

Review Form 3

Journal Name:	Journal of Advances in Microbiology
Manuscript Number:	Ms_JAMB_129847
Title of the Manuscript:	Isolation, Screening and Identification of Bacteria Isolates with Hydrocarbon Biodegradative Potentials
Type of the Article	Original Research Article

PART 1: Comments

	Reviewer's comment	Author's Feedback (Please correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Please write a few sentences regarding the importance of this manuscript for the scientific community. A minimum of 3-4 sentences may be required for this part.	This manuscript holds significant importance for the scientific community as it explores the potential of indigenous bacterial species in remediating hydrocarbon-contaminated soils, addressing a pressing environmental issue caused by petroleum pollution. By isolating and identifying bacteria such as <i>Acinetobacter rudis</i> and <i>Pseudomonas koreensis</i> with high hydrocarbon degradation capacities, the study provides a sustainable and effective solution for mitigating the adverse effects of diesel pollution on ecosystems. The integration of molecular techniques, such as 16S rRNA gene sequencing, alongside biochemical screening, ensures a robust identification of these bacterial strains, enabling further research and application in bioremediation technologies. Ultimately, the findings contribute to advancing knowledge in environmental microbiology and fostering cleaner, more resilient ecosystems.	Thanks for the comments
Is the title of the article suitable? (If not please suggest an alternative title)	The current title, "Isolation, Screening and Identification of Bacteria Isolates with Hydrocarbon Biodegradative Potentials," is clear and conveys the primary focus of the study. However, it could be made more concise and engaging while retaining its scientific relevance. Suggested Alternative Title: "Isolation and Identification of Hydrocarbon-Degrading Bacteria from Diesel-Contaminated Soils" This alternative title highlights the key aspects of the study (isolation, identification, and hydrocarbon degradation) while maintaining clarity and scientific precision.	Thanks for the comments

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<p>Is the abstract of the article comprehensive? Do you suggest the addition (or deletion) of some points in this section? Please write your suggestions here.</p>	<p>The abstract provides a good overview of the study, covering the aim, methodology, results, and conclusion. However, it could be improved for clarity and completeness by adding more specific details and making it more concise. Here are some suggestions for improvement:</p> <p><b>Additions:</b> <b>Details on Key Findings:</b> While the abstract mentions the identification of potent degraders, it does not specify the exact strains or their respective percentages. Including these details would make the findings more impactful. For example, mention <i>Acinetobacter rudis</i> and its performance (84% diesel degradation).</p> <p><b>Context and Application:</b> Highlight the significance of these findings for practical applications, such as bioremediation in environmental management, to emphasize the study's relevance.</p> <p><b>Deletions/Edits:</b> <b>Simplify Methodology:</b> The abstract provides detailed methods (e.g., the use of DCPIP and turbidity measurements). While useful, such specifics may be condensed to make room for discussing the implications of the results.</p> <p><b>Clarify Conclusion:</b> Strengthen the conclusion by emphasizing how this study contributes to the broader field of environmental microbiology and its potential impact on remediating hydrocarbon pollution.</p> <p><b>Suggested Revised Abstract:</b> This study aimed to isolate, screen, and identify bacterial species with hydrocarbon biodegradative potential from diesel-contaminated soils using redox indicators and turbidity measurements. Soil samples from a diesel-polluted site were analyzed, and eight bacterial species were identified, including <i>Acinetobacter rudis</i> and <i>Pseudomonas koreensis</i>, with <i>Acinetobacter rudis</i> achieving the highest degradation efficiency of 84% in 15 days. These findings demonstrate the potential of indigenous bacteria for effective bioremediation of petroleum-contaminated environments. The study provides critical insights into sustainable solutions for mitigating hydrocarbon pollution, contributing to advancements in environmental microbiology and ecosystem restoration.</p>	<p>All comments effected. Thank you.</p>
<p>Is the manuscript scientifically, correct? Please write here.</p>	<p>Yes, the manuscript appears to be scientifically sound. The study is methodologically robust, as it employs a combination of microbiological, biochemical, and molecular techniques to isolate, screen, and identify bacterial strains with hydrocarbon-degrading capabilities. The use of standardized methods, such as 16S rRNA gene sequencing for molecular identification and DCPIP for screening, adds reliability to the findings. The statistical analysis, including ANOVA, ensures the validity of the results.</p> <p>However, there are a few areas where clarification or additional details could strengthen the scientific rigor:</p> <ol style="list-style-type: none"><li><b>Experimental Controls:</b> The manuscript includes control setups for the experiments, but it would benefit from a more explicit discussion of how controls were used to validate the findings, particularly for the screening phase.</li><li><b>Replicates and Variability:</b> While the study mentions statistical analysis, it does not detail the number of replicates performed for the experiments. Including this information would enhance the reliability of the data.</li><li><b>Reproducibility:</b> The methodology is detailed enough for replication, but clearer documentation of environmental conditions (e.g., temperature, pH) during experiments would improve reproducibility.</li></ol>	<p>Comments have been effected, thanks.</p>

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	<p>4. <b>Limitations:</b> The manuscript does not explicitly address limitations, such as the potential influence of other environmental factors on biodegradation efficiency or the scalability of the findings for field applications.</p> <p><b>Overall Verdict:</b> The manuscript is scientifically correct, with well-structured experiments and validated findings. Addressing the above points would further solidify its scientific robustness.</p>	
<p>Are the references sufficient and recent? If you have suggestions of additional references, please mention them in the review form.</p>	<p>The references included in the manuscript are relevant and support the study's context and methodology. However, there are areas where the references could be enhanced to ensure they are comprehensive and up-to-date.</p> <p><b>Observations on Existing References:</b></p> <p>1. <b>Sufficiency:</b> The references sufficiently cover the background of hydrocarbon contamination, bioremediation, and bacterial characterization techniques. They include foundational studies as well as recent works up to 2023.</p> <p>2. <b>Recency:</b> Most references are recent, particularly the ones published between 2020 and 2023. However, some foundational references, such as Muyzer et al. (1995), are older. While these are still relevant, supplementing them with recent advancements in molecular techniques would strengthen the manuscript.</p> <p><b>Suggestions for Additional References:</b> To provide more depth and recent insights, consider including the following:</p> <p>1. Studies on advancements in 16S rRNA sequencing and its application in identifying hydrocarbon-degrading bacteria:</p> <ul style="list-style-type: none"><li>○ Louca, S., Parfrey, L. W., &amp; Doebeli, M. (2016). Decoupling function and taxonomy in the global ocean microbiome. <i>Science</i>, 353(6305), 1272–1277.</li></ul> <p>2. Research focusing on field-scale applications of hydrocarbon biodegradation:</p> <ul style="list-style-type: none"><li>○ Varjani, S., &amp; Gnansounou, E. (2017). Microbial dynamics in petroleum oilfields and their relationship with physiological properties of petroleum oil reservoirs. <i>Bioresource Technology</i>, 245, 1258–1265.</li></ul> <p>3. Recent reviews on bioremediation technologies:</p> <ul style="list-style-type: none"><li>○ Das, N., &amp; Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: An overview. <i>Biotechnology Research International</i>, 2011, 941810.</li></ul> <p><b>Summary:</b> The references are sufficient and relevant to the study. Adding recent references in the areas of molecular techniques, field-scale bioremediation applications, and microbial dynamics could further enhance the manuscript's depth and contemporary relevance.</p>	<p>Thanks for the comments Noted and corrected</p>

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<p>Is the language/English quality of the article suitable for scholarly communications?</p>	<p>The language quality of the manuscript is generally appropriate for scholarly communication, as it effectively employs technical terminology and follows a logical structure. However, certain areas would benefit from refinement to enhance clarity, precision, and adherence to academic conventions.</p> <p><b>Strengths:</b></p> <ol style="list-style-type: none"><li><b>Technical Terminology:</b> The manuscript utilizes appropriate scientific terms, making it relevant and understandable for an academic audience.</li><li><b>Structured Presentation:</b> The logical organization of sections facilitates a coherent narrative, aligning with standard practices in scientific writing.</li></ol> <p><b>Areas for Refinement:</b></p> <ol style="list-style-type: none"><li><b>Grammar and Syntax:</b> Some sentences contain minor grammatical errors or are excessively lengthy, potentially hindering readability. For instance:<ul style="list-style-type: none"><li><b>Original:</b> "This study demonstrated the availability of indigenous bacterial species with hydrocarbon biodegradative potentials in hydrocarbon-contaminated soils in our environment, to effectively and promptly to be deployed to remediate hydrocarbon contaminated soils."</li><li><b>Revised:</b> "This study identified indigenous bacterial species with hydrocarbon-degrading potential, which can be effectively deployed for the remediation of hydrocarbon-contaminated soils."</li></ul></li><li><b>Conciseness and Precision:</b> Certain sections, particularly the abstract and methodology, include redundant phrases that could be streamlined for better clarity. For example:<ul style="list-style-type: none"><li>"Positive degrader of hydrocarbon" can be revised to "Effective hydrocarbon degrader."</li></ul></li><li><b>Scholarly Tone:</b> Some phrases lack the formal tone expected in academic writing. For instance:<ul style="list-style-type: none"><li><b>Original:</b> "The medium was observed for decolourization of the blue colour of the indicator."</li><li><b>Revised:</b> "The medium was monitored for changes in the blue coloration of the indicator."</li></ul></li><li><b>Word Choice and Sentence Construction:</b> Phrasing should be revised to align with academic conventions. For example:<ul style="list-style-type: none"><li>Replace "positive degrader" with "demonstrated hydrocarbon-degrading efficacy."</li></ul></li></ol> <p><b>Recommendations:</b></p> <ol style="list-style-type: none"><li>Conduct a detailed proofreading to address grammatical issues, improve syntax, and remove redundancy.</li><li>Simplify overly complex sentences to improve readability while maintaining an academic tone.</li><li>Engage a professional language editor or utilize academic proofreading tools to ensure conformity with scholarly standards.</li></ol>	
<p><u>Optional/General</u> comments</p>	<p><b>Lacunae in the Research Article:</b></p> <ol style="list-style-type: none"><li><b>Field Applicability and Scalability:</b> While the study demonstrates laboratory-scale hydrocarbon degradation, it does not address how the findings can be scaled for field applications. Real-world environmental conditions, such as temperature fluctuations, soil heterogeneity, and the presence of competing microbial species, could influence the efficacy of these bacterial isolates.</li><li><b>Synergistic Interactions:</b> The study focuses on individual bacterial strains but does not explore the potential synergistic effects of bacterial consortia. Mixed microbial communities may exhibit enhanced degradation capabilities due to complementary metabolic pathways.</li><li><b>Impact of Environmental Factors:</b> The influence of environmental parameters such as salinity, pH, nutrient availability, and oxygen levels on the degradation efficiency of the bacterial isolates is not extensively studied.</li><li><b>Genomic Insights:</b> While molecular identification was performed using 16S rRNA sequencing, the study</li></ol>	

	<p>does not delve into the genomic or proteomic pathways responsible for hydrocarbon degradation, limiting the understanding of the metabolic mechanisms.</p> <p>5. <b>Toxicity Assessment:</b> The research does not address whether metabolites generated during the degradation process are environmentally safe or toxic, which is critical for assessing the overall efficacy and safety of bioremediation.</p> <p>6. <b>Temporal Analysis:</b> The study presents results over a 15-day period but does not examine the long-term stability or sustainability of bacterial hydrocarbon degradation.</p> <hr/> <p><b>Future Directions:</b></p> <ol style="list-style-type: none"><li><b>Field Trials:</b> Conduct large-scale field studies to validate the efficacy of the identified bacterial isolates in natural conditions, accounting for environmental variability and site-specific challenges.</li><li><b>Bacterial Consortia Studies:</b> Explore the use of microbial consortia instead of individual strains to investigate whether synergistic interactions enhance hydrocarbon degradation.</li><li><b>Omics-Based Research:</b> Employ genomics, transcriptomics, and proteomics to identify the specific genes and pathways involved in hydrocarbon degradation. This can aid in understanding the mechanisms and optimizing bacterial performance.</li><li><b>Environmental Parameter Optimization:</b> Study the effect of environmental factors such as nutrient amendments, oxygen availability, and temperature on the degradation efficiency. Optimize these conditions to enhance bacterial performance.</li><li><b>Metabolite Analysis:</b> Identify and characterize intermediate metabolites formed during hydrocarbon degradation to ensure they are non-toxic and environmentally benign.</li><li><b>Bioreactor Development:</b> Design and test bioreactors using the identified bacterial strains for controlled and efficient hydrocarbon degradation in industrial or field settings.</li><li><b>Long-Term Studies:</b> Evaluate the sustainability and long-term effectiveness of the bacterial isolates in degrading hydrocarbons over extended periods.</li><li><b>Comparative Studies:</b> Compare the efficacy of indigenous bacterial species with genetically engineered or commercial strains to assess their relative advantages and limitations.</li></ol> <p>Title Clarity:</p> <p>The title should clearly reflect the study's focus on hydrocarbon biodegradation. Consider revising it for conciseness and specificity.</p> <p>Abstract:</p> <p>The abstract is comprehensive but could benefit from a clearer structure. Consider separating the aim, methodology, results, and conclusion into distinct sections for better readability.</p>	
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	<p>Methodology:</p> <p>Ensure that the methodology section includes detailed descriptions of the techniques used, including any specific conditions or controls implemented during experiments.</p> <p>Clarify the statistical methods used for data analysis, including any software or specific tests applied.</p> <p>Results Presentation:</p> <p>Tables and figures should be clearly labeled and referenced in the text. Ensure that all tables (e.g., Table 1, Table 2) are discussed in the results section.</p> <p>Consider adding visual aids (graphs or charts) to enhance the presentation of key findings, particularly for turbidity and degradation percentages.</p> <p>Discussion:</p> <p>The discussion should connect findings to existing literature more explicitly. Compare results with similar studies to highlight the significance of your findings.</p> <p>Address any potential limitations of the study and suggest areas for future research.</p> <p>Conclusion:</p> <p>The conclusion should succinctly summarize the main findings and their implications for bioremediation practices. Consider emphasizing the practical applications of the identified bacterial species.</p> <p>References:</p> <p>Ensure that all references are formatted consistently according to the journal's guidelines. Check for completeness and accuracy of citation details.</p> <p>Language and Style:</p> <p>Review the manuscript for grammatical errors and improve the overall clarity and flow of the text. Consider using simpler language where possible to enhance accessibility.</p> <p>Ethical Considerations:</p>	
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	<p>If applicable, mention any ethical approvals obtained for the study, especially regarding environmental sampling and handling of biological materials.</p> <p>Supplementary Materials:</p> <p>If there are additional data or materials that support the findings, consider including them as supplementary materials for transparency.</p>	
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PART 2:

	<b>Reviewer's comment</b>	<b>Author's comment</b> <i>(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)</i>
Are there ethical issues in this manuscript?	<i>(If yes, Kindly please write down the ethical issues here in details)</i>	