Review Form 3

Journal Name:	**Asian Journal of Biotechnology and Bioresource Technology**
Manuscript Number:	**Ms_AJB2T_123854**
Title of the Manuscript:	**Isolation and Molecular Characterization of Industrially Significant Bacteria Obtained from Rice-Husks Dumping Sites**
Type of the Article	**Original Research Article**

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PART 1: **Review Comments**

Compulsory REVISION 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. Why do you like (or dislike) this manuscript? A minimum of 3-4 sentences may be required for this part.	This manuscript is significant for the scientific community because it addresses the pressing need for the identification and characterization of industrially significant bacteria capable of cellulose degradation, which has promising applications in biotechnology and waste management. By isolating and molecularly characterizing *Bacillus* strains from rice husk-contaminated soil, it opens new avenues for the sustainable use of agricultural waste. I appreciate the manuscript's focus on both biochemical and molecular techniques, which enhances the credibility and depth of the findings.	
Is the title of the article suitable? **(If not please suggest an alternative title)**	Yes, the title is suitable	
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.	I have suggested in the comments file.	
Are subsections and structure of the manuscript appropriate?	Yes	
Please write a few sentences regarding the scientific correctness of this manuscript. Why do you think that this manuscript is scientifically robust and technically sound? A minimum of 3-4 sentences may be required for this part.	This manuscript appears to be scientifically robust and technically sound as it follows a well-established methodology for isolating and characterizing bacteria from soil samples. The use of both biochemical and molecular techniques, including PCR and 16S rRNA sequencing, provides strong evidence for the accurate identification of *Bacillus* species. The experimental design, including the screening for cellulose utilization and the construction of phylogenetic trees, is appropriate and aligns with standard scientific protocols. Moreover, the results are presented in a clear and logical manner, with sufficient detail to allow reproducibility, further supporting the manuscript's scientific validity.	
Are the references sufficient and recent? If you have suggestions of additional references, please mention them in the review form. **-**	This has been provided in the comments file	

Minor REVISION comments **Is the language/English quality of the article suitable for scholarly communications?**	Yes, it is suitable for scholarly communications.	
Optional/General comments	The study focuses on the isolation, biochemical, and molecular characterization of industrially significant bacterial species from rice husk dumping sites in Kalambaina, Sokoto. Soil samples were collected from three different locations, and heterotrophic bacterial counts were performed to assess microbial density. Sample C, from Diga-Tsallake Kalambaina, exhibited the highest bacterial count compared to Samples A and B. Screening for cellulose utilization identified five bacterial isolates capable of degrading cellulose, with two isolates demonstrating the clearest zones of hydrolysis. Biochemical tests revealed that the isolates were Gram-positive, rod-shaped, and motile, belonging to the genus Bacillus. Molecular analysis using 16S rRNA gene sequencing confirmed the identity of two potent cellulose-degrading strains, Bacillus sp. AT-b3 and Bacillus sp. CMF 12, with percentage identity values exceeding 90%. Phylogenetic analysis further explored the evolutionary relationships of these strains with other species. The findings highlight the potential of rice husk-contaminated soils as a reservoir for industrially relevant bacteria, particularly for cellulose degradation, which could be harnessed for biotechnological applications. I. In the Abstract section, there is a minor repetition error in the phrase "novel bacteria bacteria" that needs correction. Also, "designed" should replace "design" for grammatical consistency. II. Could you provide more detail on the quantitative or qualitative assessment of cellulose degradation? III. The abstract highlights the potential of discovering novel bacteria from rice husk dumping sites, yet it's not clear if any of the bacteria identified in this study are newly discovered species or if they belong to well-known industrial Bacillus strains. Can the authors elaborate on whether any of the isolates represent novel strains, or are they strains with previously known applications? IV. The introduction cites a few studies (e.g., [1][2][3]) but lacks specific details about how these references support the claims made. Could the authors briefly summarize the key findings of these references, especially regarding rice husk output and its use in microbial fermentation? V. In the methodology section 2.1: Why was a 10 cm depth specifically chosen for sampling along with surface samples and how does this depth impact microbial community structure in comparison to deeper or shallower samples? VI. In section 3.7: The use of Congo red for detecting cellulose hydrolysis is a well-known technique. Were there any specific reasons for choosing this dye, and were other staining techniques (e.g., iodine staining) considered? How would the results differ with other methods? VII. In section 3.10.6: During the bioinformatics analysis, how were low-quality sequences filtered out before performing the BLAST search? In results and discussion section Bacteriological Analysis performed: VIII. What factors might contribute to the higher bacterial count observed in Sample C compared to Samples A and B? IX. The cellulose utilization screening showed that only five isolates were positive, with two displaying the clearest zones of hydrolysis. What factors might account for the variation in cellulose degradation efficiency between these isolates? X. The phylogenetic trees constructed for Bacillus sp. AT-b3 and Bacillus sp. CMF 12 were based on the Neighbor-Joining method. Why were this method chosen over other algorithms (e.g., Maximum Likelihood, Bayesian Inference)? Could different methods lead to variations in the inferred evolutionary relationships?	

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PART 2:

	Reviewer's comment	**Author's comment** *(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)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

Reviewer Details:

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