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REVIEWER'S REPORT

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Title: Charaterization of Producing-Bioactives Compounds Bacterial Isolates collected from soil at Brazzaville

Recommendation:
Accept as it is

Rating	Excel.	Good	Fair	Poor
Originality	√			
Techn. Quality		√		
Clarity		√		
Significance			√	

Reviewer Name: Dr. Manju M

Detailed Reviewer's Report

1. Background and Scientific Rationale

Soil ecosystems represent one of the most complex and diverse biological reservoirs on Earth, hosting a vast array of microorganisms with critical ecological and biotechnological functions. These microorganisms are involved in nutrient cycling, organic matter degradation, soil fertility maintenance, and environmental detoxification. Among soil microbes, bacteria dominate both numerically and functionally, producing numerous bioactive compounds of industrial and environmental importance. Hydrolytic enzymes and biosurfactants synthesized by soil bacteria are particularly valuable for applications in biotechnology, agriculture, and bioremediation. Understanding soil microbial diversity is therefore essential for identifying novel functional strains. This study was designed to explore these aspects in soils from Brazzaville, Republic of Congo.

2. Context of Soil Microbial Diversity in Brazzaville

Previous studies conducted in Brazzaville have demonstrated considerable microbial diversity across different urban and peri-urban soils. However, many earlier investigations focused on general biodiversity without explicitly linking soil type to functional bioactivity. Brazzaville soils are influenced by anthropogenic activities, climatic conditions, and land use patterns, which shape microbial composition. Variability in pH, organic matter content, and temperature creates ecological niches for diverse microbial populations. Despite this diversity, systematic comparisons among distinct soil categories remain limited. The present study addresses this gap by targeting well-defined soil types. This contextual framing strengthens the relevance of the investigation.

3. Objectives of the Study

The primary objective of this research was to characterize cultivable bacterial isolates from different soil categories in Brazzaville. A secondary aim was to identify dominant microbial groups and quantify their abundance using standard microbiological methods. The study further sought to evaluate the capacity of bacterial isolates, particularly Bacillus species, to produce hydrolytic enzymes such as cellulases, amylases, and lipases. Another key objective was to assess biosurfactant production using emulsification index measurements. By integrating microbiological and biochemical analyses, the study aimed to link microbial diversity with functional potential. These objectives collectively contribute to understanding soil-derived bioactives.

4. Selection and Characterization of Sampling Sites

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Four distinct sampling sites were selected to represent different soil categories within Brazzaville. These included plantation soil and ordinary soil from the Faculty of Science and Technology, landfill soil from the Massamba-Débat Stadium, and garage soil from the Bacongo district. The sites were chosen to reflect variations in land use and anthropogenic influence. GPS coordinates were recorded to ensure spatial accuracy and reproducibility. Sampling from multiple points at each site minimized spatial heterogeneity. This systematic site selection enhanced the reliability of comparative analyses.

5. Soil Sampling Strategy and Sample Preparation

Soil samples were collected aseptically from a depth of 0.5–1 cm using sterile spatulas to avoid contamination. At each site, three subsamples were collected and pooled to form a composite sample. This approach ensured representative sampling of each soil category. Samples were immediately transported to the laboratory under controlled conditions. Composite sampling reduced local variability while preserving overall site characteristics. The methodology followed established microbiological sampling standards. Such rigor ensured consistency across all sampled locations.

6. Measurement of Physical Soil Parameters

Physical parameters, including temperature and pH, were measured using a multiparameter analyzer at each sampling point. These parameters are critical determinants of microbial survival and activity. The soils exhibited temperatures ranging from 33°C to 41°C, reflecting the tropical climate of Brazzaville. pH values ranged from acidic to near-neutral (4.7–7.0), depending on soil type. Landfill soils showed greater acidity, likely due to organic waste decomposition. These physical conditions strongly influenced microbial distribution and abundance.

7. General Physicochemical Characteristics of the Soils

All sampled soils were predominantly sandy, rich in humus, and dark brown to black in color. Such characteristics indicate high organic matter content and biological activity. Sandy textures favor aeration, which supports aerobic microbial growth. The presence of humus suggests ongoing organic matter turnover. Acidic to neutral pH conditions are conducive to diverse bacterial communities. These soil attributes collectively create favorable environments for metabolically active microorganisms. The consistency of these features across sites supports comparative microbial analysis.

8. Microbial Enumeration Methodology

Microbial enumeration was conducted following the NF EN ISO 6887-1 (2017) standard for preparing stock suspensions and serial dilutions. Ten grams of soil were homogenized in physiological saline to obtain stock solutions. Decimal dilutions were prepared up to 10^{-5} to ensure countable colony numbers. Surface plating was performed using selective and non-selective media. Triplicate plates were used for each dilution to improve statistical reliability. Colony-forming units were calculated using standardized formulas. This approach ensured accurate quantification of microbial populations.

9. Identification of Microbial Groups and Genera

Several microbial groups were identified across the soil samples, including total aerobic mesophilic flora, actinomycetes, fungi, yeasts, and multiple bacterial genera. The bacterial genera identified included Bacillus, Staphylococcus, Pseudomonas, and Enterobacteriaceae. Identification relied on colony morphology, Gram staining, and biochemical tests. This culture-based approach targeted cultivable microorganisms with biotechnological relevance. Although non-cultivable microbes were not assessed, the method effectively captured dominant functional groups. The results reflect the ecological complexity of Brazzaville soils.

10. Distribution of Total Aerobic Mesophilic Flora

Total aerobic mesophilic flora (FMAT) represented the most abundant microbial group in all soil categories. Counts ranged from 1×10^6 to 3.4×10^6 CFU/g, with the highest values observed in garage soils. These high counts indicate intense microbial activity and organic matter turnover. FMAT abundance varied significantly among soil types, reflecting differences in environmental conditions. Landfill and garage soils showed particularly elevated counts due to anthropogenic inputs. This group serves as an indicator of overall soil microbial load.

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REVIEWER'S REPORT

11. Actinomycetes and Fungal Populations

Actinomycetes were consistently present across all soil types, with counts reaching up to 1.8×10^5 CFU/g. These organisms are known producers of antibiotics and extracellular enzymes. Fungi and yeasts were present at lower densities, ranging from 10^3 to 10^4 CFU/g. Their abundance was influenced by soil moisture, pH, and organic matter. Plantation and ordinary soils supported relatively higher fungal counts. The coexistence of bacteria, actinomycetes, and fungi highlights complex microbial interactions. Such diversity enhances the functional resilience of soils.

12. Abundance of Bacterial Genera

Among bacterial genera, Bacillus was one of the most dominant across all soil categories. Enterobacteriaceae, Staphylococcus, and Pseudomonas were also detected but at lower frequencies. Bacillus counts reached up to 1.3×10^5 CFU/g in garage soils. Pseudomonas exhibited the lowest abundance, particularly in highly acidic soils. Variations in abundance reflect genus-specific ecological preferences. These findings are consistent with previous studies conducted in Brazzaville. The dominance of Bacillus justified its selection for further functional analyses.

13. Comparative Microbial Profiles Across Soil Types

Distinct microbial profiles were observed for each soil category. Landfill soils exhibited high microbial loads due to organic waste accumulation. Garage soils showed elevated Bacillus and actinomycete populations, likely influenced by hydrocarbon contamination. Plantation soils supported balanced microbial communities associated with plant residues. Ordinary soils showed moderate microbial abundance with relatively higher cellulase activity. These variations demonstrate the influence of land use on microbial ecology. Comparative profiling provides insights into habitat-specific microbial functions.

14. Isolation and Purification of Bacterial Strains

A total of 59 bacterial isolates were obtained from all soil samples. Repeated subculturing ensured the purity and stability of isolates. Isolates were preserved in glycerol-supplemented TSB at 4°C for further analyses. Random selection minimized bias toward specific morphotypes. This systematic isolation strategy ensured a representative collection of cultivable bacteria. The preserved isolates constitute a valuable microbial resource. They enable reproducible biochemical and functional testing.

15. Phenotypic and Microscopic Characterization

Isolates exhibited diverse colony morphologies, including variations in color, shape, elevation, and consistency. Most isolates were rod-shaped, Gram-positive, and catalase-positive. Motility varied among isolates, reflecting physiological diversity. Bacillus isolates predominantly formed circular, dry or creamy colonies. Staphylococcus isolates were typically coccoid and clustered. These phenotypic traits supported genus-level identification. Such characterization is essential for correlating morphology with function.

16. Selection of Bacillus Isolates for Functional Screening

Nineteen Bacillus isolates were selected for detailed evaluation of bioactive compound production. This genus was prioritized due to its known capacity to synthesize extracellular enzymes and biosurfactants. Bacillus species are also robust and adaptable to diverse environmental conditions. Their spore-forming ability enhances survival in fluctuating soils. Selecting a single dominant genus allowed focused functional analysis. This strategy improved comparability across enzymatic assays.

17. Assessment of Cellulase Production

Cellulase activity was evaluated using cellulose-agar plates and Lugol's iodine staining. Clear halos around wells indicated cellulose degradation. Cellulase production varied significantly among isolates, reflecting strain-specific capabilities. Isolates MO3, MO5, MG2, and MO4 showed the highest cellulolytic activity. Lower activity was observed in isolates MD1, MD2, and MD3. These variations suggest genetic and regulatory differences among Bacillus strains. Cellulase production highlights the role of soil bacteria in plant biomass degradation.

18. Evaluation of Amylase Production

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Amylase production was assessed using starch-agar plates, with clear zones indicating starch hydrolysis. Several isolates exhibited strong amylolytic activity, including MP1, MP2, MG5, and MO3. Other isolates produced comparatively lower enzyme levels. The observed diversity reflects metabolic specialization among strains. Amylase production is ecologically important for starch degradation in soils. Industrially, such enzymes are valuable in food and fermentation processes. These findings reinforce the biotechnological potential of soil Bacillus isolates.

19. Analysis of Lipase Production

Lipase activity was detected using Tween-80 agar plates, where hydrolysis produced visible halos. Lipase production was observed in a substantial proportion of Bacillus isolates. Isolates MO3, MP1, MP3, and MG2 showed particularly strong lipolytic activity. Lipases enable the breakdown of lipid substrates commonly present in contaminated soils. Their presence is especially relevant in garage and landfill soils. These enzymes have applications in detergents, biodiesel production, and waste treatment. The results indicate functional adaptation to soil substrates.

20. Comparative Distribution of Hydrolytic Enzymes

The relative distribution of cellulase, amylase, and lipase production differed among soil categories. Landfill soils showed higher amylase and lipase production. Ordinary soils exhibited relatively higher cellulase activity. Plantation soils displayed balanced enzyme production profiles. These trends reflect substrate availability and environmental pressures. Enzyme diversity enhances microbial adaptability and ecosystem functioning. Such comparative analysis links soil type with functional microbial outputs.

21. Overall Enzymatic Production Profiles

Across all isolates, approximately equal proportions of cellulase- and lipase-producing strains were observed, while amylase production was slightly higher. This suggests a strong capacity for carbohydrate and lipid metabolism among Bacillus isolates. The coexistence of multiple enzymatic activities in single strains indicates metabolic versatility. Such multifunctional bacteria are advantageous for biotechnological exploitation. Enzymatic profiling provides a basis for selecting high-performing strains. These results highlight the functional richness of Brazzaville soils.

22. Biosurfactant Production Screening

Biosurfactant production was evaluated using the emulsification index (E24) assay. All nineteen Bacillus isolates demonstrated measurable emulsification activity. This confirms the widespread ability of soil Bacillus to produce surface-active compounds. Emulsification indices varied significantly among isolates. Strong producers included MG5, MP4, MD2, and MD5. Weaker producers showed lower but detectable activity. These findings underscore the ecological and applied relevance of biosurfactant production.

23. Interpretation of Emulsification Index Results

High emulsification indices indicate effective biosurfactant synthesis capable of stabilizing oil-water interfaces. Such activity is crucial for hydrocarbon degradation in contaminated environments. Variability among isolates suggests differences in biosurfactant structure or concentration. The presence of biosurfactant-producing strains in garage and landfill soils reflects environmental selection pressures. These compounds enhance nutrient accessibility and microbial competitiveness. The results align with previous studies reporting Bacillus-derived biosurfactants. This reinforces the genus's industrial potential.

24. Integration of Microbial Diversity and Function

The study demonstrates a clear link between microbial diversity and functional capacity in soils. Diverse bacterial communities contribute complementary enzymatic and biosurfactant activities. Soil type and land use strongly influence both composition and function. Bacillus emerges as a key functional genus across all environments studied. This integration of ecological and biochemical data strengthens the study's conclusions. It highlights soils as reservoirs of multifunctional microorganisms. Such integration is essential for applied microbial ecology.

25. Comparison with Previous Studies

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The findings are consistent with earlier studies conducted in Brazzaville, which reported similar pH ranges and dominant microbial groups. However, this study advances knowledge by explicitly linking soil category to enzymatic and biosurfactant production. Differences in microbial abundance among sites align with land-use patterns. The results corroborate previous reports on Bacillus dominance and bioactivity. Minor variations may reflect sampling locations and methodological differences. Overall, the study confirms and extends existing knowledge.

26. Environmental and Biotechnological Implications

The presence of enzyme- and biosurfactant-producing bacteria has significant environmental implications. Such microorganisms contribute to organic matter decomposition and pollutant degradation. Biosurfactants enhance hydrocarbon bioavailability, supporting bioremediation efforts. Hydrolytic enzymes have applications in agriculture, food processing, and industry. The identified strains could serve as candidates for biotechnological development. Urban soils thus represent underexplored microbial resources. Harnessing this potential could support sustainable technologies.

27. Limitations of the Study

The study focused exclusively on cultivable microorganisms, excluding non-cultivable taxa. Molecular techniques such as metagenomics were not employed. Enzyme activities were assessed qualitatively rather than quantitatively at the molecular level. Species-level identification was not performed for all isolates. Environmental parameters beyond pH and temperature were not analyzed. Despite these limitations, the study provides robust baseline data. Addressing these gaps could enhance future research.

28. Future Perspectives and Research Directions

Future studies should integrate molecular identification methods, including 16S rRNA sequencing, to refine taxonomic resolution. Quantitative enzyme assays and genetic characterization of biosynthetic pathways are recommended. Exploring synergistic interactions among microbial consortia could reveal enhanced bioactivity. Scaling up enzyme and biosurfactant production for industrial testing is a logical next step. Long-term monitoring could assess seasonal variations in microbial function. Such approaches would deepen understanding of soil microbial resources. Ultimately, Brazzaville soils hold strong potential for sustainable biotechnological applications.