alcaligenes*, which exhibited significant colour changes in the screening assay, also showed high growth rates followed by a decrease in OD_{600nm} readings, suggesting that these bacteria are efficiently utilizing pyrene as a carbon source.

The screening assay provides a rapid and cost-effective method for identifying potentially effective PAH-degrading bacteria. However, the growth kinetics and OD600 measurements offer more detailed insights into the degradation process over time, providing a better understanding of the relationship between bacterial growth and PAH degradation. The combination of both methods offers a more comprehensive evaluation of the bacterial isolates' bioremediation potential.

Consequently, the comparison between the qualitative screening assay (Table 2) and the quantitative growth assessment (Table 3) demonstrates the complementary strengths of both techniques. While the screening assay provides a useful tool for the initial identification of promising PAH-degrading bacteria, the optical density measurements offer a deeper understanding of the growth dynamics and pyrene degradation over time. The results suggest that *Citrobacter freundii* and *Pseudomonas alcaligenes* are among the most efficient PAH-degrading isolates, exhibiting both significant colour change in the screening assay and substantial growth reduction in the OD_{600nm} readings. Further studies could refine these findings and explore the metabolic pathways underlying PAH degradation in these bacterial strains.

CONCLUSION

This study demonstrates that the semi-anoxic intestinal lining of cows harbor bacterial isolates capable of degrading pyrene, a representative high-molecular-weight polycyclic aromatic hydrocarbon. Using a combination of a low-cost DCPIP redox assay and growth kinetics analysis, several isolates particularly *Citrobacter freundii* and *Pseudomonas alcaligenes* were identified as efficient PAH degraders. The consistency between qualitative and quantitative assessments validates the reliability of this screening framework for rapid identification of functionally relevant isolates. The findings highlight the gut environment as an unconventional, yet promising reservoir of metabolically versatile microbiota suited to oxygen limited environments The use of simple and affordable screening tools enhances accessibility for laboratories in resource constrained settings and supports the development of locally adapted, sustainable remediation strategies with relative speed.

REFERENCES

Adebusoye, S. A. (2023). Microbial strategies for polycyclic aromatic hydrocarbon degradation in polluted ecosystems. Environmental Advances, 12, 100342. https://doi.org/10.1016/j.envadv.2023.100342

Ajiboye, A. T., Amusa, T. O., & Akinpelu, O. A. (2020). Biodegradation of crude oil using bacterial isolates from oil-contaminated soil in Nigeria. Bioremediation Journal, 24(2), 133–142. https://doi.org/10.1080/10889868.2020.1738874

Alotaibi, M. O., Alhumaid, S., & Khan, M. A. (2024). Recent trends in bioremediation of polycyclic aromatic hydrocarbons using bacterial consortia. Science of the Total Environment, 913, 168236. https://doi.org/10.1016/j.scitotenv.2023.168236

Al-Dossary, M., Abood, S., & Al-Saad, H. (2021). Effects of physicochemical factors on pah degradation by planomicrobium alkanoclasticum. Remediation Journal, 31(2), 29-37. https://doi.org/10.1002/rem.21673

Сазонова, O., Gavrichkova, O., Ivanova, A., Petrikov, K., Streletskii, R., Sarzhanov, D. & Vetrova, A. (2022). Polycyclic aromatic hydrocarbon-degrading bacteria in three different functional zones of the cities of moscow and murmansk. Microorganisms,10(10), 1979. https://doi.org/10.3390/microorganisms10101979

Davletgildeeva, A. T., Zavarzina, D. G., Tarasov, A. L., & Merkel, A. Y. (2024). Bacterial degradation of polycyclic aromatic hydrocarbons: Pathways, enzymes and prospects. Microorganisms, 12(9), 1814. https://doi.org/10.3390/microorganisms12091814

Hennessee, C. T., & Li, Q. X. (2016). Soil microbial community structure and diversity after polycyclic aromatic hydrocarbon contamination and bioremediation. NPJ Biofilms and Microbiomes, 2(1), 1–11. https://doi.org/10.1038/npjbiofilms.2016.3

Hussain, A., Shakoor, A., & Rehman, A. (2022). Rumen microbiome: An emerging frontier for environmental bioremediation. Frontiers in Microbiology, 13, 943710. https://doi.org/10.3389/fmicb.2022.943710

Koma, D., Hasumi, F., Chung, S. Y., & Kubo, M. (2003). Biodegradation of long-chain n-paraffins from waste oil of car engine by Acinetobacter sp. Journal of Bioscience and Bioengineering, 95(6), 641–643. https://doi.org/10.1016/S1389-1723(03)80100-5

Kuppusamy, S., Kumar, V., & Megharaj, M. (2022). Biodegradation of polycyclic aromatic hydrocarbons by bacteria: Mechanisms and challenges. Chemosphere, 303, 135205. https://doi.org/10.1016/j.chemosphere.2022.135205

Liang, L., Song, X., Kong, J., Shen, C., Huang, T., & Huang, Z. (2014). Anaerobic biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by a facultative anaerobe pseudomonas sp. jp1. Biodegradation, 25(6), 825-833. https://doi.org/10.1007/s10532-014-9702-5

Li, Y., Ma, Z., Zhang, X., & Wu, J. (2021). Anaerobic biodegradation of polycyclic aromatic hydrocarbons: Microbial pathways and ecological relevance. Journal of Hazardous Materials, 413, 125378. https://doi.org/10.1016/j.jhazmat.2021.125378

Nwankwegu, A. S., Chibuike, G. U., & Ogbonna, O. O. (2022). Environmental risk and microbial remediation of petroleum hydrocarbon-contaminated soils: Advances and perspectives. Environmental Technology & Innovation, 28, 102950. https://doi.org/10.1016/j.eti.2022.102950

Mishra, M. and Das, S. (2019). Profiling marine bacterial dioxygenase gene involved in polycyclic aromatic hydrocarbon degradation. Indo Pac J Ocean Life, 1(1), 32-40. https://doi.org/10.13057/oceanlife/o010106

Oduro-Mensah, D., Amoah, C., & Darko, G. (2021). Exploring the ruminant microbiome for environmental biotechnology. Bioresource Technology Reports, 14, 100684. https://doi.org/10.1016/j.biteb.2021.100684

Roy, A. S., Baruah, R., Borah, M., Singh, A. K., Deka, M., Deka, S., Boruah, H. P. D., Saikia, N., Dutta, N., & Bora, T. C. (2002). Bioremediation potential of native hydrocarbon-degrading microorganisms in crude oil contaminated soil under different environmental conditions. Bioremediation Journal, 6(3), 241–252. https://doi.org/10.1080/10889860290075968

Sazonova, O. I., Khilyas, I. V., & Gumerov, V. M. (2022). Comparative genomics of polycyclic aromatic hydrocarbon-degrading bacteria: Pathways and adaptive strategies. Microorganisms, 10(9), 1822. https://doi.org/10.3390/microorganisms10091822

Varjani, S. J., Pandey, A., & Nitisoravut, S. (2023). Bioremediation of recalcitrant pollutants: Emerging microbial and enzymatic approaches. Bioresource Technology, 370, 128631. https://doi.org/10.1016/j.biortech.2022.128631

Yu, Z., Hu, Z., Xu, Q., Zhang, M., Yuan, N., Liu, J. & Yin, J. (2020). The luxi/luxr-type quorum sensing system regulates degradation of polycyclic aromatic hydrocarbons via two mechanisms. International Journal of Molecular Sciences, 21(15), 5548. https://doi.org/10.3390/ijms21155548

Zhao, Z., Selvam, A., & Wong, J. (2011). Effects of rhamnolipids on cell surface hydrophobicity of pah degrading bacteria and the biodegradation of phenanthrene. Bioresource Technology, 102(5), 3999-4007. https://doi.org/10.1016/j.biortech.2010.11.088

Zhou, G., Wang, Y., Li, J., & Chen, Y. (2024). High-efficiency bacterial degradation of phenanthrene and related PAHs under oxygen-limited conditions. Frontiers in Microbiology, 15, 1298472. https://doi.org/10.3389/fmicb.2024.1298472