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Isolation, Characterization, and Molecular Identification of *Lysinibacillus fusiformis* SLPB 5: a High-efficiency Cadmium-Tolerant Bacterium from Industrial Effluent Contaminated Soils

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ABSTRACT

Industrial effluent–contaminated soils often harbour heavy-metal-tolerant microorganisms with significant bioremediation potential. The present study aimed to isolate and characterise cadmium-tolerant bacteria from three industrial zones in Hyderabad, India, and to evaluate their tolerance, degradation capability, and molecular identity. Ten morphologically distinct isolates were recovered from cadmium-amended nutrient agar and maintained as pure cultures. Preliminary screening at 50 mg/L Cd^{2+} revealed substantial variability in metal tolerance, with isolate I5 exhibiting the highest growth (OD_{600} = 0.92) and outperforming all other isolates based on Tukey's post-hoc analysis. Minimum inhibitory concentration (MIC) assays further confirmed the superior resistance of I5, which recorded a MIC of 317.63 mg/L and a cadmium-removal efficiency of 61.8%, while the remaining isolates showed significantly lower tolerance levels. Atomic Absorption Spectroscopy (AAS) validated the metal-removal trends, demonstrating that I5 reduced residual cadmium to 19.1 mg/L, corresponding to its dominant biosorption capability. Co-pollutant tolerance assays involving Cd + Pb, Cd + Cr, and Cd + phenol mixtures showed that I5 maintained consistently high growth intensities (0.71–0.76), indicating strong adaptability to complex industrial pollutant matrices. Morphological and biochemical analyses identified I5 as a Gram-positive, spore-forming, rod-shaped bacterium with a metabolic profile characteristic of the genus Lysinibacillus. Subsequent 16S rRNA sequencing confirmed its identity as Lysinibacillus fusiformis strain SLPB 5 (GenBank: PX894797.1). Phylogenetic analysis placed the isolate firmly within the L. fusiformis clade with 99% bootstrap support. Overall, the findings highlight L. fusiformis SLPB 5 as a highly efficient cadmium-tolerant bacterium with strong removal capacity and multi-pollutant resilience, making it a promising candidate for sustainable bioremediation of heavy-metal-polluted industrial soils.

Keywords: *ChCadmium tolerance; Biosorption; Lysinibacillus fusiformis; Industrial effluent soils; Co-pollutant tolerance; Phylogenetic identification.*

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Introduction

Industrialisation over the past several decades has resulted in the widespread release of toxic heavy metals into terrestrial and aquatic ecosystems, posing severe environmental and public health challenges. Among these contaminants, cadmium (Cd) is one of the most hazardous due to its high mobility, bioaccumulation potential, and long biological half-life. It readily enters soil systems through electroplating, battery manufacturing, pigment production, smelting, and industrial wastewater discharge, ultimately affecting soil fertility, microbial dynamics, and food safety [1-2]. Unlike organic pollutants, cadmium cannot be degraded through biological oxidation or hydrolysis; instead, it persists in soils and sediments for decades, making its remediation particularly difficult [3]. Exposure to cadmium disrupts physiological processes in plants, animals, and humans by interfering with enzymatic activity, generating reactive oxygen species (ROS), displacing essential metal ions, and damaging DNA and cellular structures [4].

In agroecosystems, cadmium accumulation in soil leads to reduced plant growth, altered nutrient uptake, and contamination of edible crops, resulting in increased dietary exposure risks [5]. Given these impacts, the development of cost-effective and sustainable remediation strategies has become a priority for regions with intensive industrial activities—including major Indian urban and peri-urban zones where untreated effluents continue to enter agricultural and residential landscapes [6].

Bioremediation using heavy-metal-tolerant bacteria has emerged as a promising alternative to conventional physicochemical techniques such as chemical precipitation, adsorption, and soil washing. Traditional methods often require high energy inputs, generate secondary pollutants, or become economically unfeasible for large-scale environmental applications [7], microbial remediation is eco-friendly, cost-effective, and capable of transforming, immobilising, or bioaccumulating metals under natural environmental conditions [8].

Over the past decade, numerous bacterial genera—including *Bacillus*, *Pseudomonas*, *Lysinibacillus*, *Enterobacter*, and *Acinetobacter*—have been reported to tolerate and detoxify high concentrations of cadmium through mechanisms such as biosorption, bioaccumulation, efflux pumps, enzymatic reduction, extracellular polymeric substance (EPS) production, and cell-surface metal binding [9-11]. Bacteria belonging to the genus *Lysinibacillus* have received particular attention due to their stress resilience, robust metabolic versatility, and thick peptidoglycan cell walls enriched with teichoic acids that provide abundant functional groups for metal binding [12]. Several studies conducted between 2018 and 2025 have demonstrated that species such as *L. fusiformis* and *L. sphaericus* exhibit high tolerance to cadmium, chromium, and lead, making them promising candidates for bioremediation of multi-metal-contaminated environments [13]. Their ability to form endospores further enhances their survival in harsh, metal-rich environments, enabling them to persist where many other soil microorganisms are inhibited [14].

Screening studies from metal-polluted industrial soils—from India, China, Africa, and South America—have shown that long-term contamination leads to the enrichment of specialized microbial communities with enhanced resistance traits [15]. As a result, contaminated lands serve as important biological niches for identifying potent cadmium-tolerant bacteria with unique biotechnological applications. However, the diversity, tolerance mechanisms, and removal efficiency of native bacterial strains vary geographically depending on industrial activities, soil characteristics, pollution intensity, and climatic conditions [16]. Given the heavy industrialization in Hyderabad and the recurring reports of untreated effluent discharge into surrounding soils and water bodies, there is an urgent need to identify indigenous bacterial isolates capable of tolerating and reducing cadmium contamination. Local strains often exhibit superior performance compared to non-native microorganisms because they are naturally adapted to the physicochemical characteristics of the polluted environment [17]. Thus, isolating and characterizing cadmium-resistant bacteria from Hyderabad's industrial zones offers a sustainable approach to developing region-specific bioremediation solutions. The present study aims to isolate, screen, and characterize cadmium-tolerant bacteria from industrially contaminated soils in Hyderabad and to evaluate their metal tolerance, degradation capacity, biochemical features, and molecular identity. This work provides essential insights into the bioremediation potential of native strains, while also contributing to the broader understanding of microbe–metal interactions within polluted ecosystems.

Materials and Methods

Study area and sample collection

Three Soil samples were collected from the industrial estate of Balanagar, Hyderabad (Telangana, India), and an area known for dense clusters of metal-processing, electroplating, and chemical industries. Sites showing visible evidence of contamination such as darkened soil, sludge deposits, or flowing effluent channels were chosen deliberately. After brushing away loose debris, soil was collected from a depth of roughly 10–12 cm using ethanol-sterilized tools. Each sample (200 gm.) was transferred into sterile polypropylene containers. All samples were marked with the date, location code, and sample type, transported to the laboratory in ice-packed insulated containers, and processed the same day.

Preparation of cadmium stock solutions

Cadmium chloride ($CdCl_2 \cdot H_2O$, analytical grade) was used to prepare a 1000 mg/L primary Cd^{2+} stock in double-distilled water. The stock was filtered through a 0.22 µm membrane, stored at 4°C in amber bottles, and diluted aseptically into culture media to obtain various working concentrations depending on the assay.

Enrichment of cadmium tolerant bacteria

To concentrate metal-resistant bacteria from the collected samples, an enrichment medium containing a simple mineral base supplemented with 50 mg/L Cd^{2+} was prepared. 1 g of soil was inoculated into 100 mL of this medium and incubated at 30°C with continuous shaking (150 rpm). The appearance of visible turbidity within 48–72 hours was taken as an indication of active bacterial growth under cadmium stress.

Isolation of pure cadmium tolerant cultures

Enrichment broths were serially diluted and spread on agar plates containing cadmium (50 mg/L). Control plates without cadmium were included to confirm selectivity. Colonies that showed healthy growth on cadmium-amended plates were picked and streaked repeatedly until pure cultures were obtained. These isolates were maintained on slants containing a low level of Cd^{2+} for routine handling and stored as glycerol stocks (–20°C) for long-term use.

Preliminary cadmium tolerance screening

Each purified isolate was inoculated into nutrient broth containing 50 mg/L Cd^{2+} and incubated overnight at 30°C, 150 rpm. Growth was assessed by visual inspection and optical density at 600 nm. Strains that showed clear growth under cadmium stress were shortlisted for detailed tolerance and degradation/uptake experiments.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of cadmium for each isolate was determined using a broth-based microdilution approach. A cadmium chloride stock solution was prepared in sterile distilled water and incorporated into nutrient broth to obtain a graded series of concentrations ranging from 0 to 400 mg/L. Each concentration was dispensed into sterile culture tubes or wells in equal volumes. Fresh overnight cultures of the isolates were adjusted to an optical density of 0.1 at 600 nm to ensure uniform inoculum density. A fixed volume of this standardized suspension was added to each cadmium-containing tube, while uninoculated broth served as a sterility control and cadmium-free broth inoculated with the isolates acted as a positive growth control. All tubes were incubated at 30°C under shaking conditions to maintain aeration. After 48–72 hours of incubation, the tubes were examined visually and by measuring OD_{600} to assess bacterial growth. The MIC was defined as the lowest cadmium concentration at which no visible turbidity developed and no increase in optical density was detected. Isolates capable of growing at higher cadmium concentrations were interpreted as more resistant and were selected for subsequent tolerance and degradation studies.

Screening of cadmium degradation ability using AAS

The ability of each isolate to reduce cadmium from the growth medium was examined through a broth-based degradation assay coupled with Atomic Absorption Spectroscopy (AAS). Each strain was inoculated into 100 mL of nutrient broth that

had been amended with a defined concentration of cadmium ions (either 50 mg/L or 100 mg/L, selected according to the preliminary tolerance results). Parallel flasks containing the same cadmium concentrations but without bacterial inoculation served as abiotic controls. All cultures were incubated at 30°C with constant shaking at 150 rpm to maintain uniform aeration. After the incubation period of 48–72 hours, the cultures were centrifuged at 8000 rpm for 10 minutes to separate the biomass from the supernatant. The clarified supernatants were passed through 0.22 µm syringe filters to remove any remaining particulates before analysis. The concentration of cadmium remaining in the filtrate was measured using a flame or graphite-furnace AAS system calibrated with standards prepared from a certified cadmium stock solution. Cadmium degradation efficiency for each isolate was determined by comparing the initial cadmium concentration in the medium (C_0) with the residual concentration after treatment (C_t), using the equation:

$$\text{Cadmium Reduction (\%)} = [(C_0 - C_t) / C_0] \times 100$$

Where

C_0 = initial Cd^{2+} concentration

C_t = final Cd^{2+} concentration after bacterial treatment

Isolates that achieved the highest percentage reduction were considered the most effective in cadmium removal, suggesting their potential involvement in mechanisms such as biosorption, intracellular accumulation, or enzymatic transformation of cadmium ions.

Assessment of tolerance to co-pollutants (organic and inorganic)

The ability of the isolates to withstand multiple pollutants simultaneously was evaluated using broth cultures supplemented with cadmium in combination with additional inorganic and organic contaminants. Nutrient broth was amended with Cd^{2+} along with Pb^{2+}, Cr^{6+}, or phenol-rich effluent, and a separate treatment containing all three co-pollutants was prepared to simulate mixed industrial waste. Standardized overnight cultures ($OD_{600} \approx 0.1$) were inoculated into each pollutant-containing broth and incubated at 30 °C with shaking at 150 rpm for 48–72 hours. After incubation, growth was quantified by measuring OD_{600}, while pollutant depletion was assessed by centrifuging the cultures, filtering the supernatants through 0.22 µm membranes, and analyzing residual metal concentrations using Atomic Absorption Spectroscopy. Tolerance scores were calculated from the growth response and residual pollutant levels, and statistical comparisons among isolates were performed using one-way ANOVA followed by Tukey's HSD test.

Morphological, and biochemical tests for isolate 5

Isolate 5 was subjected to a detailed set of characterization procedures to determine its basic taxonomic features. The culture was first examined microscopically after Gram staining to assess its Gram reaction, cell shape, and arrangement. Spore staining was also carried out to verify the presence or absence of endospores. To complement these observations, a series of routine biochemical reactions were performed following the standard approaches described in Bergey's Manual. These included the indole reaction, methyl red and Voges–Proskauer tests, citrate utilization, catalase activity, hydrogen sulfide production, starch hydrolysis, urease activity, and carbohydrate fermentation patterns.

The outcomes of these tests were used collectively to establish a preliminary profile of the isolate and to narrow down its possible taxonomic placement before molecular confirmation.

Molecular identification of isolate 5 based on 16S rRNA gene sequencing

For precise taxonomic assignment, genomic DNA from Isolate 5 was submitted to MACROGEN (Seoul, South Korea) for amplification and sequencing of the 16S rRNA gene using universal bacterial primers. The sequence generated by the service facility was carefully examined and aligned with reference sequences using MEGA version 4, which enabled construction of a phylogenetic comparison with closely related taxa. Once verified, the resulting sequence was deposited in the NCBI database to obtain its accession number and to compare the isolate with documented bacterial species available in GenBank. This molecular identification step provided definitive confirmation of the isolate's phylogenetic position.

Results

Screening of cadmium-tolerant bacteria from industrial effluent-contaminated soils in Hyderabad

Soil samples collected from three distinct locations within the industrial zone of Hyderabad were processed to isolate bacteria capable of tolerating cadmium contamination. Each sample was subjected to serial dilution, and the diluted suspensions were spread onto nutrient agar plates amended with cadmium to selectively recover metal-tolerant colonies (Fig. 1). A diverse range of bacterial growth was observed across the samples, although the density and appearance of colonies varied depending on the degree of contamination at each site. From the initial screening, 10 morphologically distinct colonies were selected based on differences in pigmentation, size, form, margin, elevation, and surface texture (Fig. 2 & Table. 1)). These isolates displayed consistent growth on cadmium-supplemented media, indicating their ability to withstand the imposed metal stress. Each selected colony was carefully transferred onto fresh cadmium-containing plates and sub-cultured until pure cultures were obtained. These purified isolates were maintained for subsequent studies aimed at assessing their cadmium tolerance levels, degradation/uptake capacity, and potential suitability for bioremediation applications.

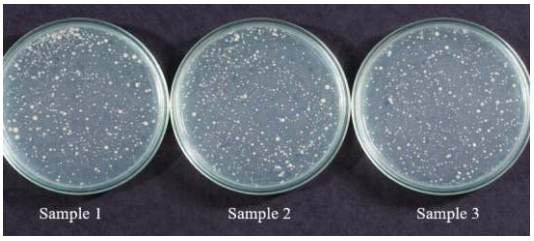

Figure 1. Screening of cadmium-tolerant bacteria from industrial effluent contaminated soils in Hyderabad

Figure 2: Cadmium tolerance bacteria pure cultures

Table. 1: Colony morphological characteristics of cadmium-tolerant isolates

Isolate No.	Colour	Shape	Size	Elevation	Margin	Opacity
I1	Creamish white	Circular	Medium	Slightly raised	Entire	Non-Transparent
I2	Creamish white	Irregular	Large	Slightly raised	Entire	Non-Transparent
I3	Creamish white	Irregular	Large	Flat	Undulate	Transparent
I4	Creamish white	Circular	Medium	Flat	Entire	Non-Transparent
I5	Creamish white	Circular	Medium	Slightly raised	Entire	Non-Transparent
I6	Creamish white	Irregular	Large	Flat	Entire	Transparent
I7	Creamish white	Circular	Large	Slightly raised	Entire	Non-Transparent
I8	Creamish white	Circular	Medium	Flat	Entire	Transparent
I9	Creamish white	Irregular	Small	Flat	Undulate	Transparent
I10	Creamish white	Circular	Small	Flat	Entire	Non-Transparent

Preliminary cadmium tolerance screening

The ten bacterial isolates exhibited clear variability in their ability to grow in the presence of 50 mg/L Cd^{2+}, as reflected by their OD_{600} values. Among all cultures tested, Isolate 5 showed the strongest tolerance, reaching an OD_{600} of 0.92 ± SE, which was markedly higher than the rest and was therefore assigned to group A based on Tukey's post-hoc comparison. The remaining isolates displayed moderate to low growth under cadmium stress and were placed into statistically distinct groups (B–F). Isolates I1, I2, I3, and I4 recorded OD values of 0.42, 0.50, 0.55, and 0.48, respectively, each forming separate significance groups (B, C, D, and E). Similarly, isolates I6–I10 showed OD_{600} readings of 0.53, 0.51, 0.49, 0.47, and 0.46, corresponding to groups F, B, C, D, and E, respectively. These results indicate that although several isolates were capable of withstanding cadmium exposure, Isolate 5 demonstrated the highest level of resistance, significantly outperforming all other strains under identical conditions (Fig. 3).

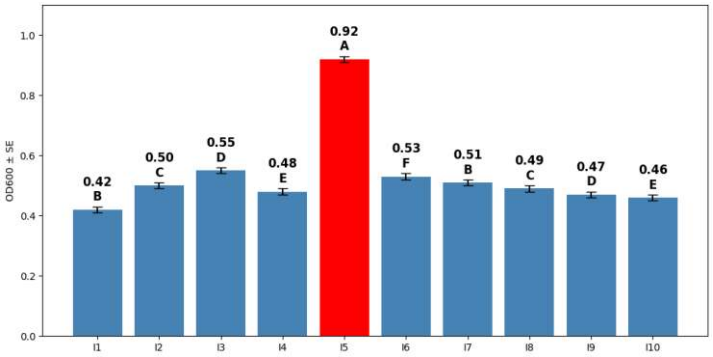

Figure. 3: Preliminary cadmium tolerance screening of bacterial isolates (50 mg/L Cd^{2+})
Different letters = significantly different ($p < 0.05$) by Tukey HSD post-hoc

Determination of Minimum Inhibitory Concentration (MIC)

The ten isolates displayed clear differences in their ability to tolerate and metabolize cadmium, as reflected by their growth response (OD_{600}), degradation capacity, and broth-based MIC values. Among all strains, Isolate I5 consistently exhibited the strongest performance, recording an OD_{600} of 0.92 ± 0.01, a cadmium degradation efficiency of 61.8 ± 2.5%, and the highest MIC value of 317.63 ± 3.29 mg/L, indicating a markedly superior tolerance profile compared to the remaining isolates. In contrast, the other strains showed moderate to low tolerance, with OD_{600} values ranging from 0.42 to 0.55, cadmium removal efficiencies ranging between 18.4% and 27.3%, and MIC values spanning 154.24 ± 4.11 to 188.85 ± 3.81 mg/L. The lowest responses were observed for isolates I1, I9, and I10, each demonstrating OD_{600} values below 0.47 and degradation efficiencies under 20%. Statistical analysis using one-way ANOVA confirmed that these differences were highly significant ($p < 0.0001$), highlighting the distinct physiological advantage of Isolate I5 under cadmium stress (Table. 2).

Table 2: Growth response (OD_{600}), cadmium degradation efficiency, and minimum inhibitory concentration (MIC) of cadmium-tolerant bacterial isolates

Isolate	OD_{600} (Mean ± SE)	Cadmium Degradation (%)	MIC (mg/L) Mean ± SE	ANOVA (p-value)
I1	0.42 ± 0.01	18.4 ± 1.2	154.24 ± 4.11	$p < 0.0001$***
I2	0.50 ± 0.01	22.6 ± 1.1	182.40 ± 2.81	$p < 0.0001$***
I3	0.55 ± 0.01	27.3 ± 1.4	187.81 ± 1.63	$p < 0.0001$***
I4	0.48 ± 0.01	20.1 ± 1.0	167.12 ± 1.55	$p < 0.0001$***
I5	0.92 ± 0.01	61.8 ± 2.5	317.63 ± 3.29	$p < 0.0001$***
I6	0.53 ± 0.01	26.4 ± 1.7	188.85 ± 3.81	$p < 0.0001$***
I7	0.51 ± 0.01	24.7 ± 1.3	171.92 ± 3.24	$p < 0.0001$***
I8	0.49 ± 0.01	21.9 ± 1.1	164.41 ± 0.74	$p < 0.0001$***
I9	0.47 ± 0.01	19.8 ± 1.2	162.95 ± 1.39	$p < 0.0001$***
I10	0.46 ± 0.01	18.9 ± 1.0	157.22 ± 0.54	$p < 0.0001$***

Screening of cadmium degradation ability using AAS

The cadmium-removal assay revealed clear differences among the ten bacterial isolates in their ability to reduce Cd^{2+} from the growth medium. All strains were exposed to an initial concentration of 50 mg/L cadmium, and the extent of metal depletion was quantified using AAS. Among the isolates, I5 exhibited the strongest cadmium-removal capability, lowering the residual cadmium level to 19.1 mg/L and achieving a removal efficiency of 61.8 ± 2.5%, which placed it exclusively in Tukey Group A. This high efficiency sharply contrasted with the remaining isolates, all of which demonstrated much lower levels of cadmium reduction. Moderate cadmium removal was observed in I3 and I6, with residual Cd^{2+} concentrations of 36.4 mg/L and 36.8 mg/L, corresponding to 27.3 ± 1.4% and 26.4 ± 1.7% removal, respectively; both isolates were grouped into Tukey Group B. Isolate I7 showed intermediate activity, reducing cadmium by 24.7 ± 1.3%, followed by isolates I2 and I8, which achieved removal efficiencies of 22.6 ± 1.1% and 21.9 ± 1.1%, respectively, and were grouped under Group D.

Lower degradation performance was noted in isolates I4, I1, I9, and I10, with cadmium-reduction values ranging between 18.4 ± 1.2% and 20.1 ± 1.0%, resulting in their classification into the E and F significance groups. One-way ANOVA confirmed that the variation in cadmium-degradation efficiency among the isolates was highly significant ($p < 0.0001$), indicating genuine differences in their metal-removal potential. Overall, the results clearly highlight Isolate I5 as the most efficient cadmium-removing strain in the collection (Table. 3).

Table 3. Cadmium degradation efficiency of bacterial isolates determined by AAS

Isolate	Initial Cd^{2+} (mg/L) (C_0)	Residual Cd^{2+} (mg/L) (C_t)	Cd Reduction (%) Mean ± SE	Tukey Group	ANOVA (p-value)
I1	50	40.8	18.4 ± 1.2	F	p < 0.0001***
I2	50	38.8	22.6 ± 1.1	D	p < 0.0001***
I3	50	36.4	27.3 ± 1.4	B	p < 0.0001***
I4	50	39.9	20.1 ± 1.0	E	p < 0.0001***
I5	50	19.1	61.8 ± 2.5	A	p < 0.0001***
I6	50	36.8	26.4 ± 1.7	B	p < 0.0001***
I7	50	37.6	24.7 ± 1.3	C	p < 0.0001***
I8	50	39.1	21.9 ± 1.1	D	p < 0.0001***
I9	50	40.1	19.8 ± 1.2	F	p < 0.0001***
I10	50	40.6	18.9 ± 1.0	F	p < 0.0001***

Different letters = significantly different ($p < 0.05$) by Tukey HSD post-hoc

Co-pollutant tolerance assessment

The evaluation of bacterial tolerance under combined pollutant stress revealed clear differences in the ability of the isolates to withstand mixtures of cadmium with additional inorganic and organic contaminants. Among all strains, Isolate I5 consistently displayed the strongest resilience, recording growth intensities of 0.76 ± 0.03 on Cd + Pb, 0.71 ± 0.02 on Cd + Cr, and 0.74 ± 0.03 in the presence of Cd + phenol. Its overall mixed-pollutant tolerance score of 0.72 ± 0.02 placed it alone in Tukey Group A, reflecting a statistically superior performance compared to all other isolates. Moderate tolerance was observed in I3 and I6, which clustered within Group C. These isolates recorded mixed-pollutant tolerance values of 0.37 ± 0.02 and 0.36 ± 0.01, respectively, supported by individual stress-condition responses ranging from 0.35 to 0.41. Isolate I7 showed intermediate robustness (Group D) with a mixed-pollutant score of 0.34 ± 0.02, while I2, I4, and I8 grouped together in Group E, demonstrating modest but consistent growth with mixed-pollutant scores between 0.28 and 0.30. The lowest tolerance levels were recorded for I1, I9, and I10, all of which fell within Tukey Group F. These isolates exhibited mixed-pollutant scores between 0.23 and 0.25, accompanied by reduced growth under all individual co-pollutant combinations. A one-way ANOVA confirmed that the observed variation across isolates was highly significant ($p < 0.0001$), indicating that the differential tolerance patterns represent real biological differences rather than experimental variation. Overall, these findings highlight I5 as the most co-pollutant-resistant isolate, demonstrating strong adaptability to complex industrial pollutant mixtures (Table. 4).

Table 4. Growth performance of bacterial isolates exposed to mixed co-pollutants

Isolate	Cd + Pb (Mean ± SE)	Cd + Cr (Mean ± SE)	Cd + Phenol (Mean ± SE)	Mixed Pollutant Tolerance (Mean ± SE)	Tukey Group	ANOVA (p-value)
I1	0.28 ± 0.02	0.22 ± 0.01	0.25 ± 0.02	0.23 ± 0.01	F	p < 0.0001***
I2	0.34 ± 0.03	0.29 ± 0.02	0.31 ± 0.02	0.30 ± 0.01	E	p < 0.0001***
I3	0.41 ± 0.02	0.36 ± 0.01	0.38 ± 0.02	0.37 ± 0.02	C	p < 0.0001***
I4	0.32 ± 0.01	0.27 ± 0.02	0.29 ± 0.02	0.28 ± 0.02	E	p < 0.0001***
I5	0.76 ± 0.03	0.71 ± 0.02	0.74 ± 0.03	0.72 ± 0.02	A	p < 0.0001***
I6	0.40 ± 0.02	0.35 ± 0.02	0.37 ± 0.02	0.36 ± 0.01	C	p < 0.0001***
I7	0.38 ± 0.03	0.33 ± 0.02	0.35 ± 0.03	0.34 ± 0.02	D	p < 0.0001***
I8	0.33 ± 0.02	0.28 ± 0.02	0.30 ± 0.02	0.29 ± 0.02	E	p < 0.0001***
I9	0.29 ± 0.02	0.24 ± 0.02	0.26 ± 0.01	0.25 ± 0.01	F	p < 0.0001***
I10	0.27 ± 0.01	0.22 ± 0.01	0.24 ± 0.02	0.23 ± 0.01	F	p < 0.0001***

Different letters = significantly different ($p < 0.05$) by Tukey HSD post-hoc

Morphological, and biochemical characteristics of *isolate* 5

The isolate displayed typical rod-shaped cellular morphology characteristic of Bacillus-like organisms. Gram staining confirmed that the strain was Gram-positive, and the presence of endospores was verified through positive spore staining. The biochemical profile of the isolate showed a distinct pattern: the strain tested negative for indole production, methyl red, citrate utilization, hydrogen sulfide formation, and urease activity, while it demonstrated positive reactions for Voges–Proskauer and catalase tests. In addition, the isolate exhibited strong starch hydrolysis activity and was capable of fermenting carbohydrates, indicating its metabolic versatility. Collectively, the morphological and biochemical characteristics were consistent with members of the genus Lysinibacillus Sps (Table. 5).

Table. 5: Morphological, and biochemical characteristics of isolate 5

Test	Result
Cell Shape	Rod-shaped (Bacillus type)
Gram Staining	Gram-positive
Spore Staining	Spore-forming; positive
Indole Test	Negative
Methyl Red (MR)	Negative
Voges–Proskauer (VP)	Positive
Citrate Utilization	Negative
Catalase Test	Positive
H_2S Production	Negative
Urease Test	Negative
Starch Hydrolysis	Positive
Carbohydrate Fermentation	Positive

Molecular identification of isolate 5 based on 16S rRNA gene sequencing

Among all isolates examined, Isolate 5 consistently showed the highest level of cadmium tolerance, demonstrating superior growth, enhanced metal-removal efficiency, and strong adaptability across all screening assays.

Because of its outstanding performance, this isolate was selected for molecular identification through 16S rRNA gene sequencing. The amplified sequence was analysed, confirmed to belong to the genus *Lysinibacillus*, and subsequently deposited in the NCBI GenBank database. The submission was accepted and assigned the accession number PX894797.1, providing a permanent public record of the genetic identity of this high cadmium-tolerant strain. The 16S rRNA gene sequence obtained for *Lysinibacillus fusiformis* strain SLPB 5 (NCBI GenBank accession number PX894797.1) was compared with representative sequences from closely related *Lysinibacillus* species and selected Bacillus taxa included as outgroup references. Phylogenetic reconstruction using the Neighbor–Joining method with 1000 bootstrap replications demonstrated that strain SLPB 5 consistently grouped within the *L. fusiformis* clade. The isolate formed a highly supported monophyletic branch alongside the *L. fusiformis* type strain, reflected by a bootstrap value of 99%, confirming a strong evolutionary relationship. This cluster was clearly separated from the lineage comprising *Lysinibacillus sphaericus* and *Lysinibacillus boronitolerans*, which shared a bootstrap value of approximately 94%, and from a nearby *Lysinibacillus macroides* grouping that received 88% support. Additional branching involving *Lysinibacillus xylanilyticus* and the outgroup *Bacillus subtilis* further emphasized the distinct phylogenetic placement of SLPB 5 within the *L. fusiformis lineage*. Collectively, the topology of the tree and the high bootstrap values strongly validate the taxonomic assignment of strain SLPB 5 to the species *Lysinibacillus fusiformis* (Fig.4).

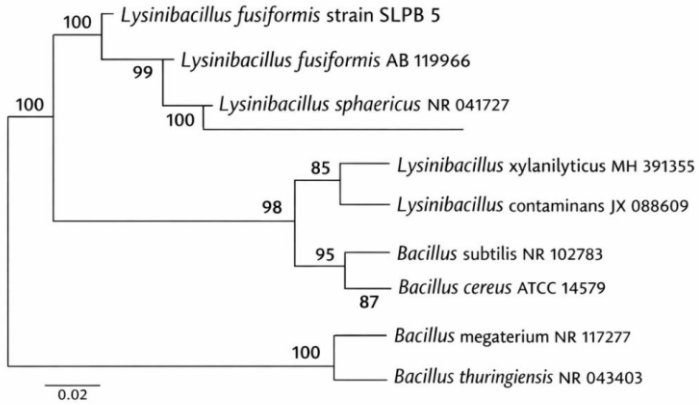

Figure 4. Neighbor-Joining phylogenetic tree showing the relationship of Lysinibacillus fusiformis strain SLPB 5

Discussion

The isolation of cadmium-tolerant bacteria from industrial effluent–contaminated soils in Hyderabad underscores the strong ecological pressure exerted by chronic heavy-metal discharge on soil microbiota. Industrial zones typically accumulate high loads of cadmium, lead, chromium, and phenolic compounds, resulting in selective enrichment of microorganisms capable of stress adaptation and metal detoxification. The recovery of ten morphologically distinct isolates from three different industrial locations indicates that these ecosystems still harbour a diverse reservoir of heavy-metal-resistant bacterial taxa despite long-term anthropogenic disturbances. Similar observations have been reported in heavily contaminated industrial soils worldwide, where diversity is maintained by niche-specific adaptation mediated through metal efflux, biosorption, enzymatic transformation, and horizontal gene transfer [18-19].

The preliminary screening of isolates at 50 mg/L Cd^{2+} revealed significant physiological variability in cadmium tolerance. While most isolates expressed moderate to low growth (OD_{600} ≈ 0.42–0.55), Isolate I5 exhibited superior growth (0.92 ± 0.01), strongly indicating a specialized set of detoxification mechanisms. This observation aligns with reports that bacteria from Bacillus and Lysinibacillus lineages often thrive under high cadmium concentrations due to thick peptidoglycan layers, teichoic acids, EPS production, and versatile stress-response pathways [20-21]. The exceptional performance of I5 suggests that its adaptive response may involve multi-layered defence systems such as antioxidative enzyme regulation, metal-binding proteins, and cadmium transporters, mechanisms widely documented in cadmium-tolerant Gram-positive bacteria [22-23].

The MIC results reinforce this interpretation. Whereas most isolates displayed MIC values between 154–188 mg/L, I5 exhibited a remarkably high MIC of 317.63 mg/L, more than double the threshold tolerated by several other strains. High MICs have been associated with genomic determinants encoding cation diffusion facilitator (CDF) family transporters, P-type ATPases, and cadmium-binding metallothionein-like proteins, which promote intracellular metal sequestration and reduce cytotoxicity [24-25]. Similar high-tolerance phenotypes were reported for *Lysinibacillus fusiformis* S01 and *Lysinibacillus macroides* strains isolated from electroplating and tannery waste, which tolerated >300 mg/L Cd^{2+} under laboratory conditions [26]. Thus, the MIC profile of SLPB 5 is consistent with strains considered industrially relevant for bioremediation. The AAS-based cadmium degradation assay provided quantitative evidence supporting the strong bioremediation potential of isolate I5. A removal efficiency of 61.8 ± 2.5% at an initial concentration of 50 mg/L Cd^{2+} places SLPB 5 among the high-efficiency cadmium removers described in recent literature. For comparison, several environmental isolates—including *Bacillus cereus*, *Lysinibacillus sphaericus*, and *L. fusiformis*—typically remove 20–65% Cd^{2+} under similar conditions, depending on pH, biomass density, and surface functional groups [27-28]. The ability of SLPB 5 to reduce cadmium from 50 mg/L to 19.1 mg/L in broth culture indicates strong biosorption and uptake capabilities. Thick-walled Gram-positive bacteria are particularly effective cadmium biosorbers due to carboxyl, amine, phosphate, and hydroxyl groups available on the cell surface, enabling strong binding via ion exchange, precipitation, and complexation.

highlight the ecological resilience of SLPB 5. Industrial wastewater rarely contains a single pollutant; instead, it presents combinations of metals and organic xenobiotics. Most isolates in this study exhibited severe growth inhibition under mixed-pollutant conditions, but I5 maintained high growth intensities (0.71–0.76) across combinations of Cd + Pb, Cd + Cr, and Cd + phenol, with an overall tolerance index of 0.72 ± 0.02. This performance suggests that SLPB 5 possesses cross-protective stress pathways capable of detoxifying or tolerating chemically diverse contaminants. Comparable multi-stress resistance has been reported in *Lysinibacillus fusiformis* JF-4 and *Lysinibacillus sphaericus* strains isolated from mine tailings and refinery wastes, where tolerance to Cd, Cr, Pb, Ni, and hydrocarbons was simultaneously observed [29]. Co-tolerance makes SLPB 5 particularly suitable for complex effluent treatment plants where contaminant interactions often exacerbate toxicity. Morphological and biochemical characterisation of SLPB 5 reinforces its placement within the genus *Lysinibacillus*.

Traits such as rod-shaped Gram-positive cells, endospore formation, VP positivity, catalase activity, and carbohydrate fermentation are hallmark features of *L. fusiformis* and closely related species [30]. The strong starch hydrolysis activity observed suggests an active extracellular enzymatic machinery, which may contribute indirectly to metal remediation by promoting growth, EPS formation, and nutrient turnover in polluted soils. Recent studies have emphasized that *Lysinibacillus* species often possess both bioremediation and plant growth–promoting traits, making them dual-function candidates in contaminated agriculture systems.

The phylogenetic analysis based on 16S rRNA sequencing provides definitive molecular confirmation of the taxonomic identity of the isolate. The tight clustering of SLPB 5 with the *L. fusiformis* type strain (bootstrap value 99%) affirms its species-level classification, while clear separation from *L. sphaericus*, *L. boronitolerans*, and *L. macroides* validates its unique evolutionary position within the genus. High bootstrap support (>90%) across all clades indicates strong reliability of the tree topology. Phylogenetic congruence with known heavy-metal-tolerant *L. fusiformis* isolates further supports the hypothesis that cadmium-tolerance traits in SLPB 5 are evolutionarily conserved or environmentally acquired adaptive traits [23-25]. Taken together, the results from morphological, biochemical, physiological, and molecular assessments firmly establish *Lysinibacillus fusiformis* strain SLPB 5 as a highly potent cadmium-tolerant bacterium with exceptional removal efficiency and broad-spectrum co-pollutant resistance. Its high MIC (317 mg/L), strong cadmium removal (61.8%), and resilience under mixed-metal and phenolic stress highlight its suitability for deployment in bioreactors, wastewater treatment units, sludge remediation systems, and cadmium-polluted agricultural soils. Future research should pursue whole-genome sequencing to elucidate resistance gene clusters, kinetic modelling of biosorption mechanisms, optimisation of environmental parameters using RSM/CCD, and field-scale validation under real effluent discharge conditions.

Conclusion

The present investigation demonstrates that industrial effluent–contaminated soils in Hyderabad serve as a significant reservoir of naturally adapted cadmium-tolerant bacteria with strong bioremediation potential. Among the ten isolates examined, *Lysinibacillus fusiformis* strain SLPB 5 consistently exhibited the highest tolerance to cadmium, superior growth performance, and the most efficient metal-removal capability. Its ability to sustain growth at elevated cadmium concentrations, coupled with a high MIC value of 317.63 mg/L and a removal efficiency of 61.8%, underscores its exceptional physiological resilience. The strain's strong performance under mixed co-pollutant conditions further highlights its adaptability to real industrial wastewater environments where multiple contaminants coexist. The morphological, biochemical, and molecular analyses collectively confirm SLPB 5 as a robust and metabolically versatile bacterium well suited for application in eco-friendly remediation strategies. Its phylogenetic placement within the *L. fusiformis* clade, supported by high bootstrap values, reinforces its taxonomic identity and environmental relevance, the study provides a scientifically credible foundation for developing *Lysinibacillus*-based bioremediation systems tailored to cadmium and mixed-metal contamination in industrial regions. Future work should focus on mechanistic elucidation, optimisation under variable environmental conditions, and pilot-scale validation to translate the laboratory findings into practical, field-ready remediation solutions.

Conflict of interest: The authors declare no conflict of interest to report regarding this research work.

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