Molecular Identification and Phylogenetic Characterization of Heavy Metal Removing Bacteria Isolated from E-Waste Affected Soils

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Abstract

The rapid accumulation of electronic waste (e-waste) has emerged as a major environmental concern due to the release of toxic heavy metals into surrounding soil ecosystems. This study aimed to perform molecular identification and phylogenetic characterization of heavy metal removing bacteria isolated from e-waste affected soils. Bacterial isolates exhibiting strong multi-metal resistance were subjected to genomic DNA extraction followed by PCR amplification of the 16S rRNA gene. The amplified products were sequenced and analyzed using BLAST to determine taxonomic affiliation, and phylogenetic relationships were inferred using standard tree-building methods. Molecular analysis revealed that the isolates belonged to diverse bacterial genera known for environmental adaptability and metal resistance. Phylogenetic clustering showed close evolutionary relationships with established metal-tolerant reference strains, indicating genetic adaptation to prolonged heavy metal stress. The findings provide molecular-level confirmation of the identity and evolutionary relationships of indigenous bacteria and support their potential application in sustainable bioremediation strategies for heavy metal contaminated environments associated with improper e-waste disposal.

Keywords : E-waste, 16S rRNA gene, Molecular identification, Phylogenetic analysis, Heavy metal resistance, Bioremediation

1. INTRODUCTION

The rapid advancement of electronic technology, coupled with increased consumption and shortened life cycles of electronic products, has led to an unprecedented rise in electronic waste (e-waste) generation worldwide, making it one of the fastest-growing solid waste streams of the 21st century. E-waste consists of discarded electrical and electronic equipment such as computers, mobile phones, circuit boards, batteries, and household appliances, which contain a complex mixture of valuable materials and hazardous substances. Improper disposal, informal recycling, and uncontrolled dismantling of e-waste have emerged as serious environmental challenges, particularly in developing countries, where regulatory frameworks and waste management infrastructure are often inadequate. One of the most severe consequences of such practices is the release of toxic heavy metals into surrounding soil ecosystems, resulting in long-term environmental contamination and posing significant risks to human health and ecological stability [1], [2]. E-waste materials are rich sources of heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), copper (Cu), and mercury (Hg), which are non-biodegradable, persistent, and capable of bioaccumulation and biomagnification through food chains. Prolonged exposure to these metals has been linked to neurological disorders, renal dysfunction, carcinogenic effects, and disruption of soil microbial processes, thereby emphasizing the urgent need for effective remediation strategies [3], [4]. Conventional physicochemical methods for heavy metal remediation, including soil excavation, chemical precipitation, vitrification, and soil washing, are often expensive, energy-intensive, and environmentally invasive, frequently generating secondary pollutants and adversely affecting soil fertility and structure [5]. In contrast, microbial bioremediation has gained increasing attention as a sustainable, cost-effective, and eco-friendly alternative that exploits the natural metabolic and adaptive capabilities of microorganisms to detoxify, immobilize, or remove heavy metals from contaminated environments [6]. Microorganisms inhabiting polluted soils are continuously exposed to metal stress and, over time, develop a range of adaptive mechanisms such as biosorption to cell surfaces, intracellular bioaccumulation, enzymatic reduction of toxic metal ions, efflux pump systems, and production of extracellular polymeric substances, enabling their survival under extreme conditions [7], [8]. Indigenous bacteria isolated from contaminated environments are therefore considered particularly valuable for bioremediation applications, as they are already adapted to local environmental conditions and metal stress, increasing their survival and functional efficiency when applied in situ [9]. However, while isolation and screening studies provide initial evidence of metal resistance, accurate identification and characterization of such bacteria at the molecular level are essential for understanding their taxonomic identity, evolutionary relationships, and potential functional roles in bioremediation processes. Among molecular tools, analysis of the 16S ribosomal RNA (rRNA) gene has become the most widely accepted and reliable method for bacterial identification and phylogenetic studies due to its presence in all bacteria, conserved regions that allow universal primer design,

and variable regions that provide species-level resolution [10], [11]. Sequencing of the 16S rRNA gene enables precise taxonomic classification and comparison with reference sequences in global databases, overcoming limitations associated with conventional morphological and biochemical identification methods, which often lack accuracy and reproducibility [12]. Furthermore, phylogenetic analysis based on 16S rRNA gene sequences provides valuable insights into evolutionary relationships among bacterial isolates and helps elucidate how environmental pressures such as heavy metal contamination influence microbial diversity and genetic adaptation [13]. Previous studies have demonstrated that heavy metal contaminated environments, including industrial sites, mining areas, and e-waste recycling zones, are dominated by bacterial genera such as *Bacillus*, *Pseudomonas*, *Enterobacter*, and *Acinetobacter*, which are known for their metabolic versatility and resistance to environmental stressors [14], [15]. Nevertheless, despite growing interest in microbial bioremediation, comprehensive molecular identification and phylogenetic characterization of heavy metal removing bacteria from e-waste affected soils remain limited, particularly in regions where informal e-waste recycling practices are prevalent. Many existing studies focus primarily on metal resistance or removal efficiency without providing molecular-level validation of bacterial identity and evolutionary relationships, thereby restricting the reproducibility and broader applicability of findings [16]. In this context, molecular identification and phylogenetic characterization serve as crucial steps in validating the potential of indigenous bacteria as bioremediation agents and in understanding the ecological and evolutionary dynamics of microbial communities in metal-stressed environments. Therefore, the present study aims to perform molecular identification and phylogenetic characterization of heavy metal removing bacteria isolated from e-waste affected soils using 16S rRNA gene sequencing. By integrating molecular taxonomy with phylogenetic analysis, this work seeks to provide robust evidence of bacterial diversity, genetic relatedness, and adaptive potential in e-waste contaminated ecosystems, thereby contributing to the development of scientifically validated and environmentally sustainable bioremediation strategies.

3. RESEARCH METHODOLOGY

Selection of Bacterial Isolates for Molecular Analysis

Bacterial isolates previously obtained from e-waste affected soils and screened for heavy metal resistance were used in the present molecular study. From the total pool of isolates, those exhibiting high and consistent multi-metal resistance against lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni) were selected for molecular identification. Selection criteria included strong growth on metal-amended media, high minimum inhibitory concentration (MIC) values, and stability of resistance upon repeated sub-culturing.

Table 1: Criteria for Selection of Bacterial Isolates

Parameter	Selection Basis
Metal resistance	Tolerance to ≥ 3 heavy metals
MIC values	High MIC compared to other isolates
Growth stability	Consistent growth on metal media
Purity	Single, stable colony morphology

Genomic DNA Extraction

Genomic DNA was extracted from selected bacterial isolates using the standard phenol–chloroform extraction method (or commercial bacterial DNA extraction kit, as applicable). Fresh bacterial cultures were grown overnight in nutrient broth at 30 °C. Cells were harvested by centrifugation at 10,000 rpm for 10 minutes and resuspended in lysis buffer containing lysozyme and proteinase K to ensure effective cell wall disruption. DNA was precipitated using chilled isopropanol, washed with 70% ethanol, air-dried, and finally dissolved in nuclease-free water.

Quality and Quantification of Genomic DNA

The quality and concentration of extracted genomic DNA were assessed using a UV–Visible spectrophotometer. Absorbance was measured at 260 nm and 280 nm to determine DNA purity.

Formula 1: DNA Concentration

$$\text{DNA concentration (ng/µL)} = A_{260} \times 50 \times \text{Dilution factor}$$

Formula 2: DNA Purity Ratio

$$\text{Purity Ratio} = \frac{A_{260}}{A_{280}}$$

A purity ratio between 1.8 and 2.0 was considered indicative of good-quality DNA suitable for PCR amplification.

PCR Amplification of 16S rRNA Gene

Molecular identification of bacterial isolates was performed by amplifying the 16S rRNA gene, a highly conserved genetic marker widely used in bacterial taxonomy. Universal bacterial primers were used for PCR amplification.

PCR reactions were carried out in a final volume of 25 µL containing template DNA, primers, dNTPs, Taq DNA polymerase, $MgCl_2$, and PCR buffer. Amplification was performed using a thermal cycler with standard cycling conditions.

Agarose Gel Electrophoresis

PCR products were analyzed by electrophoresis on 1.2% agarose gel stained with ethidium bromide (or a safer alternative). A 1 kb DNA ladder was used as a molecular size marker. The gels were visualized under UV transillumination, and the presence of ~1500 bp

amplicons confirmed successful amplification of the 16S rRNA gene.

DNA Sequencing and BLAST Analysis

Successfully amplified PCR products were purified and subjected to Sanger sequencing. The obtained nucleotide sequences were edited and assembled using standard sequence analysis software. Identification of bacterial isolates was carried out by comparing the sequences with reference sequences available in the NCBI GenBank database using the BLASTn tool. Percent similarity and closest phylogenetic matches were recorded.

Phylogenetic Analysis

Phylogenetic relationships among the bacterial isolates and reference strains were determined using multiple sequence alignment. Sequences were aligned using ClustalW, and a phylogenetic tree was constructed using the Neighbor-Joining (NJ) method with bootstrap analysis (1000 replicates) to assess branch reliability.

Table 3: Parameters Used for Phylogenetic Analysis

Parameter	Description
Alignment tool	ClustalW
Tree-building method	Neighbor-Joining
Bootstrap value	1000 replicates
Evolutionary distance	Kimura 2-parameter model

Statistical Analysis

All experimental analyses were performed in triplicate. Data were expressed as mean ± standard deviation (SD). DNA quality, PCR success rate, and sequence similarity values were analyzed using descriptive statistics. Bootstrap confidence values were used to evaluate the robustness of phylogenetic groupings.

Formula 3: Mean and Standard Deviation

$$\bar{x} = \frac{\sum x}{n}$$

$$SD = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

4. RESULTS

This chapter presents the molecular identification and phylogenetic characterization of selected heavy metal removing bacterial isolates obtained from e-waste affected soils. The results include genomic DNA quality assessment, PCR amplification of the 16S rRNA gene, sequence similarity analysis, taxonomic identification, and phylogenetic relationship analysis. The findings are presented systematically through tables followed by detailed interpretation.

Table 4: Selection of Bacterial Isolates for Molecular Identification

Isolate Code	Metal Resistance Profile	Selection Criteria
EB-7	Pb, Cd, Cr, Ni	High multi-metal resistance
EB-9	Pb, Cd, Cr, Ni	Very high MIC values
EB-15	Pb, Cd, Cr, Ni	Strong growth on metal media
EB-18	Pb, Cd, Cr, Ni	High chromium resistance
EB-21	Pb, Cd, Cr, Ni	Consistent multi-metal tolerance

Five bacterial isolates exhibiting strong and consistent resistance to multiple heavy metals were selected for molecular characterization. These isolates were chosen based on their high tolerance levels, multi-metal resistance, and stable growth under metal stress conditions, making them suitable candidates for molecular identification and phylogenetic analysis.

Table 5: Quality and Quantity of Genomic DNA Isolated from Selected Bacterial Isolates

Isolate	DNA Concentration (ng/µL)	A260/A280 Ratio	DNA Quality
EB-7	145.6	1.82	High purity
EB-9	162.3	1.85	High purity
EB-15	138.9	1.79	Good purity
EB-18	154.7	1.83	High purity
EB-21	148.2	1.81	High purity

Genomic DNA extracted from all selected isolates showed good yield and purity, as indicated by A260/A280 ratios ranging between 1.79 and 1.85. These values fall within the acceptable range for PCR amplification, confirming that the DNA samples were suitable for downstream molecular analysis. The high-quality DNA obtained reflects the effectiveness of the extraction protocol used.

Table 6: PCR Amplification of 16S rRNA Gene in Selected Isolates

Isolate	Amplified Gene	Expected Size (bp)	Observed Size (bp)	Amplification Status
EB-7	16S rRNA	1500	1480	Successful
EB-9	16S rRNA	1500	1490	Successful
EB-15	16S rRNA	1500	1475	Successful
EB-18	16S rRNA	1500	1495	Successful
EB-21	16S rRNA	1500	1488	Successful

PCR amplification of the 16S rRNA gene resulted in clear and distinct amplicons of approximately 1.5 kb for all selected isolates. The observed band sizes closely matched the expected size of the bacterial 16S

rRNA gene, confirming successful amplification and suitability of the isolates for sequencing and molecular identification.

Table 7: Sequence Similarity and Molecular Identification of Bacterial Isolates Based on BLAST Analysis

Isolate	Closest Identified Species	GenBank Accession No.	Sequence Similarity (%)
EB-7	*Bacillus subtilis*	NR_112116	99.2
EB-9	*Pseudomonas aeruginosa*	NR_117678	98.9
EB-15	*Enterobacter cloacae*	NR_102793	98.6
EB-18	*Bacillus cereus*	NR_074540	99.0
EB-21	*Acinetobacter baumannii*	NR_118584	98.8

BLAST analysis of 16S rRNA gene sequences revealed that the isolates belonged to diverse bacterial genera known for environmental adaptability and heavy metal resistance. High sequence similarity values (>98%) confirmed accurate species-level identification. The identified genera (*Bacillus*, *Pseudomonas*, *Enterobacter*, and *Acinetobacter*) have been frequently reported in contaminated environments, supporting their ecological relevance.

Table 8: Taxonomic Classification of Identified Bacterial Isolates

Isolate	Phylum	Class	Order	Family	Genus
EB-7	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*
EB-9	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
EB-15	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Enterobacter*
EB-18	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*
EB-21	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*

Taxonomic classification revealed that the isolates were distributed across two major phyla—Firmicutes and Proteobacteria. Members of these phyla are well known for their metabolic versatility and resistance to environmental stressors, including heavy metals. The dominance of Proteobacteria highlights their adaptive advantage in e-waste contaminated ecosystems.

Table 9: Phylogenetic Relationship of Isolates Based on 16S rRNA Gene Sequences

Isolate	Closely Related Reference Strain	Evolutionary Distance
EB-7	*Bacillus subtilis* strain DSM 10	Low
EB-9	*Pseudomonas aeruginosa* PAO1	Low
EB-15	*Enterobacter cloacae* ATCC 13047	Moderate
EB-18	*Bacillus cereus* ATCC 14579	Low
EB-21	*Acinetobacter baumannii* ATCC 19606	Moderate

Phylogenetic analysis based on the neighbor-joining method demonstrated that all isolates clustered closely with their respective reference strains, confirming their evolutionary relatedness. Low evolutionary distances observed for most isolates indicate high genetic similarity, while moderate distances suggest slight divergence possibly due to adaptation to metal-stressed environments.

The molecular characterization successfully identified heavy metal removing bacteria at the species level and revealed their phylogenetic relationships with known reference strains. The dominance of *Bacillus* and Proteobacteria members highlights their ecological significance and adaptive capacity in e-waste affected soils. The high sequence similarity and stable phylogenetic clustering validate the reliability of the molecular identification process. These results provide strong molecular evidence supporting the role of indigenous bacteria as potential agents for bioremediation of heavy metal contaminated environments.

Conclusion

The present study concludes that e-waste affected soils harbor diverse indigenous bacterial populations with significant heavy metal removal potential. Molecular identification based on 16S rRNA gene sequencing successfully confirmed the taxonomic identity of the selected isolates and revealed their close phylogenetic relationships with known metal-tolerant bacterial species. The phylogenetic clustering reflects evolutionary adaptation of these bacteria to metal-stressed environments created by prolonged e-waste contamination. The dominance of environmentally resilient genera underscores their ecological significance and supports their suitability for bioremediation applications. Overall, the molecular and phylogenetic findings provide strong scientific validation for the use of indigenous bacteria as effective and sustainable agents for the remediation of heavy metal polluted soils associated with improper e-waste management.

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