



Isolation and Characterization of Antibiotic-Degrading Bacteria from Hospital and Industrial Waste-Contaminated Soil

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Manuscript details:

Received: 09.04.2026
Accepted: 09.05.2026
Published: 30.05.2026

Cite this article as:

Anuja S. Zine, Jadhav M.A., Rutuja R. Dahibhate, Alka V. Pawar, Megha S. Dhotre and Vaishnavi B. Nagve (2026) Isolation, Identification, and Characterization of Soil Fungi for Extracellular Enzyme Production, *Int. J. of Life Sciences*, 14 (1A):151-155.
<https://doi.org/10.5281/zenodo.20653903>

Available online on <http://www.ijlsci.in>
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)



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ABSTRACT

Antibiotic contamination of soil and water environments has emerged as a serious global concern due to its ecological toxicity and contribution to the spread of antimicrobial resistance. Large quantities of antibiotics are continuously released into the environment through hospital effluents, pharmaceutical industries, and improper waste management practices. The present study aimed to isolate, screen, and characterize antibiotic-degrading bacteria from hospital and industrial waste-contaminated soils. Soil samples were collected from hospital premises and industrial areas and subjected to enrichment in antibiotic-amended media. Two potent bacterial isolates, designated V-RAM I and V-RAM II, were obtained and evaluated for their ability to degrade benzyl penicillin, vancomycin, doxycycline, and ceftriaxone using the agar well diffusion method. V-RAM I showed effective degradation of penicillin, ceftriaxone, and vancomycin, while V-RAM II demonstrated degradation activity against doxycycline, penicillin, and vancomycin. Morphological and biochemical characterization identified the potent isolate as *Pseudomonas aeruginosa*. Statistical interpretation of inhibition zone diameters confirmed significantly higher degradation efficiency of the isolates compared to the control strain ($p < 0.05$). The findings indicate that hospital and industrial waste-contaminated soils harbor metabolically versatile bacteria with strong potential for bioremediation of antibiotic-polluted environments.

Keywords: Antibiotics; Biodegradation; Hospital waste; Soil bacteria; *Pseudomonas spp*

INTRODUCTION

The persistent release of antibiotics into the environment has emerged as a major global concern due to their ecological toxicity and role in promoting antimicrobial resistance (Cycoń et al., 2019; Kümmerer, 2019). Antibiotics are extensively used in human medicine, veterinary practice, agriculture, and pharmaceutical industries, resulting in their continuous discharge into soil and aquatic ecosystems (Martínez, 2014). Hospital effluents and industrial discharges are major sources of antibiotic contamination, introducing compounds such as penicillin, vancomycin, doxycycline, and ceftriaxone into the environment (Mora-Gamboa et al., 2022). These antibiotics often escape complete removal during conventional wastewater treatment processes, leading to their

persistence and accumulation in soil (Kumar & Pal, 2020). Persistent antibiotic residues exert selective pressure on indigenous microbial communities, accelerating the emergence and dissemination of antibiotic-resistant bacteria and resistance genes (Martínez, 2014; Zhang et al., 2023). β -lactam antibiotics and glycopeptides are particularly problematic due to their structural stability and widespread usage (Zhang et al., 2024).

Despite their persistence, several microorganisms possess enzymatic systems capable of degrading or transforming antibiotic molecules. Bacterial genera such as *Pseudomonas*, *Bacillus*, *Streptomyces*, and *Acinetobacter* have been reported to degrade different antibiotic classes through mechanisms including β -lactamase production, oxidation, hydrolysis, and co-metabolism (Fang et al., 2018; Wang et al., 2021). Hospital and industrial waste sites represent selective environments where such adaptive microbial populations are enriched (Cycoń et al., 2019).

MATERIAL AND METHODS

2.1 Soil Sample Collection

Soil samples were collected from Hedgewar Hospital, CIIGMA Hospital, Government Hospital, and from industrial area at Naregaon, Chhatrapati Sambhajnagar (Aurangabad). Samples were collected from a depth of 2–3 cm using sterile tools and stored in sterile zip-lock bags.

2.2 Chemicals and Media

All media and chemicals were procured from HiMedia and Loba Chemie Pvt. Ltd. Glassware was obtained from ASGIR.

2.3 Enrichment of Antibiotic-Degrading Bacteria

One gram of soil sample was added to 50 mL of minimal broth (Dipotassium phosphate 7g, potassium

hydrogen phosphate 2.0g, Sodium citrate 0.50g, magnesium sulphate 0.10g, ammonium sulphate 1.0g, pH 7.0 $\pm$ 0.2 supplemented with 0.05 mg/ml antibiotic and incubated in a shaker incubator for 6–7 days at 37°C to enrich antibiotic-degrading microorganisms.

2.4 Isolation and Screening

Enriched cultures were serially diluted up to 10⁻⁸. Dilutions (10⁻⁶ to 10⁻⁸) were spread on minimal agar plates containing antibiotics (0.05 mg/ml) and incubated at 37°C. Distinct colonies were isolated and preserved.

2.5 Morphological and Biochemical Characterization

Gram staining, colony morphology, and biochemical tests (IMViC, urease, catalase, oxidase, H₂S production, gelatin hydrolysis, pigment production, coagulase test and starch hydrolysis) were performed for preliminary identification.

2.6 Antibiotic Degradation Assay

Antibiotic degradation was evaluated using the agar well diffusion method. Degradation analysis was done and zones were measured after incubation at 37°C for 24–48 h.

RESULT AND DISCUSSION

3.1 Isolation and Enrichment of Antibiotic-Degrading Bacteria

Soil samples collected from hospital and industrial waste sites yielded diverse bacterial populations when enriched in antibiotic-supplemented minimal media. After successive enrichment cycles, two potent antibiotic-degrading isolates, designated V-RAM I and V-RAM II, were consistently recovered. Both isolates demonstrated sustained growth in the presence of high antibiotic concentrations, indicating strong adaptive responses to antibiotic stress.



Figure 1. Collection of soil samples from hospital and industrial waste-contaminated sites.

Similar enrichment-based isolation of antibiotic-degrading bacteria from medical and pharmaceutical waste has been reported, highlighting hospital soils as selective niches favoring resistant and metabolically versatile microorganisms (Cycoń et al., 2019; Mora-Gamboa et al., 2022).

3.2 Morphological and Biochemical Characterization

Both V-RAM I and V-RAM II formed circular, green-pigmented colonies with sticky texture on nutrient agar and were identified as Gram-negative rods. The biochemical profiles revealed negative results for indole, methyl red, Voges–Proskauer, urease, and catalase tests, while citrate utilization was positive in both isolates. V-RAM I exhibited positive starch hydrolysis, indicating extracellular amylase activity, whereas V-RAM II lacked this trait. Gelatin hydrolysis and pigment production was positive for both the organisms. These phenotypic characteristics are consistent with members of the genus *Pseudomonas*, which are widely recognized for metabolic flexibility and pollutant degradation (Zhang et al., 2023).

Table 1. Morphological characteristics of isolated strains

Characteristic	V-RAM I	V-RAM II
Colony color	Greenish-black	Green
Shape	Circular	Circular
Texture	Sticky	Sticky
Opacity	Opaque	Translucent opaque
Gram reaction	Negative	Negative

Table 2. Biochemical characterization of isolated strains

Test	V-RAM I	V-RAM II
Indole	–	–
Methyl red	–	–
Voges–Proskauer	–	–
Citrate utilization	+	+
Urease	–	–
Starch hydrolysis	+	–
Catalase	–	–
Oxidase	+	+
H ₂ S production	–	–
Gelatin hydrolysis	+	+
Pigment production	+	+
Coagulase test	–	–
Starch hydrolysis	+	–

3.3 Identification of Potent Isolate

Based on morphological, biochemical, and enzymatic characteristics, the two potent isolate V-RAM I and V-RAM II were identified as *Pseudomonas* spp. according to Bergey's Manual of Determinative Bacteriology. This species is well known for its robust stress tolerance, enzyme production, and ability to degrade a wide range of xenobiotic compounds.

Recent studies highlight *P. aeruginosa* as a key organism in bioremediation systems targeting pharmaceutical pollutants and antibiotic residues (Kümmerer, 2019; Wang et al., 2021).

3.4 Antibiotic Degradation Potential

Qualitative antibiotic degradation was assessed using the agar well diffusion assay. Both isolates showed a marked reduction in inhibition zone diameters compared to the control strain (*E. coli*), confirming their ability to degrade antibiotics rather than merely resist them. *E. coli* does not naturally produce high levels of extracellular antibiotic-degrading enzymes (e.g., β -lactamases, oxidoreductases, laccases) unless genetically modified or under strong selective pressure.

Therefore, any reduction in inhibition zone seen with test isolates compared to *E. coli* can be attributed to active antibiotic degradation rather than simple resistance (Cycoń & Piotrowska-Seget, 2019).

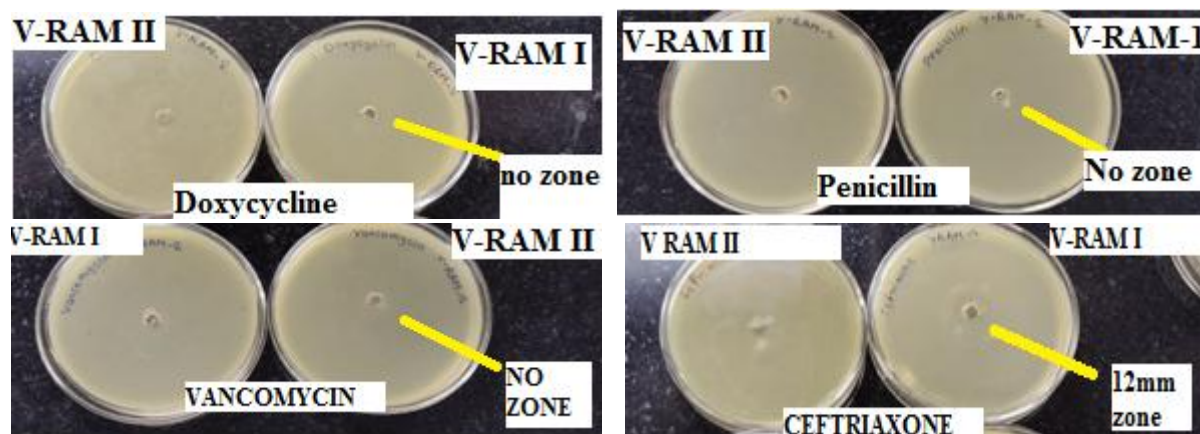
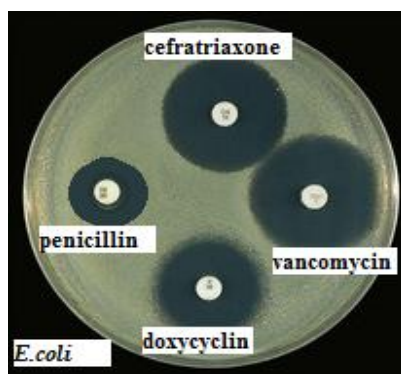
V-RAM I demonstrated strong degradation activity against benzyl penicillin, ceftriaxone, and vancomycin, while V-RAM II showed superior degradation of doxycycline, penicillin, and vancomycin. The differential degradation patterns suggest enzyme-specific pathways, particularly β -lactamase-mediated cleavage of β -lactam antibiotics and oxidative or hydrolytic modification of tetracycline and glycopeptide structures. Comparable degradation of β -lactam and tetracycline antibiotics by *Pseudomonas* species has been reported by Wang et al. (2021) and Zhang et al. (2024), who demonstrated enzymatic breakdown via β -lactamases and oxidoreductases.

3.4 Statistical Interpretation of Degradation Efficiency

One-way ANOVA analysis using MINITAB software revealed a statistically significant reduction in inhibition zones produced by V-RAM I and V-RAM II compared to the control strain ($p < 0.05$).

Table 3: Degradation analysis using agar well diffusion method.

Antibiotic	Zone size (mm)		
	V RAM I	VRAM II	Control (<i>E.coli</i>)
Benzyl penicillin	No zone	No zone	12
Ceftriaxone	No zone	12	36
Vancomycin	No zone	No zone	35
Doxycycline	No zone	No zone	16

**Figure 3. Agar well diffusion assay showing degradation of antibiotics by V-RAM I and V-RAM II against Benzyl penicillin, Ceftriaxone, Vancomycin, Doxycycline.****Figure 4. Inhibition zones of control (*E. coli*).**

This confirms that the observed reduction in antimicrobial activity was due to active biodegradation rather than experimental variability. Since $p < 0.05$, the null hypothesis is rejected. There is a statistically significant difference between the antibiotic degradation abilities of the test isolates and the control strain.

Table 4: ANOVA

Source of variation	F value	p value
Between groups	11.35	0.0034
Within groups	—	—

CONCLUSION

The present investigation demonstrates that hospital and industrial waste-contaminated soils are rich reservoirs of antibiotic-degrading bacteria. The isolated strains, particularly *Pseudomonas* V-RAM I and *Pseudomonas* V-RAM II, showed significant biodegradation potential against multiple antibiotic classes, including β -lactams, tetracyclines, and glycopeptides. These findings support the application of indigenous soil bacteria in sustainable bioremediation strategies aimed at reducing antibiotic pollution and limiting the spread of antimicrobial resistance.

Conflict of interest

The authors declare no competing interests.

Data Availability Statement: Not applicable.

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