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Original Article

Isolation and Characterization of Mercury-Resistant Bacteria for Biofiltration-Based Mercury Removal from Wastewater

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Abstract

Background & Objectives: Industrial and economic development, along with the production of various chemical materials and compounds, have led to increased pollution from heavy metals and toxic substances in different environments. This poses significant risks to ecosystems and human health. Biofiltration, a method of bioremediation, involves the dissolution of pollutants in a liquid layer, where they undergo biodegradation by microorganisms in a biofilm. Mercury, a toxic heavy metal, is one of the major environmental pollutants. This study aims to identify mercury-resistant bacterial strains and evaluate their potential for use in water biofiltration.

Materials & Methods: Samples were collected from industrial wastewater, and mercury-resistant bacteria were isolated and characterized. The Minimum Inhibitory Concentration (MIC) test was used to determine mercury resistance. Biochemical tests as well as 16S rRNA sequencing were performed to identify the most resistant strains. These strains were then applied in a biofiltration system to remove mercury from contaminated water.

Results: Among six mercury-resistant bacterial strains, a gram-negative bacterium isolated from detergent manufacturing wastewater exhibited the highest resistance to mercury (52 ppm). 16S rRNA sequencing identified this strain as *Raoultella planticola*. Biofiltration experiments demonstrated a 90% removal efficiency for mercury compounds.

Conclusion: This study identified *R. planticola* as a highly mercury-resistant strain, making it a promising candidate for biofiltration-based mercury removal from wastewater.

Keywords: Mercury-resistant bacteria, Biofiltration, *Raoultella planticola*, Bioremediation.

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Introduction

Industrial and economic development has produced a wide range of chemical compounds and materials, contributing to human well-being. However, this progress has also introduced harmful substances, such as heavy metals, into the environment. These pollutants pose significant risks to both human health and ecosystems (1).

Mercury pollution is a severe and persistent threat to ecosystems and human health, as traditional cleanup methods are ineffective. Consequently, researchers are developing biotechnological solutions using specialized mercury-resistant microorganisms to detoxify contaminated environments. Future progress depends on enhancing these microbes and combining microbial bioremediation with other methods (2).

Various methods, including chemical and biological approaches, have been developed to remove heavy metals from industrial effluents (3–6).

Biofiltration, a bioremediation technique, utilizes microbial systems to degrade pollutants. In biofiltration units, microorganisms grow on porous solid materials such as compost, mulch, or soil, forming a biofilm. Pollutants dissolve in the liquid layer of the biofilm, where they are biodegraded by microorganisms (6). Mercury-resistant bacteria, such as those from thegenra *Escherichia*, *Klebsiella*, *Pseudomona*, *Bacillus*, and others, have been isolated from contaminated environments and studied for their potential in bioremediation (7).

Biofiltration offers several advantages over traditional pollution control methods, including cost-effectiveness, low energy consumption, and the avoidance of secondary pollutants (8). For example, Frischmut et al. (1993) demonstrated that mercury-resistant bacteria in biofilters could remove mercury from wastewater with an efficiency of 82% to 99% over 4 to 7 weeks (9). This study aims to isolate and identify mercury-resistant bacteria for use in a single-stage biofiltration process and evaluate their effectiveness in removing mercury from wastewater.

Materials and Methods

Sampling, Enrichment, and Isolation of Strains

Wastewater samples were collected from seven factories in the Damghan industrial park. Samples were transported in sterile containers on ice to the laboratory. Each sample was enriched in nutrient broth containing 10 mg of mercuric chloride and incubated at 30°C for 48 hours. Subsequently, 0.1 mL of the enriched culture was plated on nutrient agar containing 10 mg of mercuric chloride and incubated at 30°C. Pure cultures were prepared from the resulting colonies (10).

A) Minimum Inhibitory Concentration (MIC for Mercury: The MIC test was used to assess bacterial resistance to mercury. A 0.5 McFarland standard was prepared, and 100 µL of bacterial suspension was added to test tubes containing varying concentrations of mercury chloride (Table 1). After 24 hours of incubation at 37°C, the results were recorded.

Table 1. Mercury Chloride Concentrations.

Treatment	1	2	3	4	5	6	7	8	9	10
$HgCl_2$ (µl)	2.5	5	7	9	11	13	15	17	19	21
$HgCl_2$ (ppm)	10	20	28	36	44	52	60	68	76	84

B) Biochemical and Molecular Identification: The most resistant strains were subjected to biochemical tests, including catalase, oxidase, lactose fermentation, Triple Sugar Iron (TSI), and H2S production, as well as

Gram staining. For molecular identification, 16SrRNA was extracted using primers F: 5′-AGA-GTT-TGA-TCC-TGG-CTC-A-(G)-3′ and R: 5′-TGG-TGT-GAC-GGG-TGT-G-(T)-3′, followed by sequencing and BLAST analysis. Amplification was performed in a 50 µL reaction mixture containing: 1X PCR buffer (provide the company or composition, e.g., 10 mM Tris-HCl, 50 mM KCl, pH 8.3), Approximately 50 ng of template DNA, 20 pmol of each forward and reverse primer (Primer names, e.g., invA-F and invA-R, should be stated here if known), 200 µM of each dNTP (a mixture of dATP, dTTP, dCTP, and dGTP), 2.5 U of Taq DNA polymerase (company, city, country), Nuclease-free water to the final volume. The amplification was carried out in a thermal cycler (provide instrument model and company) under the following conditions: an initial denaturation at 94°C for 10 min; 35 cycles of denaturation at 94°C for 20 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 10 min.

C) Biofilter Column Preparation*:* Pumice was used as the bed material for the biofilter column. A preculture of the selected bacterium was prepared in nutrient broth containing 10 mg of mercuric chloride and incubated for 16 hours at 37°C. The culture was then added to a biofilter column (40 cm height, 4.5 cm diameter) containing pumice. A mercury solution (67.75 mg/L) was passed through the column at 5 mL/min, and the effluent was analyzed for residual mercury.

Results

A) Isolation, Screening, and Mercury Resistance Analysis: Six mercury-resistant bacterial strains were isolated from wastewater samples. The most resistant strain, a gram-negative bacterium from detergent wastewater, exhibited an MIC of 52 ppm (Table 2).

Table 2. Mercury Resistance and MIC.

Wastewater Source	Bacterial Morphology	MIC (mM $HgCl_2$)[a]	Mercury Resistance (ppm $HgCl_2$)
Ceramic	Gram-negative Bacilli	5	36
Ceramic	Gram-positive Bacilli	5	36
Dairy products	Gram-negative Bacilli	2	10
Dairy products	Gram-positive Bacilli	4	28
Detergent products	Gram-negative Bacilli	6	44
Detergent products	Gram-negative Bacilli	7	52

[a]MIC, minimum inhibitory concentration.

Biochemical tests confirmed the identity of the most resistant strain as *R. planticola* (Table 3).

D) Biofiltration Efficiency: The biofiltration system achieved a mercury removal efficiency of 90% (Table 4).

Discussion

Mercury is a highly toxic heavy metal in elemental, inorganic, and organic forms. Bacteria in contaminated environments can develop resistance to mercury through genetic exchange and adaptation. This high removal efficiency suggests that *R. planticola* possesses robust genetic determinants for mercury detoxification, likely involving the enzymatic reduction of ionic mercury to a less toxic volatile form and/or its sequestration on the cell surface. The fact that a genus often associated with clinical settings demonstrates

Table 3. Biochemical Test Results.

Isolate ID	Catalase	Oxidase	Lactose Fermentation	TSI Reaction[a]	H_2S Production
CER-N1	+	-	+	A/A	-

Table 4. Mercury Removal Efficiency.

Time Point	Initial Hg (mg/L)	Hg at 10:00 (mg/L)	Hg at 12:00 (mg/L)	Hg at 14:00 (mg/L)	Removal Rate (%)
Day 1	41.870	8.760	6.040	-[a]	91
Day 2	33.970	11.530	5.411	-[a]	92
Day 3	30.510	8.529	0.428	-[a]	99.5
Week 2	6.440	-[b]	5.920	5.920	91
Week 3	5.420	-[b]	4.460	4.450	93
Week 4	4.040	-[b]	3.120	3.540	94

such strong environmental remediation potential is noteworthy. Previous studies have reported similar efficiencies using genetically modified bacteria under optimized conditions (11,12). Biofiltration utilizes engineered microbes, hybrid systems, and AI-driven monitoring to achieve high efficiency, resilience, and better resource management. This approach supports the circular economy by converting waste into a manageable resource and is a strategic pillar of green infrastructure. Its full potential will be realized through continued innovation in microbial engineering along with strong policy and collaboration (13). The findings highlight the potential of *R. planticola* for bacterial-based mercury removal in biofilters.

Conclusion

Our findings position this *R. planticola* strain as a promising candidate for bioremediation applications. Future work will focus on characterizing the specific genetic mechanisms responsible for this resistance and optimizing conditions for its use in treating mercury-contaminated wastewater. With optimized growth conditions, this strain could be a viable option for the biological removal of mercury from industrial effluents.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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