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Molecular characterization of heavy metal-tolerant bacteria from agricultural soil in Ago-Iwoye, Ogun State, Nigeria

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Abstract

This study aimed to isolate and characterize heavy metal-tolerant bacteria from agricultural soil in Ago-Iwoye, Ogun State, Nigeria. They were isolated from soil samples collected and screened for tolerance to different concentrations of lead (Pb), copper (Cu), and cadmium (Cd). Heavy metal-tolerant bacteria were characterized utilizing phenotypic and molecular techniques. All the isolates were able to grow and tolerate different concentrations of the heavy metals, while four isolates identified as *Bacillus cereus*, *Pseudomonas aeruginosa*, *Atlantibacter hermannii*, and *Enterobacter quasihormaechei* exhibited a high degree of tolerance to Pb, Cu, and Cd with minimum inhibitory concentrations of 1500 mg/L of Pb, 1000 mg/L of Cu, and 700 mg/L of Cd. These results highlight the high potential of these bacteria as bioremediation tools for heavy metal-contaminated soil.

Keywords: Heavy metal, tolerance, bacteria, agricultural soil.

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Introduction

Heavy metals are a group of metallic elements that have high atomic weights and densities; they include lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), chromium (Cr), and others (Genchi et al., 2020). These metals are naturally occurring, but their concentration and availability in the environment have significantly increased due to human activities such as industrial processes, mining, and using products containing heavy metal like pesticides (Bakulski et al., 2020).

Heavy metals in the environment pose serious concerns due to their toxic effects on living organisms and ecosystems (Alengebawy et al., 2021). They persist in the soil, posing significant threats to human health (Sarwar et al., 2020). Once heavy metals enter the soil, they last for long periods and are not easily degraded or removed (Bansod et al., 2017; Verma, 2021).

Soil pollution with toxic metals has become an alarming treat to the agricultural sector. Many of these metals are environmentally stable and can persist in the soil because they are not biodegradable. Consequently, they can enter the food chain and bioaccumulate in plants and animals, resulting in chronic adverse effects on human health (Khan et al., 2015). Heavy metals can further lead to damage to vital organs such as the kidneys and liver and can also cause cancers, neuropathy, and changes in blood composition in humans (Rehman et al., 2018).

Heavy metals find their way into agricultural soils through the contaminated water used for irrigation, pesticides and fertilizers, and farming (Sandeep et al., 2019). The toxic effects of heavy metals on soil microorganisms can impair essential ecological processes, such as nutrient cycling and organic matter decomposition. These disruptions can cascade effects on ecosystems' overall functioning and biodiversity. Effective soil remediation strategies are crucial to address the risks associated with heavy metal contamination (Saha et al., 2020). Traditional methods, such as excavation and land-filling, can be expensive and disruptive, often displacing contaminated soil to another location. In contrast, bioremediation, which utilizes living organisms to degrade or immobilize pollutants, offers a more sustainable and cost-effective approach (Huq et al., 2020). Microorganisms play a crucial role in the biogeochemical cycling of heavy metals and have the potential to remediate contaminated soils. Some bacteria have developed mechanisms to tolerate and detoxify heavy metals, making them promising candidates for bioremediation strategies (Choudhary & Sharma, 2017).

Heavy metal-tolerant bacteria are found in various environments, including contaminated soils, sediments, wastewater, and mine tailings (Du et al., 2023). These bacteria exhibit diverse metal tolerance capabilities, enabling them to survive and thrive in high metal concentrations (Forsyth et al., 2018). The variety of heavy metal-tolerant bacteria is reflected in their taxonomic diversity, encompassing various genera such as *Pseudomonas*, *Bacillus*, *Cupriavidus*, and *Rhizobium* (Bhende et al., 2022).

The presence of heavy metal-tolerant bacteria offers promising opportunities for developing effective bioremediation strategies (Mani & Kumar, 2014). Their ability to solubilize, chelate, transform, and accumulate heavy metals provides valuable tools for reducing the environmental impact of metal contamination (Chatterjee et al., 2023). Harnessing the capabilities of these bacteria has made it possible to mitigate the risks associated with heavy metal pollution and restore the health and balance of contaminated environments (Choudhary & Sharma, 2017). The isolation and identification of heavy metal-tolerant bacteria from contaminated soil can provide valuable insights into microbial diversity, their physiological capabilities, and their potential for use in bioremediation processes (Pandey et al., 2019). Therefore, the present study strived to isolate and identify heavy metal-tolerant bacteria from soil.

Research Methods

1. Sampling site

The study area employed for this research was the Tree Crop Nursery Development Project site of the College of Agricultural Sciences, Olabisi Onabanjo University, located in Ago-Iwoye. The area is situated between latitudes $6^0$55'N and $7^0$00'N and longitudes $3^0$45'E and $4^0$05'E. Ago-Iwoye is only two kilometers from Oru and five kilometers from Ijebu-Igbo, the other two towns in Ijebu-North Local Government Area of Ogun State, Nigeria.

2. Collection of soil samples

Soil samples were collected from a depth of 0 cm to 15 cm using a sterile soil auger at 8-10 locations on the farm. These were bulked to create a composite sample, which was subsequently transported in a sterile polythene bag to the Department of Microbiology laboratory for analysis.

3. Isolation of bacteria

The bacteria were isolated from the soil samples utilizing the pour plate technique. A serial dilution was performed by adding 1g of the soil to 9 ml of sterile distilled water to achieve a 10^{-1} dilution using a sterile pipette under aseptic conditions. Further dilutions up to 10^{-10} were carried out by transferring 1 ml from previous dilutions into 9 ml of sterile distilled water. A 1 ml aliquot of the 10^{-6} dilution was poured into sterile Petri dishes, over which molten nutrient agar (Oxoid UK®) was added, swirled, and allowed to set. Incubation occurred at 35°C for 24-48 hours.

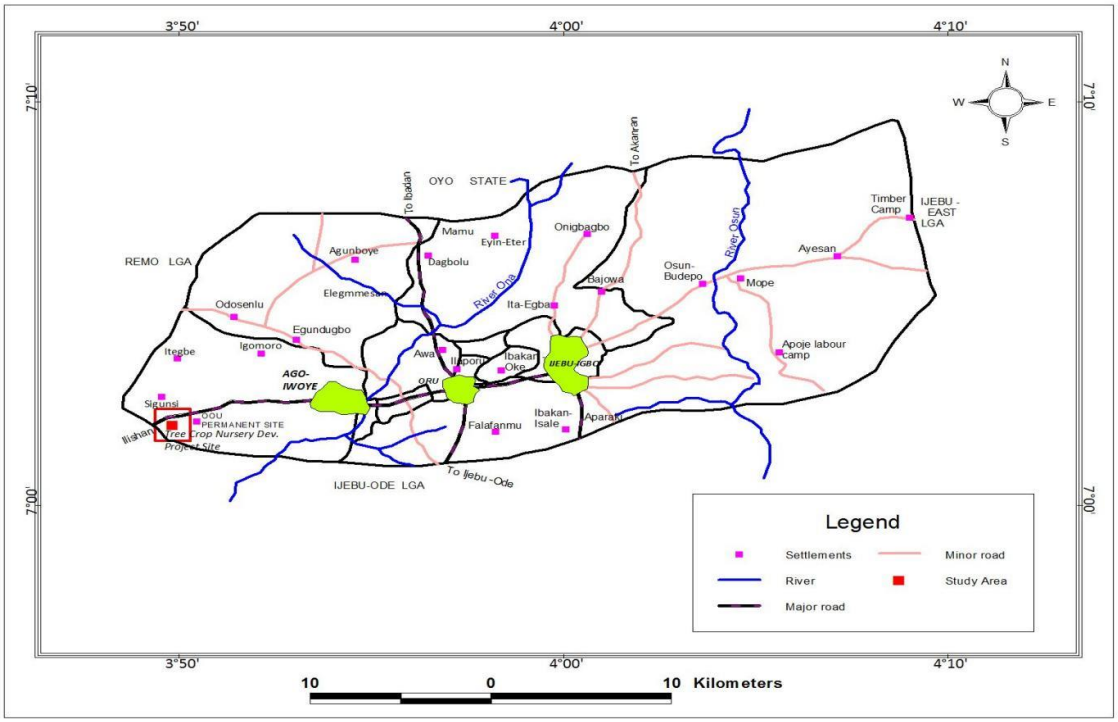
4. Screening for heavy metal tolerance of the isolated bacteria

The bacterial isolates were screened for heavy metal tolerance utilizing the agar dilution method. Three heavy metals—lead, copper, and cadmium—were used in their salt forms: lead nitrate ($Pb(NO_3)_2$), copper sulphate ($CuSO_4$), and cadmium nitrate ($Cd(NO_3)_2$). Fresh cultures of each bacterium were

inoculated onto nutrient agar plates supplemented with increasing concentrations (0.5-1500 mg/L) of the aforementioned heavy metals individually. The plates were incubated at 37°C for 24-48 hours. The lowest concentration at which bacteria failed to grow was considered the Minimum Inhibitory Concentration (MIC) (Rahman et al., 2022).

Figure 1

Map of Ijebu North Local Government showing the experimental site at Ago-Iwoye, Ogun State, Nigeria (Source: Department of Geography, Olabisi Onabanjo University, Ago-Iwoye, Ogun State).



5. Morphological and biochemical characterization of heavy metal-tolerant bacteria

Heavy metal-tolerant bacteria were characterized to the genus level based on morphological, cellular, and biochemical characteristics as described in Bergey's Manual of Systematic Bacteriology (1984). For further analysis, pure bacteria isolates were maintained on nutrient agar at 4°C.

6. Molecular Characterization of heavy metal-tolerant bacteria: Genomic DNA extraction

The genomic DNA of the isolates was extracted utilizing a Zymo Genomic DNA

Extraction Kit per the manufacturer's protocol.

7. PCR amplification of 16S rRNA and bacterial strain identification

The 16S ribosomal ribonucleic acid (16S rRNA) gene sequence of the bacterial isolates were amplified using polymerase chain reaction (PCR) with universal primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT). The initial denaturation step was performed at 94˚C for 5 minutes, followed by subsequent cycles of denaturation at 94˚C for 45 seconds, annealing at 56˚C for 30 seconds, extension at 72˚C for 45 seconds, and a final extension at 72˚C for 7 minutes. A total of 35 cycles were conducted, and the temperature after the final extension was maintained at 4˚C.

Low-quality nucleotide bases were trimmed, and the sequence was converted to FASTA format for comparison against recorded sequences on the GenBank database using the Basic Local Alignment Search Tool (BLAST) algorithm on the National Center for Biotechnology Information (NCBI) platform.

8. Phylogenetic Analysis of identified bacterial isolates

Sequences matching those in the GenBank database were employed as reference sequences. Multiple sequence alignments were conducted utilizing the recovered reference sequences with the Clustal W algorithm on *MEGA 11* software version 11.0.13. The phylogenetic tree was computed using the neighbor-joining method, 1000 bootstrap replications, and the maximum composite likelihood model. This analysis involved 10 nucleotide sequences (including the recovered reference sequences). All ambiguous positions were removed for each sequence pair (pairwise deletion option).

Research Results and Discussion

Isolation of bacteria and screening for heavy metal tolerance

16 bacteria were isolated from soil samples and screened for heavy metal tolerance. Four isolates designated as X1, X2, X3, and X4 exhibited high tolerance to high lead, copper, and cadmium concentrations. The tolerance pattern to the heavy metals was Pb > Cu > Cd. Furthermore, the tolerance was highest for Pb and comparatively lower for Cd. In comparison to a previous research by Krishna et al. (2012), while both studies observed lead as the most tolerated metal, the present study results diverged with a distinct pattern of heavy metal tolerance (Pb > Cu > Cd), contrasting with the previous study order (Pb > Zn > Ni > Cu > Cd). Additionally, this study revealed narrower discrepancies that might stem from variations in sampling environments, microbial communities, or experimental conditions, highlighting the intricacies of bacterial responses to heavy metals and the need for further exploration (Kumar et al., 2022; Li et al., 2022; Sharma et al., 2023).

Assessment of Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) of the heavy metals ranged from 600 mg/L to 1500 mg/L, compared to the broader spectrum reported by Krishna et al. (2012). The MIC was highest for Pb and lowest for Cd, as shown in Table 1.

Table 1
Minimum Inhibitory concentration of heavy metal-tolerant bacteria

Bacterial isolates	Minimum Inhibitory Concentration (mg/L)		
	Pb	Cu	Cd
X1	1500	1000	700
X2	1500	800	600
X3	1500	800	600
X4	1500	1000	600

Morphological and biochemical characterization of heavy metal-tolerant bacteria

Presented in Table 2 are the morphological, Gram-stain reactions, biochemical characterizations, and carbohydrate utilization test results of the four heavy metal-tolerant bacteria isolated from the soil samples. The bacteria were identified to the genus level as *Pseudomonas spp.*, *Atlantibacter spp.*, *Enterobacter spp.*, and *Bacillus spp.* Kepel et al. (2021) also isolated heavy metal-tolerant bacteria belonging to the genera *Pseudomonas* and *Bacillus* from sediments of Manado Bay in Indonesia. All the bacteria isolated in this study exhibited multiple heavy metal tolerances, a common phenomenon among heavy metal-tolerant bacteria isolated in previous investigations (Ndiaye et al., 2023; Li et al., 2023). The diversity of heavy metal-tolerant bacteria was reflected in their taxonomic diversity, encompassing various genera such as *Pseudomonas*, *Bacillus*, *Cupriavidus*, and *Rhizobium* (Li et al., 2019).

Molecular characterization of heavy metal-tolerant bacteria from farmland soil

Table 3 displays the molecular characterization of heavy metal-tolerant bacteria obtained from farmland soil.

Table 2
Morphological and biochemical characteristics and carbohydrate utilization test of heavy metal-tolerant bacteria from farmland soil

Bacterial isolates	X1	X2	X3	X4
Morphological Characteristics				
Colony color	Greenish	Cream	Cream	Cream
Margin	Entire	Entire	Entire	Entire
Opacity	Translucent	Translucent	Opaque	Opaque
Gram Nature	Negative	Negative	Negative	Positive
Cell shape	rod	rod	rod	rod
Biochemical Characteristics				
Catalase	+	+	+	+
Citrate	-	-	+	+
Indole	-	+	-	-

Utilization of Carbohydrate				
Glucose	-	+	+	+
Lactose	-	+	+	-
Sucrose	-	+	+	+
H_2S forming	-	-	-	-
Gas Production	-	+	+	+
Suspected Organisms	*Pseudomonas spp.*	*Atlantibacter spp.*	*Enterobacter spp.*	*Bacillus spp*.

Notes: -: Negative; +: Positive; spp: species

Isolate X1 was identified as *Pseudomonas aeruginosa* with a percentage identity of 93.03%. Isolate X2, identified as *Atlantibacter hermannii*, showed a high percentage identity of 99.85%. Isolate X3 was identified as *Enterobacter quasihormaechei*, demonstrating a percentage identity of 88.18%. Isolate X4 was identified as *Bacillus cereus*, with a percentage identity of 98.32%. Presented in Plate 1 is the agarose gel electrophoresis for identification of the heavy metal-tolerant bacteria.

Table 3

Molecular characterization of heavy metal-tolerant bacteria isolated from farmland

Isolate code	Identified organism	Percentage identity (%)	Reference strain accession number
X1	*Pseudomonas aeruginosa*	93.03	NR_117678.1
X2	*Atlantibacter hermannii*	99.85	NR_104940.1
X3	*Enterobacter quasihormaechei*	88.18	NR_180451.1
X4	*Bacillus cereus*	98.32	NR_115526.1

Phylogenetic relationship between bacterial isolates

Figure 1 portrays the phylogenetic tree, which demonstrates the similarity in sequences to those recovered from the GenBank database. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. All isolates shared a common ancestor. The phylogenetic tree distributed the sequences into three major clades labeled A, B, and C. In clade A, it was shown that isolates B7 and X6 were closely related to *Bacillus cereus*. In clade B, it was shown that isolate X1 was closely related to *Pseudomonas aeruginosa*. In clade C, isolate X4 was closely related to *Atlantibacter hermannii*.

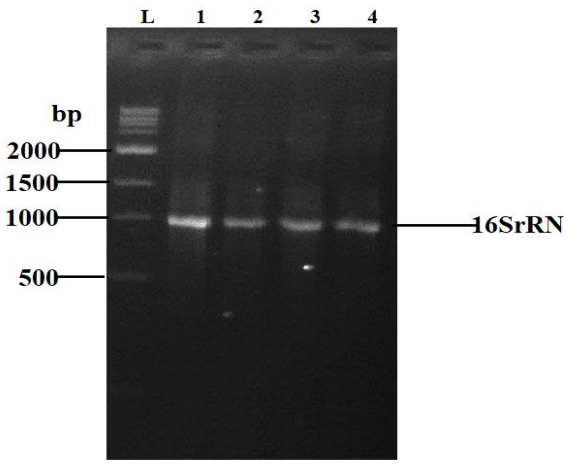
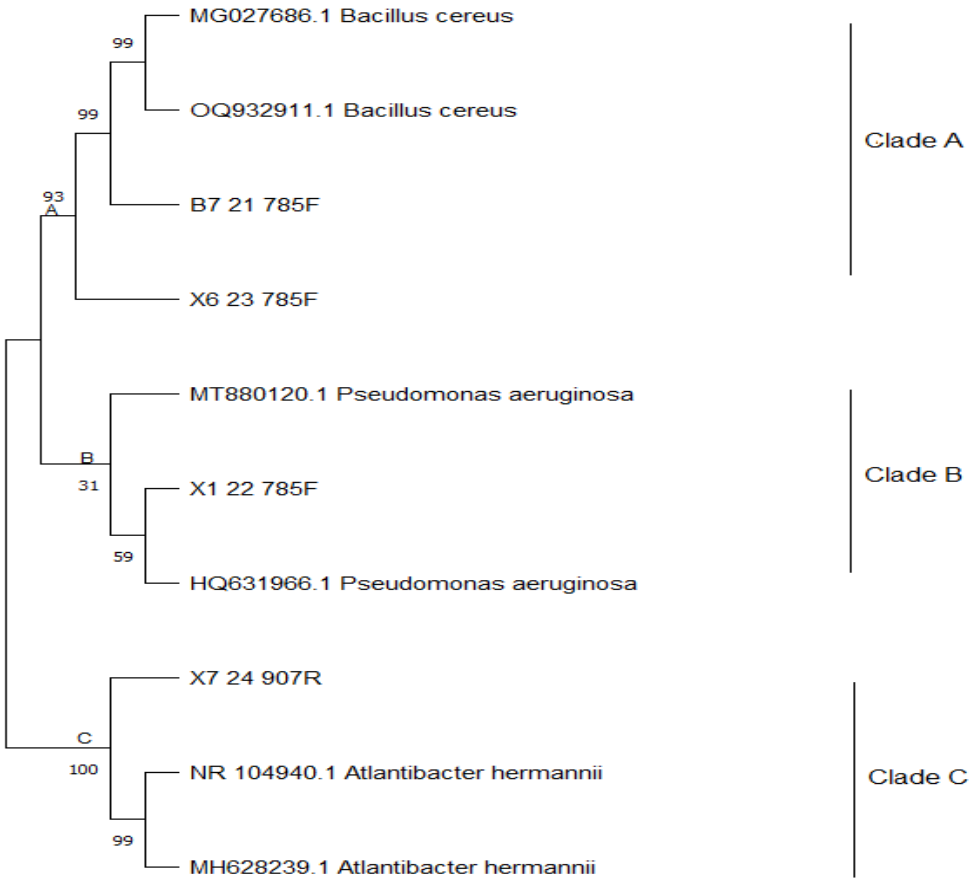


Plate 1 *Agarose gel electrophoresis for the identification of bacterial isolates*

Notes: Lane 1: DNA ladder; Lane 2: *Pseudomonas aeruginosa*; Lane 3: *Atlantibacter hermannii*; Lane 4: *Enterobacter quasihormaechei*; Lane 5: *Bacillus cereus*

Figure 2
Phylogenetic tree showing the relationship between bacterial isolates and the most closely related strains from GenBank



Conclusion

In conclusion, this study successfully isolated and characterized heavy metal-tolerant bacteria from Ago-Iwoye, Ogun State, Nigeria. Identified strains, including *Bacillus cereus*, *Pseudomonas aeruginosa*, *Enterobacter quasihormaechei*, and *Atlantibacter hermannii*, exhibited substantial tolerance to lead (Pb), copper (Cu), and cadmium (Cd).

With minimum inhibitory concentrations as high as 1500 mg/L for lead, 1000 mg/L for copper, and 700 mg/L for cadmium, these bacteria indicated significant potential for bioremediation in heavy metal-contaminated soils. Additionally, the findings emphasized their applicability as effective tools for sustainable environmental management in agricultural areas, addressing the challenges of heavy metal pollution.

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