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ISOLATION, SCREENING AND CHARACTERIZATION OF CADMIUM TOLERANT BACTERIA FROM INDUSTRIAL EFFLUENT

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ABSTRACT

Soil and water contamination with cadmium (Cd) is a severe concern for the developing world due to its non-biodegradability and significant potential to damage the ecosystem and associated services. Industries such as mining, manufacturing, building, etc., rapidly produce a substantial amount of Cd, posing environmental risks. Microremediation generally relies on the metabolic potential of microorganisms such as bacteria, fungi and algae, that have been reported to have a high tolerance for metals. Bacteria are reported to have a significant bioremediation capability in which hazardous pollutants act as a source of energy for cellular development and growth. The present study is therefore aimed at isolation, screening and characterization of cadmium tolerant bacterial strains from the effluent of West coast paper mill, Dandeli. Samples were collected asceptically and subjected to elemental analysis to detect the presence of cadmium. Physiochemical parameters, including Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), CO_2 and Total Dissolved Solids (TDS), were assessed. Four cadmium resistant bacterial strains (C1, C2, C3, and C4) were isolated, screened and identified upto the genus level by cultural, microscopic and biochemical characteristics to assess their metal tolerance potential. Among them, C2 strain exhibited significant cadmium tolerance and was subjected to molecular characterization and identified as *Klebsiella pneumoniae*. *Klebsiella pneumoniae* was subjected to MIC with various concentrations of cadmium (0.005, 0.010, 0.015, 0.020, 0.025, and 0.030 mg/mL) and was observed that 0.025 mg/mL showed optimum growth which was explored for further analysis. Antibiotic susceptibility test revealed that *Klebsiella pneumoniae* was sensitive to cefixime and ciprofloxacin, indicating potential safety in environmental applications. The results highlight the promising use of *Klebsiella pneumoniae* in the bioremediation of cadmium-contaminated aquatic environments.

KEYWORDS: Cadmium tolerance, *Klebsiella pneumonia*, industrial effluents.

INTRODUCTION

Industrialization has raised our living standard very high but it has also destroyed our environment. Tons of industrial wastes are released in our environment every year. This is especially true for developing countries like China and India (Faezi*et al*., 2010). This industrial waste may contain toxic and carcinogenic elements and cadmium is one of them. It is non-biodegradable and has no known role in cellular metabolism. It is accumulating in our environment as a result of its extensive release from different industrial processes such as mining and smelting of ores, electroplating, manufacturing plastics, colour pigments and phosphate fertilizers, United States Environmental Protection Agency (EPA) has set cadmium oral uptake at 0.5µg/Kg/Day in drinking water and 1µg/Kg/Day in food. Higher concentrations may cause ill health effects in human including skeletal and cardiovascular dysfunctions, lungs, liver and kidney damage and reproductive problems. No treatment for cadmium toxicity has been approved so far. There is an

urgent need to protect and conserve our environment by reducing heavy metal pollution or else it would be soon out of our control to protect environment. Several studies have reported the use of microorganisms to eliminate heavy metals from the environment as less expensive, cost effective and environmentally friendly strategy (Hussain *et al*., 2015).

Cadmium is a metallic element and in periodic table placed in group II B (relative atomic mass: 112.41 and atomic number: 48). This element normally exists in white silver and soft form. It is normally not present in pure form in the environment it usually forms complex oxides with copper ore, lead, zinc, carbonates and sulfides. This metal cannot be detected by taste and odor. Cadmium sulphate and cadmium chloride are more soluble in water than cadmium oxide. Cadmium (Cd) is a heavy metal contaminant in the environment. Extensive data suggest Cd is the most toxic heavy metal and it is included in the black list of several international agreements established to regulate the input of Cd into the environment. Cd can enter the human food chain through plants, smoking materials, and diet. Cd is carcinogenic, embryotoxic, teratogenic, and mutagenic and may cause hyperglycaemia, reduced immunopotency, and anaemia, due to its interference with iron metabolism (Rafatullah*et al*., 2014). Cadmium is known to bind with essential respiratory enzymes causing oxidative stress and cancer.

Cadmium tolerant bacteria (CdtB) have evolved, and these have become crucial for bioremediation purposes. When the microorganisms consume waste, they convert the waste into nontoxic by products and in the process of this conversion they actually produce many metabolites to degrade the complex waste into simple compounds this is because microorganisms have developed many tolerant mechanisms to survive the presence of toxic heavy metals in their environment. Among the mechanisms developed by microbes include, metal sorption, extracellular precipitation uptake and accumulation, mineralization and enzymatic oxidation or reduction to a non-toxic form, and efflux of heavy metals from the cell (Halimoon*et al*., 2015).

They remove cadmium ions via complexation by exopolysaccharides, adsorption to cell surfaces, binding with bacterial cell envelopes, biosynthesis of metallothioneins, intracellular accumulation, precipitation, transformation to volatile compounds and formation of other proteins that trap the cadmium. Microorganisms play an important role in removal of cadmium from the environment. In the past years many studies have been carried out for the isolation of cadmium resistant bacterial strains. In this regard, Abbas, 2014 reportd that cadmium accumulation ability among bacteria e.g, *Pseudomonas putida, Escherichia coli, Pseudomonas aeruginosa, Pseudomonas syringae, Pseudomonas fluorescens, Comamonas testosterone, Staphylococcus aureus, Alcaligenes eutrophus, Gluconobacteroxydans, Bacillus subtilis, Staphylococcus lugdunensis, Alcaligenes xylosoxidans, Ralstoniametallidurans, Lactobacillus plantarum, Serratia liquefaciens, Klebsiella planticola, Paenibacillus sp. and Bacillus thuringiensis* have been studied the most.

The current low world market price of cadmium motivates the development of new applications that by time may develop into new sources of emissions to the environment not covered by existing regulation. Therefore, decontamination of these pollutants through bioremediation process and other biotechnological means are prerequisite for any future decision by the governments.

MATERIALS AND METHODS

Sample collection and analysis

Effluent sample was collected aseptically in a sterilized screw cap bottle from the crocodile pond of the West Coast Paper Mill. Elemental and physiochemical parameters like BOD, COD, Dissolved Oxygen, Carbon dioxide, pH, temperature were analyzed.

Isolation and screening of heavy metal-resistant bacteria

Bacteria were isolated from industrial effluent by spread plate technique. The collected effluent was spread on the agar plates (Luria Bertani) using spreader. Plates containing bacterial cultures were incubated at 37°C for 24h. Another set of agar was prepared by adding test heavy metal (Cd 0.01g/100ml). After the incubation bacterial colonies having variable morphology were selected to obtain their pure culture.4 colonies having different morphological features are selected and the resulting bacterial colonies were purified by further subculturing in the same media through the streak plate method.

Effect of cadmium on bacterial growth

Effect of Cd^{2+} on the growth of bacterial strain was determined by growing in the presence (0.025 1mM Cd^{2+}) as well in the absence (control) of Cd^{2+}. Growth in each culture was observed every 4 h by taking absorbance of an aliquot (1mL) at 600nm. Growth curves were plotted with time verses absorbance. Resistance of bacterial isolate against heavy metal was determined by growing it in MS broth supplemented with metal ions. Stock solutions of 1mM concentration of heavy metal ions salts.

Physical and biochemical characterization

Bacterial isolate was identified on the basis of colony morphology, gram staining and different biochemical tests such as IMViC tests, catalase test, oxidase test, Starch hydrolysis test, Indole test, etc.

Molecular characterization

For molecular characterization 16SrRNA gene was amplified through polymerase chain reaction (PCR).

Evolutionary analysis by Maximum Likelihood method the evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model.

Determination of optimum growth conditions

Optimum growth conditions of bacterial isolate were determined with respect to temperature and pH. To determine optimum temperature, isolate was grown in LB broth at different incubating temperatures by taking pH constant and to determine optimum pH broths of different pH were taken.

Antibiotic sensitivity

Assessment of antibiotic resistance heavy metal-resistant bacteria associated with antibiotic resistance were tested employing disc diffusion assays using Nutrient agar media. For this test, two antibiotics [Ciprofloxacin and Cephalexin] were selected that have wide-spectrum application.

RESULTS AND DISCUSSION

Physicochemical characteristics of industrial effluent

Some physicochemical properties of industrial effluent were measured at the time of sampling. Temperature of the sample was 27°C, pH was 7.2. The other characters were tested in the laboratory such as Dissolved oxygen was 10.4mg/L. Biological oxygen demand was 10.2mg/L. Chemical oxygen demand was 26.1mg/Land Carbon dioxide was 12.58mg/L.

Table 1: Physicochemical analysis of the sample.

Sl. No.	Parameters	Result
1.	Temperature	27°C
2.	pH	7.2
3.	Dissolved Oxygen (DO)	10.4 mg/L
4.	Biological Oxygen Demand (BOD)	10.2 mg/L.
5.	Chemical Oxygen Demand (COD)	26.1mg/L
6.	Carbon dioxide (CO_2)	12.58 mg/L.

Elemental analysis of industrial effluent

Elemental analysis of heavy metals from effluents: Cadmium and lead were analyzed using atomic absorption spectroscopy, The concentration of cadmium was estimated to be 0.012 mg/l in the sample, hence we proceeded with for further analysis.

Table 2: Elemental analysis of the sample.

Sl. No.	Parameters	Units	Test method	Result
1.	Cadmium	mg/L	IS 3025 (Part 41)	0.012 mg/L
2.	Lead	mg/L	IS 3025 (Part 47)	-0.075

Isolation of Cadmium tolerant bacteria from the effluent sample

The LB (Luria-Bertani) agar media used for microbial isolation from industrial effluent samples. Most of the plates exhibit numerous bacterial colonies of varying sizes, shapes, and distributions, indicating a diverse microbial population present in the effluent.

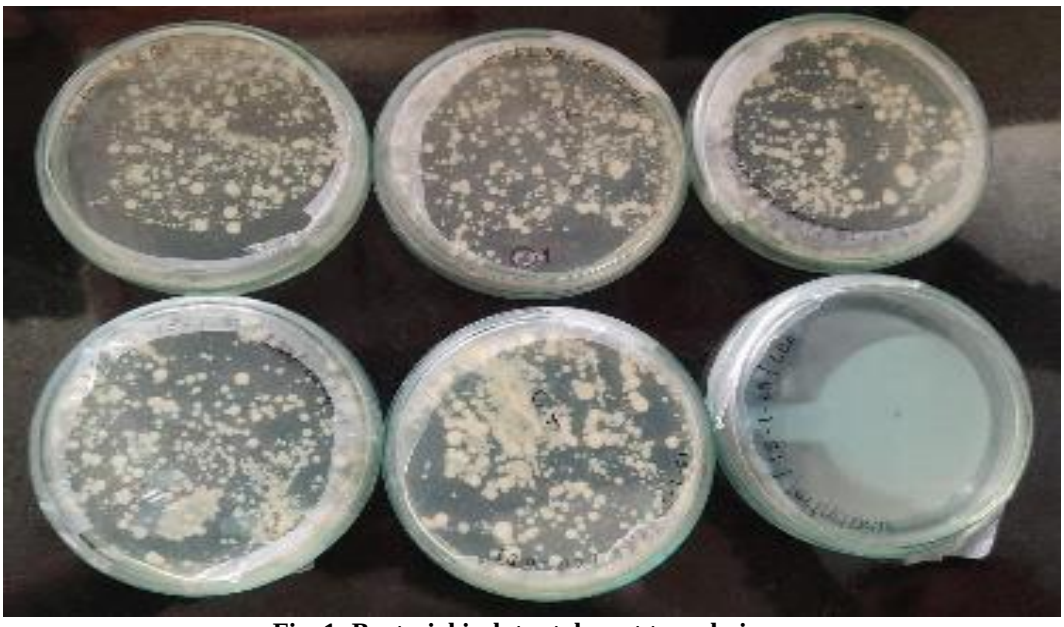


Fig. 1: Bacterial isolates tolerant to cadmium.

Screening of heavy metal tolerance bacteria

Based on the colony morphology four different isolates were observed and named as C1, C2, C3, C4 strains.

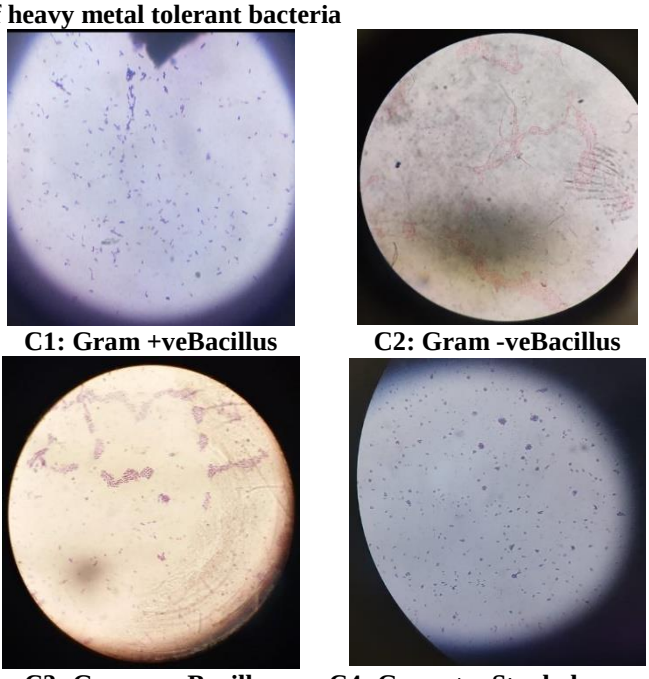
C1 **C2** **C3** **C4**

Fig. 4: Pure cultures of isolates tolerant to Cadmium.

Table 3: Colony Morphology of Bacterial Isolates.

Colony morphology	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Surface	Smooth	Smooth	Smooth	Smooth
Texture	Mucoid	Mucoid	Mucoid	Mucoid
Elevation	Raised	Slightly raised	Raised	Raised
Optical texture	Slightly convex	Slightly convex	Slightly convex	Slightly convex
Pigmentation	White	Creamish white	White	Translucent
Margin	Entire	Entire	Entire	Entire

Microscopic observation of heavy metal tolerant bacteria



C1: Gram +veBacillus **C2: Gram -veBacillus**

C3: Gram -ve Bacillus **C4: Gram +veStaphylococcus**

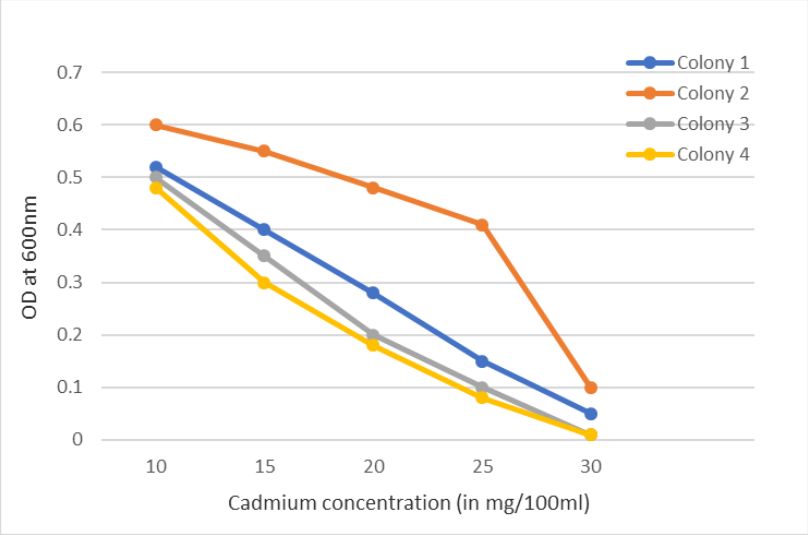
Fig. 4: Microscopic observation of the bacterial isolates.

Biochemical Characteristics of the bacterial isolates

Table 3: Biochemical characteristics of cadmium tolerant bacteria.

Biochemical test	Colony 1	Colony 2	Colony 3	Colony 4
Indole test	-	-	-	-
Methyl red test	-	-	-	+
Voges Proskauer test	+	+	-	+
Citrate utilization test	+	+	+	+
Catalase test	+	+	+	+
Oxidase test	+	-	+	-
Starch hydrolysis test	+	+	-	-
Urease test	+	+	+	+
Cellulose test	+	+	+	-

Based on the macroscopic, microscopic and biochemical characterization, the organisms were classified upto genus level as *Bacillus, Klebsiella, Pseudomonas* and *Staphylococcus*.



Graph 1: Optimization of cadmium.

In the optimization of cadmium tolerance, the growth response of four bacterial colonies was analyzed. Among them, the C2 colony exhibited the highest tolerance to cadmium stress. Growth was observed in the C2 colony up to a concentration of 25 mg/100 ml of cadmium. In contrast, the other three colonies showed reduced or no growth at this concentration. This was confirmed through UV-Visible spectroscopy by measuring the optical density of bacterial cultures. The C2 colony maintained a higher absorbance value, indicating better growth under cadmium exposure. These findings suggest that the C2 colony possesses enhanced cadmium resistance compared to the other isolates.

Molecular identification of the Isolate

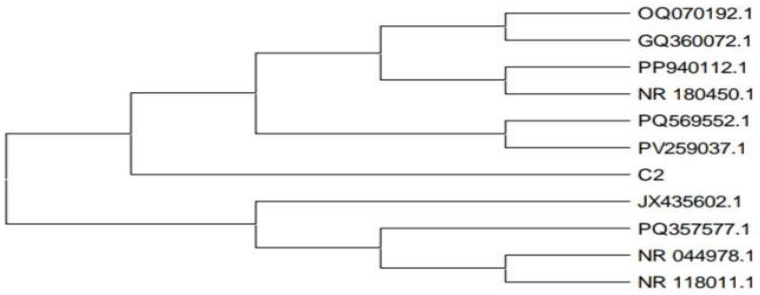
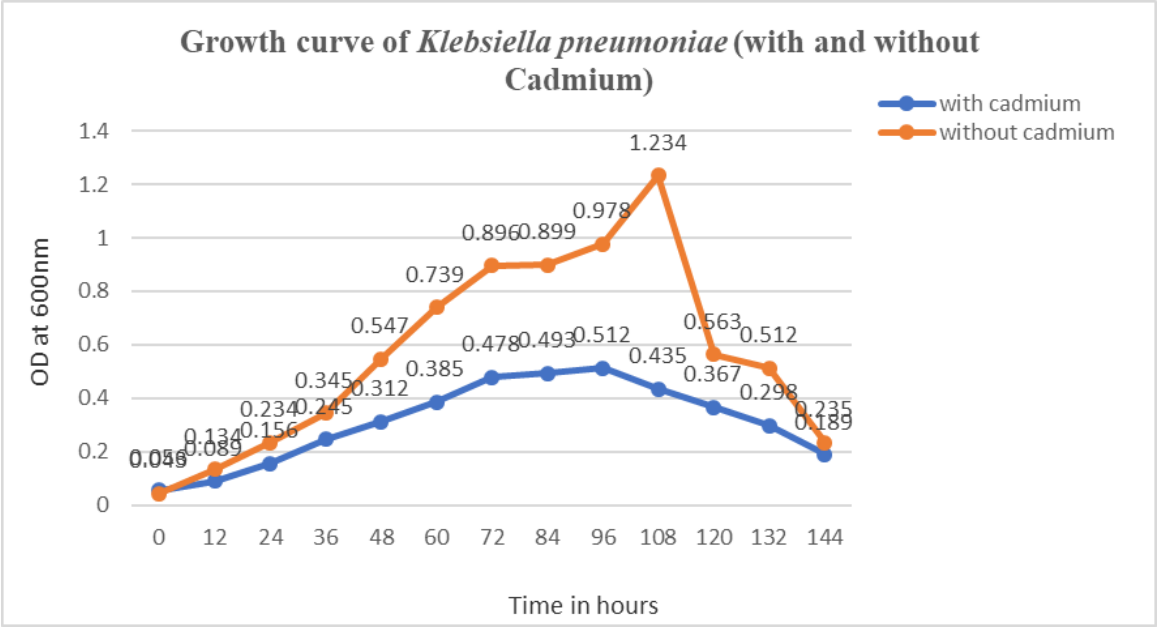


Fig. 6: Phylogenetic tree.

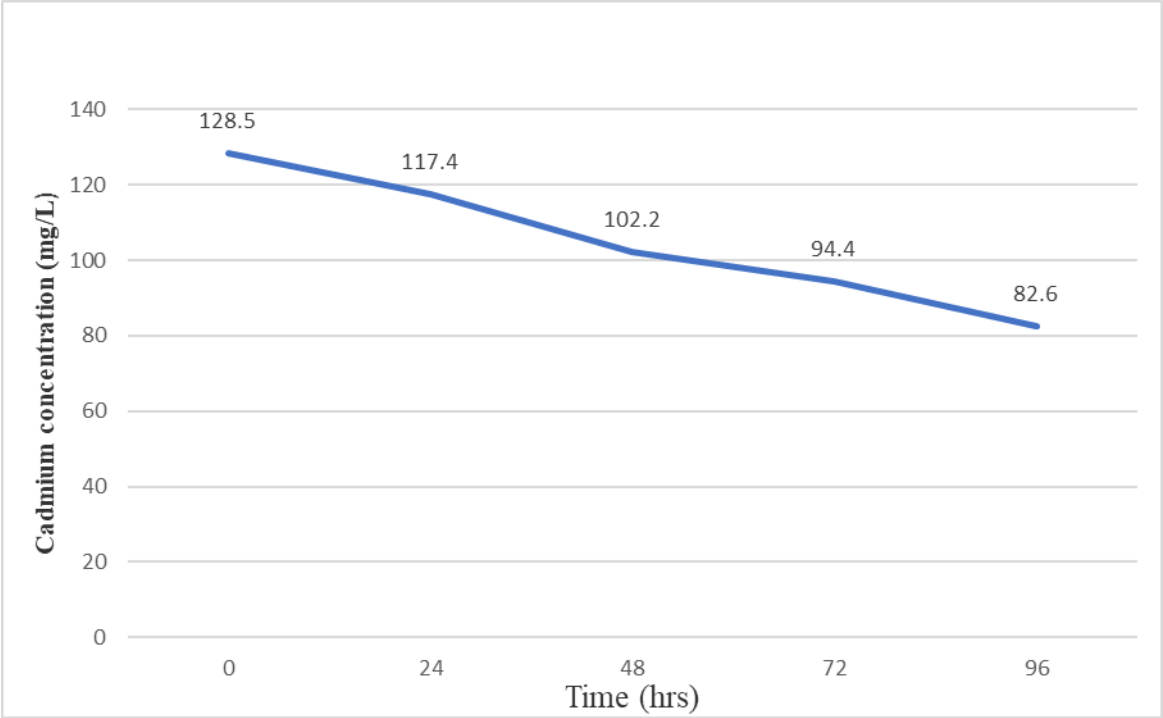
Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Klebsiella pneumoniae strain zg2010	2549	2549	99%	0.00%	99.36%	JX435602.1
Klebsiella pneumoniae strain BLKA1	2558	2558	100%	0.00%	99.29%	PQ357577.1
Enterobacter roggenkampii strain Mapoly Kareem-2024	2558	2558	100%	0.00%	99.29%	PQ569552.1
Enterobacter mori strain DW1	2555	2555	100%	0.00%	99.29%	OQ070192.1
Enterobacter sp. Pp9c	2555	2555	100%	0.00%	99.29%	GQ360072.1
Enterobacter asburiae strain 10A	2547	2547	100%	0.00%	99.15%	PP940112.1
Enterobacter asburiae strain 10B	2547	2547	100%	0.00%	99.15%	PP940111.1
Enterobacter cloacae subsp. dissolvens strain LMG 2683	2538	2538	100%	0.00%	99.01%	NR_044978.1
Enterobacter wuhouensis strain WCHEs120002	2536	2536	100%	0.00%	99.01%	NR_180450.1
Enterobacter cloacae subsp. dissolvens strain ATCC 23373	2532	2532	100%	0.00%	98.94%	NR_118011.1

Fig. 3: Sequences producing significant alignments.

Growth Curve of *Klebsiella pneumonia*



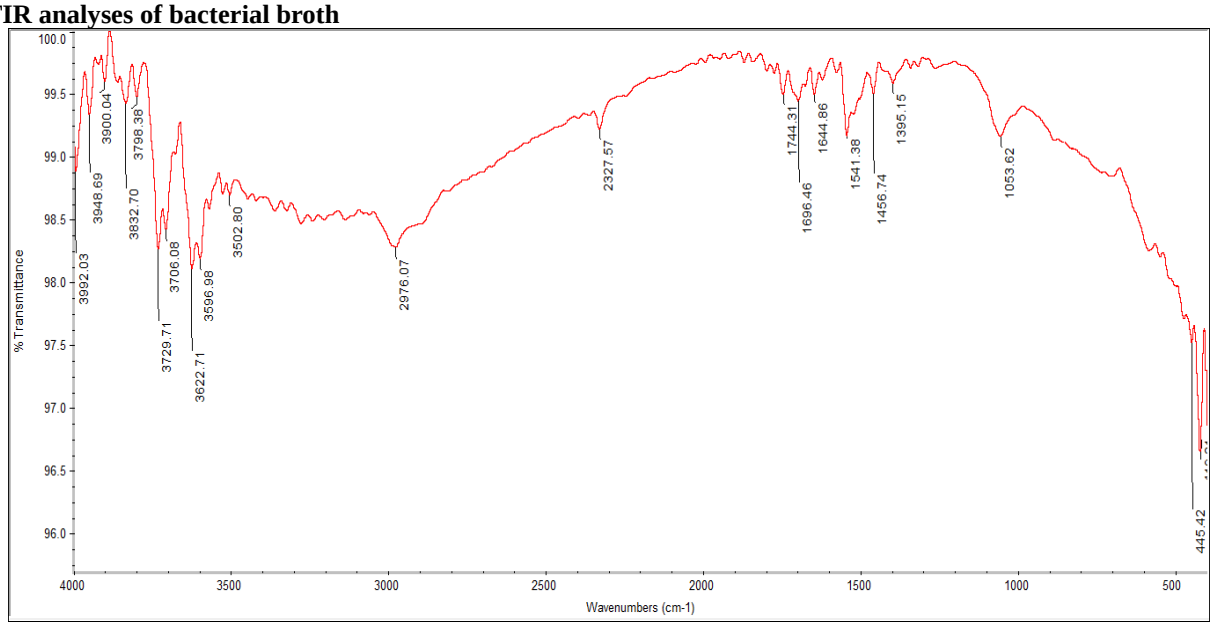
Graph 4: Growth phases of *Klebsiella pneumoniae* with and without cadmium.



Graph 7: Biosorption of cadmium by *Klebsiella pneumoniae* at different hours.

The tolerance of cadmium by the C2 colony was assessed over a period of 72 hours using Atomic Absorption Spectroscopy (AAS). Initially, the cadmium concentration in the broth was recorded at 128.5 mg/L at 0 hours. After 24 hours of incubation with the C2 colony, a slight decrease in cadmium concentration was observed, dropping to 117.4 mg/L. As the exposure period increased, a more significant reduction was noted, with the concentration reaching 102.2 mg/L at 48 hours. By the end of 72 hours, the cadmium concentration further decreased to 94.4 mg/L, indicating active uptake or biosorption by the bacteria. By the end of 96 hours, the cadmium concentration further decreased to 82.6 mg/L, indicating active uptake or biosorption by the bacteria. This progressive reduction demonstrates the metal tolerance and potential cadmium-removal ability of the C2 isolate. The trend observed suggests that the highest removal efficiency occurred between 48 and 72 hours. Overall, C2 shows promising potential for application in heavy metal bioremediation, particularly for cadmium-contaminated environments.

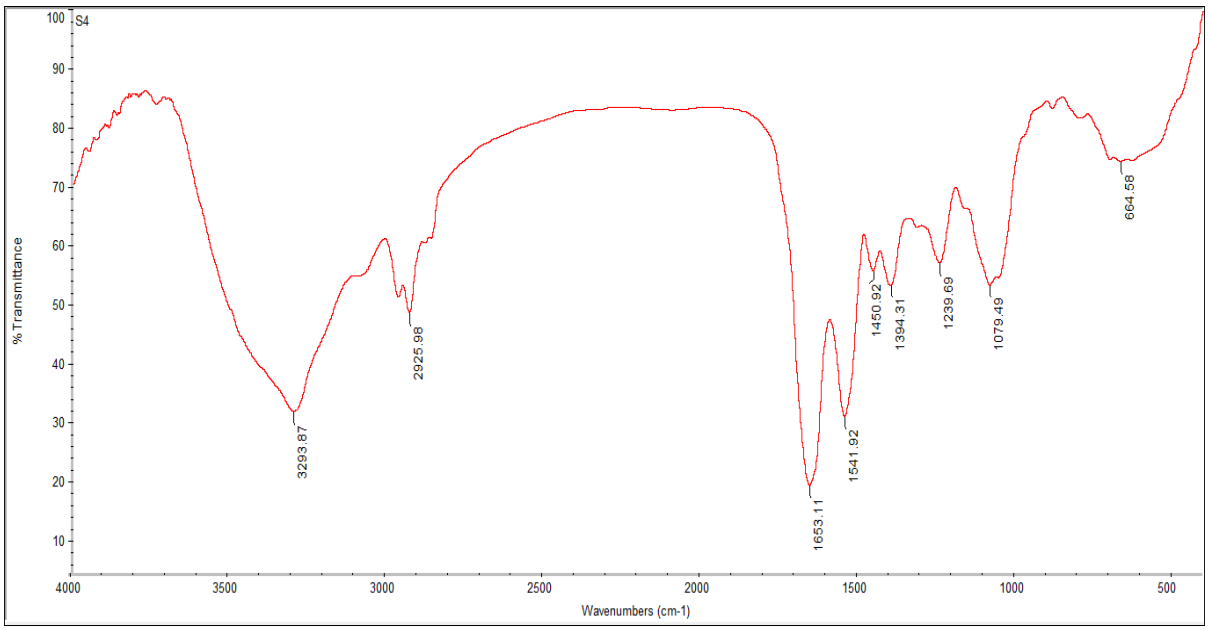
FTIR analyses of bacterial broth



Graph 8: FTIR chromatogram of minimal broth inoculated with C2 Control.

Table 8: FTIR spectral analysis of minimal broth inoculated with C2 Control.

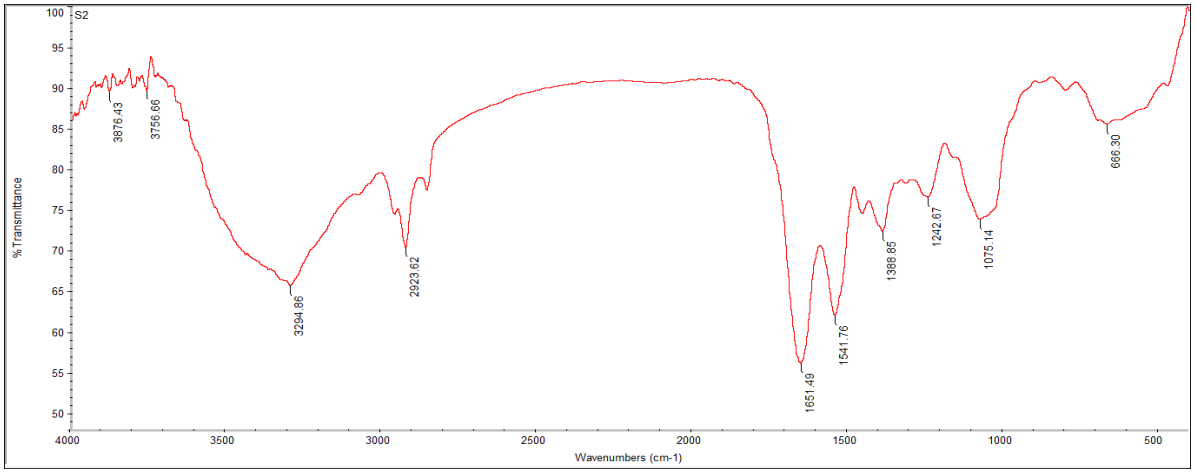
Absorption	Appearance	Group	Compound class
3622.71	Medium sharp	O-H (Streching)	Alcohol
3596.98	Medium sharp	O-H (Streching)	Alcohol
3502.8	Medium	N-H (Streching)	Primary amine
2976.07	Strong broad	O-H (Streching)	Carboxylic group
2327.57	Strong	O=C=O (Streching)	Carbon dioxide
1744.31	Strong	C=O (Streching)	Esters
1696.46	Strong	C=O (Streching)	Primary amide
1644.86	Strong	C=N (Streching)	Imine/ Oxime
1541.38	Strong	N=O (Streching)	Nitro compound
1456.74	Medium	C-H (bending)	Alkane
1395.15	Medium	O-H (bending)	Carboxylic acid
1053.62	Medium	C-N (Streching)	Amine



Graph 9: FTIR chromatogram of minimal broth inoculated with C2 after incubation at 24 hours.

Table 9: FTIR spectral analysis of broth inoculated with C2 (with cadmium, at 24 hours).

Absorption	Appearance	Group	Compound class
3294.86	Strong broad	O-H (Streching)	Alcohol
2923.62	Strong broad	N-H (Streching)	Amine salt
1651.49	Weak	C-H (bending)	Aromatic compound
1541.76	Strong	N-O (Streching)	Nitro compound
1388.85	Medium	C-H (bending)	Aldehyde
1242.67	Medium	C-N (Streching)	Amine
1075.14	Strong	C-O (Streching)	Primary alcohol
666.3	Strong	C-Cl (Streching)	Halo compound



Graph 8: FTIR chromatogram of broth inoculated with C2 (with cadmium, at 48 hours).

Table 9: FTIR spectral analysis of broth inoculated with C2 (with cadmium, at 48 hours).

Absorption	Appearance	Group	Compound class
3293.87	Strong broad	O-H (Streching)	Alcohol
2925.98	Strong broad	N-H (Streching)	Amine salt
1653.11	Strong	C=N (Streching)	Imide/ Oxime
1541.92	Strong	N-O (Streching)	Nitro compound
145092	Medium	C-H (bending)	Alkane
1394.31	Strong	S=O (Streching)	Sulphonyl chloride
1239.69	Strong	C-O (Streching)	Alkyl aryl ether
1079.49	Strong	C-O (Streching)	Primary alcohol
664.58	Strong	C-Cl (Streching)	Halo compound

FTIR measurement of the C2 isolate exposed to cadmium was conducted to identify the functional group associated with cadmium interaction. The FTIR absorption spectra of C2 isolate in the presence of cadmium revealed several characteristic peaks indicating various functional groups. The peaks from 3622.71 to 664.58 corresponds to multiple functional groups.

Scanning Electron Microscopy (SEM) analysis of Cadmium tolerant bacteria (*Klebsiella pneumoniae*)

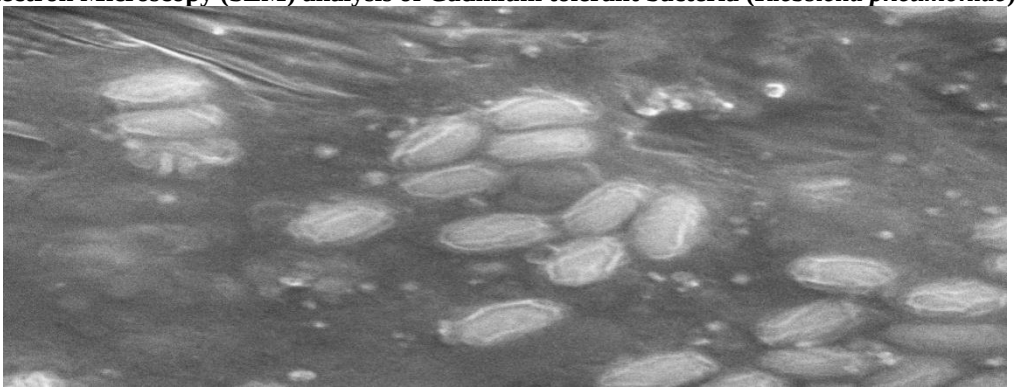


Fig. 14: Sem images of the *Klebsiella pneumonia.*

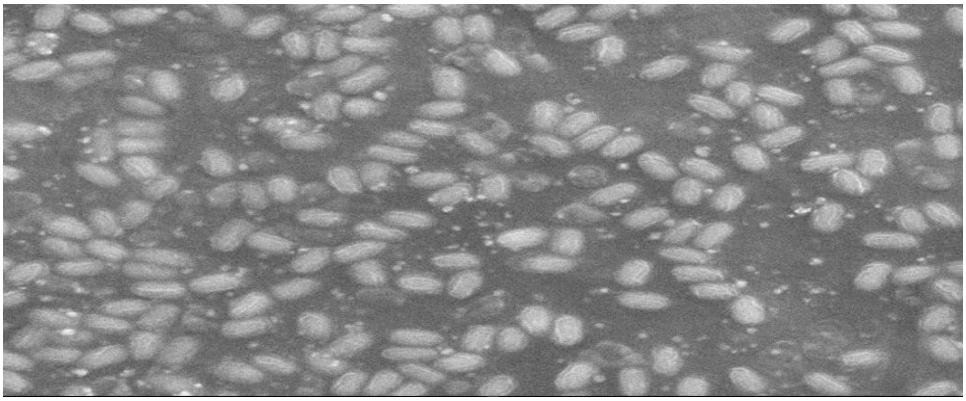


Fig. 15: SEM images of *Klebsiella pneumoniae* in the minimal media after 24 hrs of incubation.

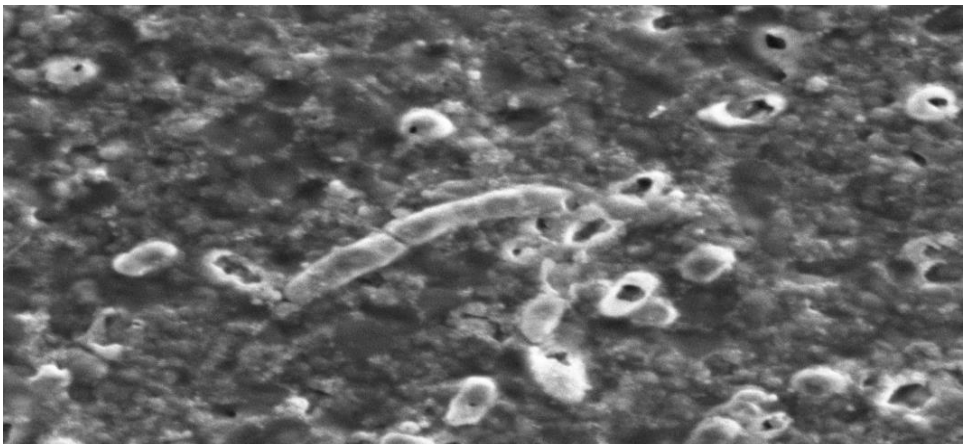


Fig. 16: SEM images of *Klebsiella pneumoniae* in the minimal media after 48 hrs of incubation.

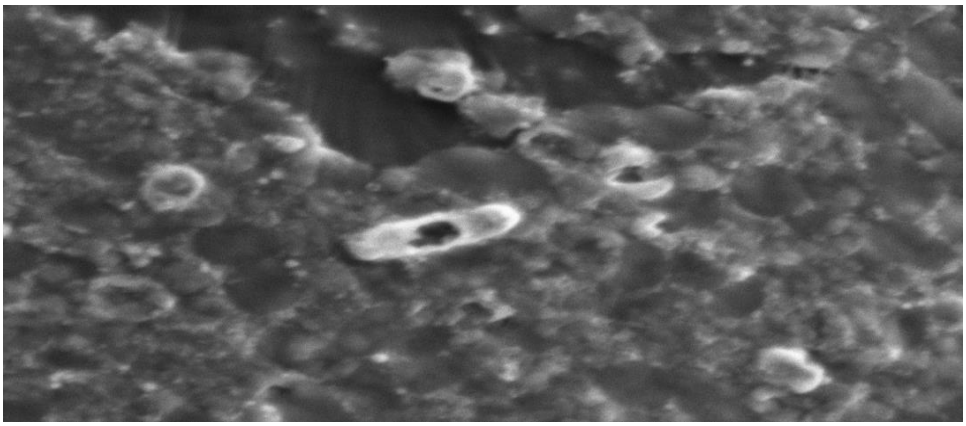


Fig. 17: SEM images of *Klebsiella pneumoniae* in the minimal media after 72 hrs of incubation.

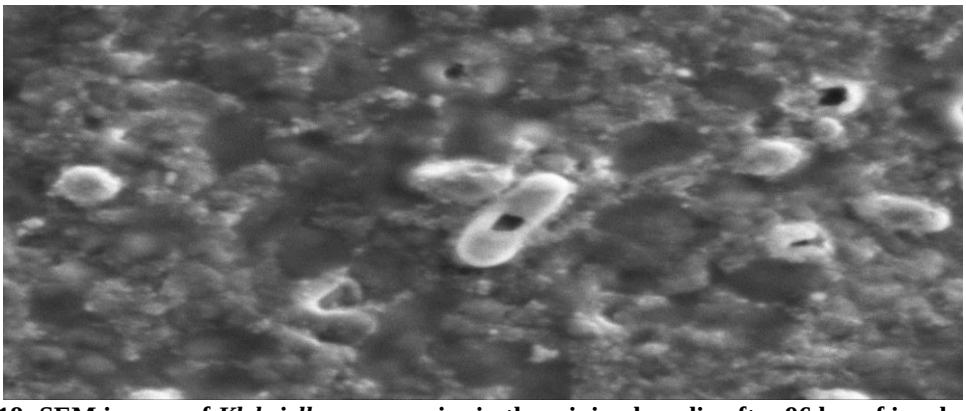
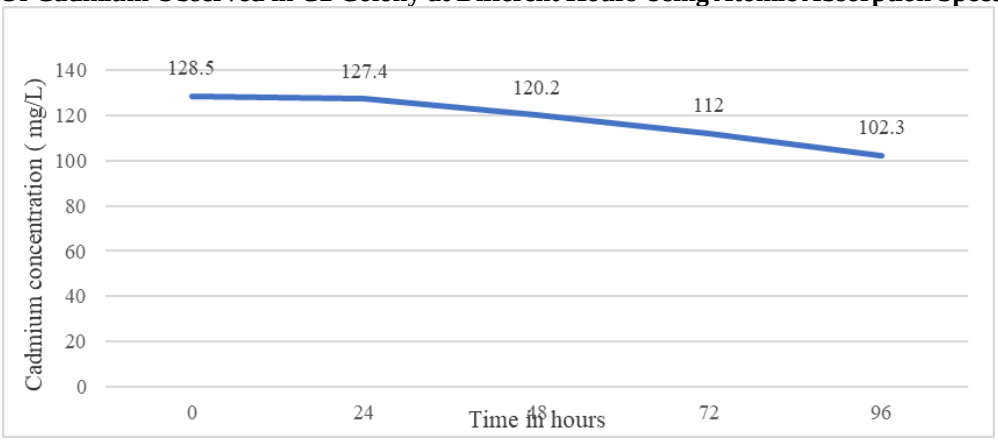


Fig. 18: SEM images of *Klebsiella pneumoniae* in the minimal media after 96 hrs of incubation.

Biosorption Of Cadmium Observed in C2 Colony at Different Hours Using Atomic Absorption Spectroscopy



Graph 7: Bisorption ability of *Klebsiella pneumoniae* at different hours.

The tolerance of cadmium by the C2 colony was assessed over a period of 72 hours using Atomic Absorption Spectroscopy (AAS). Initially, the cadmium concentration in the broth was recorded at 128.5 mg/L at 0 hours. After 24 hours of incubation with the C2 colony, a slight decrease in cadmium concentration was observed, dropping to 127.4 mg/L. As the exposure period increased, a more significant reduction was noted, with the concentration reaching 120.2 mg/L at 48 hours. By the end of 72 hours, the cadmium concentration further decreased to 112 mg/L, indicating active uptake or biosorption by the bacteria. This progressive reduction demonstrates the metal tolerance and potential cadmium-removal ability of the C2 isolate. The trend observed suggests that the highest removal efficiency occurred between 48 and 72 hours. Overall, C2 shows promising potential for application in heavy metal bioremediation, particularly for cadmium-contaminated environments.

Fig. 19: *Klebsiella pneumoniae* showing resistance to Azithromycin

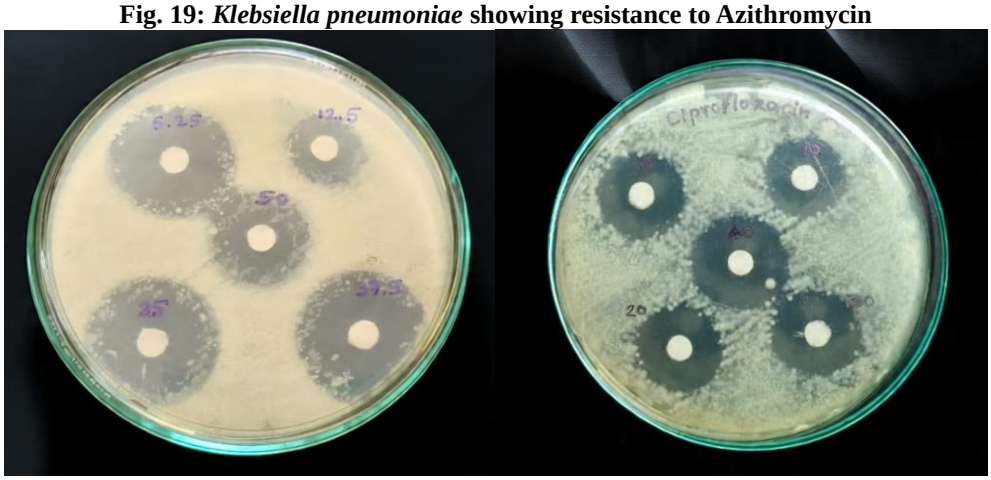


Fig. 20: *Klebsiella pneumoniae* showing sensitivity to Ciprofloxacin and Cephalexin

The study on the isolation and characterization of cadmium (Cd)- tolerant bacteria from industrial effluents provides valuable insights into the potential of microbial bioremediation for heavy metal pollution.

Heavy metal pollution of soil and wastewater is a significant environmental problem (Cheng *et al*., 2003). Wastewaters from the industries and sewage sludge applications have permanent toxic effects to human and the environment (Rehman *et al*., 2008). Cadmium (Cd) is a nonessential element, but poisonous for plants, animals and humans. Cadmium is one of the most toxic pollutants of the surface soil layer, released into the environment by mining and smelting activities, industries, incineration of plastics and batteries, land application of sewage sludge, and burning of fossil fuels (Tang *et al*., 2006).

The industries effluents are rich source of heavy metals and is likely that the bacteria residing in it must be resistant to heavy metals. The present study was therefore initiated with the aim to isolate and identify heavy metal, resistant bacteria from industrial effluent contaminated sites of Dandeli, Karnatak.

In most of the studies the heavy metal tolerant bacteria identified are Bacillus *sp*., *Aeromonas hydrophila*, *Exibacteriumprofundum*, *Bacillus cereus* and *Exiguobacterium sp*., (Pandit *et al*., 2022). *Staphylococcus aureus*, *Bacillus subtilis. Pseudomonas aeruginosa*, *Alcaligenes xia*, Citrobacter (Shim *et al*2015)., Proteus mirabilis (Isham *et al*, 2015). *Arthobacterviscous*, *Acidithiobacillusferrooxidants*, *Acidophiliumsymbioticum* (Chakravarty and Banerjee 2012), Bacillus cereus RC-1(Huang *et al*., 2014) *Lactobacillusrhamnosus*, LC-707, *Propionibacterium freundenreichii*, *Lactobacillus rhamnosus*, Tsukamurellapaurometabola A155, P aeruginosa B237 and C. taiwanensis E 324 which were isolated from zinc mines of Thailand could bioaccumulate the 16.89mg g^{-1} of cadmium (Limcharoensuk *et al*. 2015). However, the present study revealed the presence of four different isolates C1, C2, C3 and C4 which were tolerant to Cadmium and were identified based on its colony morphology, biochemical and morphological characteristics. All the four isolates were subjected to bioremediation of cadmium. Among the four isolates, the C2 strain demonstrated the most promising results, suggesting its potential significance in cadmium resistance and metabolic versatility. Based on this preliminary observation, the C2 colony was selected for further molecular identification employing 16S rRNA gene sequencing, a widely accepted and highly specific method for bacterial taxonomy and phylogeny. The 16S rRNA gene contains conserved and variable regions that allow for both universal primer binding and species-level differentiation (Janda and Abbott, 2007; Clarridge, 2004) The isolate underwent genomic DNA extraction, followed by PCR amplification of the16S rRNA gene. The presence of a single discrete band of approximately 1500bp confirmed successfully amplification. Sequencing using both forward and reverse primers yield high-quality reads, which were aligned to produce a consensus sequence. This sequence was then used for BLAST analysis against the NCBI GenBank database, a standard approach for comparing unknown sequence to known reference data (Altschul *et al*., 1990). The BLAST results showed a 99.29-99.36% similarity with several *Klebsiella pneumoniae* strains, particularly zg2010(JX435602.1) and BLKA1(PQ357577.1), strongly indicating the identity of the isolate as *K. pneumoniae* .Although similar identity values were observed with some Enterobacter species, further phylogenetic analysis and distance matrix comparisons using MEGA11 software confirmed that the closest evolutionary relationship was with *K. pneumoniae* (Kumar *et al.,* 2018) The phylogenetic tree constructed using the maximum likelihood method with the Tamura-Nei model showed that sample C2 clustered tightly with *k. pneumoniae*, supporting the BLAST findings. The small evolutionary divergence values (0.002-0.003) between C2 and reference *Klebsiella* strains further reinforce the identification.

The earlier studies reported that the *Enterococcus faecalis* isolated from petrochemical wastewater were involved in cadmium absorption. The different bacterial strains and their cadmium removal capacity varied with various experimental conditions. The current studies focused on to evaluate the cadmium bioremediation potential of the isolated *Klebsiella pneumoniae* strain (C2), a quantitative analysis of residual cadmium concentration was performed using Atomic Absorption Spectroscopy (AAS) over a period of 96 hours. These findings support the potential application of this strain in bioremediation of cadmium- contaminated environment.

The ability of microbial stains to grow in the presence of heavy metals would be helpful in the waste water treatment where microorganisms are directly involved in the decomposition of organic matter in biological processes for waste water treatment, because often the inhibitory effect of heavy metals is a common phenomenon that occurs in the biological treatment of waste water and sewage (Filali *et al*., 2000). In the present study high degree of heavy metals resistance associated with multiple antibiotic resistances was detected in industrial effluent bacteria.

The isolated *Klebsiella pneumoniae* C2 strain was subjected to antibiotic susceptibility testing using the standard paper disc diffusion method. Three antibiotics were tested: ciprofloxacin, cephalexin, and azithromycin. The results demonstrated clear zones of inhibition for ciprofloxacin and cephalexin, indicating that the strain was sensitive to these antibiotics. However, no zone of inhibition was observed for azithromycin, suggesting a lack of sensitivity or possible resistance. The absence of azithromycin activity could not be fully explained in our initial investigation. However, based on available

literature, *Klebsiella pneumoniae* is known to exhibit intrinsic resistance to macrolide antibiotics such as azithromycin. This resistance is often attributed to the bacterium's outer membrane barrier and the presence of efflux pumps that prevent effective intracellular accumulation of the drug. (Das *et al*., 2006). These factors likely contributed to the observed lack of azithromycin efficacy against the C2 strain in our study.

The number of antibiotics used in the experiment restricted the ability to compare a broader resistance profile particularly when contrasted with *Pseudomonas aeruginosa,* which was tested with a wider range of antibiotics in parallel studies. The limited antibiotic panel used for K. pneumoniae may have affected the comprehensiveness of our resistance analysis *P. aeruginosa* is inherently resistant to many antimicrobial agents, mainly due to the synergy between multi-drug efflux system or a type1 AmpC β-lactamase and low outer membrane permeability. (Das *et al*., 2006). Antibiotic susceptibility was confirmed by disk diffusion technique on Muller-Hinton medium (Becton Dickinson Microbiological Systems, Cockysville, MD), performed according to the Clinical Laboratory Standard Institute (CLSI) guidelines (Gencer*setal*,.] Quality control strains of *Pseudomonas species* NCTC-10662 was used to validate the results of the antimicrobial discs. Among the aminoglycosides, amikacin has the highest sensitivity against *Klebsiella pneumoniae* , which is in corroboration with an earlier report published from India (Mohanty 2006) Amikacin was designed as a poor substrate for the enzymes that bring about inactivation by phosphorylation, adenylation or acetylation. The present results are in line with earlier suggestion made by Fontaine and Hoadley, (2000) that combined expression of antibiotic resistance and metal tolerance may not be a chance phenomenon but rather the results of selection by metals present in an environment. It has also been asserted that heavy metals, disinfectants, antibacterials and antimicrobial – all can select for different kinds of bacteria, including those resistant to lifesaving antibiotics (Moken *et al*., 2007).

FT-IR analysis of cadmium resisting bacterial cells in the presence and absence of heavy metal salts (cadmium chloride) showed strong adsorption behaviour of certain microorganism towards metal ions is a function of the chemical structures present on the biomass. The Fourier Transform Infra-Red (FT-IR) analysis of the bacteria is required to know the chemical bonds that played a role in the adsorption of metal. Alcohol and carboxylic acid groups (O-H stretching at ~3300 cm^{-1} and ~2976 cm^{-1}) were prominent across all sample, but the broadening of peaks in S1 to S4 indicates hydrogen bonding or metal ion complexation possibly due to cadmium interaction with hydroxyl or carboxyl groups on bacterial cell walls. Peaks at ~1650-1653 cm^{-1} correspond to C=N or amide groups, which showed slight intensity variation in cadmium- treated samples, potentially indicating alteration in protein conformation or expression response to cadmium stress. The presence of strong N-O stretching peaks (~1540cm^{-1}) in all spectra implies consistent nitro compound features, but their shift or increased intensity in cadmium- exposed samples may reflect a stress induced upregulation of nitro- reductase pathways, as seen in metal detoxification systems (Gadd, 2000). The FT-IR analysis revealed substantial biochemical shifts in functional group expressions between cadmium-exposed and unexposed bacterial samples. The emergence or disappearance of characteristic peaks, particularly involving hydroxyl, amide, nitro, and ether groups, demonstrates that cadmium- resistant bacteria undergo functional and structural adaptation, likely involving metal chelation, protein modification, and surface polysaccharide interaction mechanism, our observation align with previous findings indicating that cadmium- resistant bacteria secrete metal-binding biomolecules, including protein, peptides, and polysaccharides to neutralize toxic effects (Rajkumar *et al.*, 2010).

The present study utilizes scanning electron microscopy (SEM) to observe the morphological changes in *Klebsiella pneumoniae*. The SEM image, captured at magnifications ranging from x7000 to x13000, reveal distinct morphological alterations indicative of cellular stress in the cadmium-treated groups. Morphological characteristics without cadmium exposure, *Klebsiella pneumoniae* exhibited typical short rod-shaped cells with smooth, intact surfaces and a relatively uniform size distribution. The bacterial cells appeared healthy, densely packed, and well structured, which is consistent with normal physiological conditions. These observations are aligned with previous studies showing that *K. pneumoniae* forms regular rods in optimal growth environment (Podschun and Ullmann, 1998). In contrast, cadmium-exposed samples showed notable morphological changes: irregular and elongated shapes, pitting or shrinkage of the cell surface, formation of lysed structure, Reduced density and aggregation of cells. These changes reflect membrane damage, intracellular stress, and possible leakage of cytoplasmic content, suggesting cytotoxic effects of cadmium. Cd ions are known to induce oxidative stress, inhibit enzymatic functions, and disrupt membranes integrity in gram- negative bacteria (Bruins *et al*., 2000) in similar studies on *Pseudomonas aeruginosa,* the organism has shown relatively greater resistance to cadmium toxicity. SEM images of *P. aeruginosa* under cd stress typically exhibit: minor distortion in shape. Maintenance of cellular integrity longer than *k. pneumoniae*. Enhanced biofilm formation as a protective mechanism. This enhanced tolerance in pseudomonas is attributed to its efficient efflux systems (e.g., CzcBA system) for heavy metal ions. Siderophores production aiding in metal chelation. Robust oxidative stress response and quorum sensing system (Hauser, 2009; Teiztel *et al*., 2009). In contrast K. pneumoniae lacks similarly efficient metal detoxification mechanisms, rendering it more susceptible to cadmium-induced stress.

In the present study, *Klebsiella pneumonia* was identified as the most potent strain resistant to cadmium demonstrating Cd adsorption and removal at Ph7 and temperature of 37^{o} C. (Qian *et al*., 2022) reported *Bacillus cereus* strains C9 and C27 as highly resistant to cadmium and demonstrated their significant potential for Cd removal. The findings align with previous research that highlights the potential of *Bacillus* species in remediating heavy metal contamination through multiple mechanisms, including adsorption, metal binding, and siderophore production and that the mechanisms underlying the cadmium resistance of these strains are multifaceted. Renu *et al*. (2022) reported that cell wall modifications also play a crucial role. The cell walls of *Bacillus cereus* strains C9 and C27 might be modified to bind cadmium ions more effectively, preventing them from entering the cytoplasm and causing damage.

Strains C9 and C27 achieved peak Cd adsorption at moderate concentrations (70 µM) and acidic pH (4.5), with strain C9 demonstrating a slightly higher adsorption efficiency than strain C27. These results are consistent with previous studies, which reported optimal Cd adsorption for *Bacillus cereus* in acidic conditions (pH 6.5–7.0), due to the protonation of functional groups on the bacterial surface, enhancing electrostatic attraction to Cd ions (Todorova *et al*., 2019). However, adsorption efficiency declined at higher concentrations (90 µM), possibly due to site saturation and competitive interactions, a phenomenon also noted by Zhao *et al*. (2017). Compared to *Bacillus megaterium*, which reached a maximum Cd adsorption of 1.3 mg/g (Fang *et al*., 2016), strain C9 exhibited higher adsorption, highlighting its potential as a more effective bioremediatory. The adsorption kinetics also mirrored findings in *Bacillus thuringiensis*, where extended incubation improved Cd removal rates up to 36–48 h, as reported by Çolak *et al*., (2011). Moreover, the decline in adsorption efficiency could also be related to the cadmium resistance mechanisms of the strains. When cadmium concentrations are extremely high, the strain may activate additional detoxification mechanisms such as the production of stress proteins (Khan *et al*., 2016). These proteins can bind to cadmium ions in the cytoplasm, reducing their free concentration and toxicity. This process, however, might divert the strain's resources from the adsorption process, leading to a decrease in adsorption efficiency. Microorganisms can rapidly interact with extracellular metals and play an important role in metal tolerance through metal dispersion, transport, immobilization, and transformation (Lu *et al*., 2023).

The effect of initial pH on cadmium uptake by strains C9 and C27 was studied within the pH range of 6.0 to 7.0. Cd uptake significantly decreased as pH increased, the maximum Cd removal for both strains was observed at pH 7.5, suggesting it as the optimal pH for biosorption. The reduction in Cd uptake with increasing pH is attributed to the competition between metal ions and hydroxide ions (OH^{-}), which can result in the precipitation of metal hydroxides and decrease the availability of Cd. At lower pH, the increased concentration of hydrogen ions (H^{+}) enhances metal ion binding, improving biosorption (Wen *et al*., 2018) Further research is needed to explore the mechanisms behind this enhanced biosorption. This pattern, with rapid initial adsorption followed by stabilization, is typical of biosorption processes, as seen in other studies like those on Pb^{2+} by *Bacillus* species (Çolak *et al*., 2011). The initial rapid uptake is driven by the availability of binding sites, while the stabilization reflects the exhaustion of these sites and the attainment of equilibrium.

While many studies have reported Cd resistance and adsorption capabilities in *Bacillus* species, exhibited unique advantages, including higher adsorption capacities, significant siderophore activity, and robust salt tolerance. For instance, Junpradit *et al*. (2021) reported Cd removal efficiencies of up to 24% in *E. cloacae*, which is lower than the maximum removal rate of 32.27% observed for strain C27. Moreover, *Bacillus cereus* strains studied by Zhou *et al*. (2024) showed comparable resistance to Cd. Our results highlight the competitive edge of *Klebsiella pneumoiae* for application in bioremediation, particularly in multi-metal contaminated environments. Additionally, their physiological versatility makes them suitable for diverse ecological conditions, further expanding their practical utility. Future research should focus on unravelling the genetic and molecular mechanisms underlying their metal resistance and optimizing their application in field-scale remediation projects. Incorporating these strains into bioremediation systems could further enhance their efficacy by leveraging various microbial interactions.

CONCLUSION

The successful isolation and characterization of heavy metal-resistance bacteria from industrial effluents underscore the immense potential of indigenous microbial populations in bioremediation application. The morphological, biochemical, and structural adaptations observed in response to heavy metal stress, particularly cadmium, affirm metal the resilience and versatility of this organism. Based on the characterization of these strains and comparisons of their 16S rDNA sequences, the organism was identified as *Klebsiella pneumonia*. These findings not only contribute to our understanding of microbial resistance mechanism but also open eco-friendly solutions for industrial wastewater treatment. Future studies focusing on genomics and proteomic profiling, as well as pilot-scale application, are warranted to further harness these bacterial strains for environment sustainability and pollution control.

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