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Genomic analysis of heavy metal-resistant *Enterobacter sp.* PGRG2 isolated from electronic waste of NCR region, India

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Abstract

Heavy metal pollution has come under increased scrutiny, due to its bioaccumulation and highly persistent nature. Conventional techniques for remediating heavy metals from soil may release additional toxic compounds into the environment. Therefore, the method of remediation using microorganism and plants is being preferred now over other physio-chemical methods. In this study, interaction between metals and microbes was studied for removing lead (Pb), cadmium (Cd) & nickel (Ni) from heavy metal contaminated soil. The main objective of this investigation was to isolate, identify and assess the soil microbes having the potential for remediation of lead, cadmium & nickel at high concentration. PGRG2 was isolated from E-waste contaminated site and its tolerance against lead, cadmium & nickel were determined on nutrient agar. Genomic sequencing of the organism was performed with the Illumina Novaseq600 platform. PGRG2 tolerated 1000ppm of Pb, 750ppm of Cd & 200ppm of Ni. The genome revealed genes responsible for metal transport/resistance and genes involved in EPS production. This study highlights the tolerance capacity of isolated bacteria and the predicted genes involve in transportation and resistance of lead, cadmium, nickel and other heavy metals present in the genome of PGRG2.

Keywords: Bioaccumulation, Environment, Heavy metal, Microbes, Remediation, Resistance

Introduction

Increase in development and urbanization is directly proportional to the increment in the pollution in the developing countries (Hao et al., 2020). This is because some sectors in developing countries do not follow proper precautions and protocol during the use or disposal of some hazardous and toxic substances (Ferronato and Torretta (2019)). The contamination of heavy metals, petroleum hydrocarbons, antibiotics, plastics and many more is spreading in the environment rapidly because of which the concentration of contaminants in soil, water and air is increasing continuously (Naidu et al., 2021). From soil, water and air these toxic components are also reaching to the living organisms which result in various kind of misfunctioning in the physiological functions and tissue damage in humans and plants (Alengebaww et al., 2021). The spreading of these harmful substances in the environment is posing a critical threat (Siddiqua et al., 2022). In order to fight this threat, there is a need of some cleanup strategies which are less expensive, environment friendly and easy to apply. One such strategy for this purpose is the remediation of contaminated environment is by using plants and microorganisms (Bala et al., 2022).

This study addresses the issue of heavy metal contamination in the soil. Heavy metal contamination is a significant issue as it is a non-biodegradable material and can cause harm to the environment (Mitra et al., 2022). All heavy metals are harmful above the permissible limit but lead, cadmium and nickel are recognized as dangerous heavy metals among all the metals present in E-waste. The emission of Pb, Cd and Ni into the environment is an escalating global problem, presenting serious health hazards, especially for children. Intake of these metals can cause behavioral problems, cognitive disorders, hearing impairment, and permanent brain damage in children (Olufemi et al., 2022). In adults, exposure of Pb can cause detrimental effect on heart, reproductive system and blood pressure. Lead has a half-life of approximately 30 days in blood, whereas its half-life in bones ranges from 20 to 30 years (Collin et al., 2022). Chronic exposure to nickel and its compounds can lead to their accumulation in the human body, potentially causing various health issues, including lung fibrosis, kidney and cardiovascular diseases, and respiratory tract cancer (Genchi et al., 2020).

It is therefore important to remediate lead and cadmium contaminated soil. Various physio-chemical technologies like landfill, chemical precipitation, excavation, thermal treatment, etc. are being commonly used for this purpose (Priya et al., 2023). Due to certain limitations like low rate

of remediation and high cost these methods are not preferred. Further, these treatments are not eco-friendly and can damage soil (Igolima et al., 2022). In-contrast, bioremediation can be regarded as a possible substitute for the remediation of lead from the soil. Due to the rapid pace of mutation and evolutionary adaptability, the application of microorganisms for lead toxic elimination has been used for their adaptable existence in extremophilic conditions (Mathivanan et al., 2021). Remediation using microbes is abundantly used as it is a cost effective and environment friendly option even when used on a large scale (Wang et al., 2022). This method provides stability over wide pH and temperature range, also minimizes the generation of waste created during detoxification process of contaminated soil (Romantschuk et al., 2023).

Techniques for bioremediation using bacteria are: - biosorption, bioaccumulation, bioleaching, biotransformation, biomineralization, biodegradation as well as by altering the genetic composition of microbes using genetical modification techniques (Pande et al., 2022). Microorganisms like bacteria, fungi, and algae adapt themselves to manage heavy metals, and convert harmful heavy metal configurations into safer alternatives that can persist into the environment without harming it (Avangbenro and Babalola (2017)). Numerous bacterial species are recognized for their ability to remediate heavy metals, with *Pseudomonas sp.* W6 being particularly noted for its lead biosorption capabilities (Kalita and Joshi (2017)). *Kleibsilla pneumoniae* Kpn555 obtained from coffee waste residue were tested for the tolerance capacity and biosorption activity of lead and was found to tolerate up to 900 mg/L of lead (Martis et al., 2021). Similarly, it is reported that *K.michiganensis* R19 can be used to recover lead (Pb) ions from multi-metal solution (Shameer 2016).

This study sought to isolate and identify bacterial strains with the potential to remediate Pb, Cd and Ni pollution in soil. The biochemical features were used to characterize the strains. Remediation potential and effect of pH on remediation potential of selected strains were also studied. Further, a genome of new strain *Enterobacter sp.* PGRG2 was investigated for the presence of genes involve in heavy metal resistance.

Materials and methods

Soil sample collection

The soil samples were procured from areas around electronic product manufacturing companies/factories and nearby scrap heaps. Three E-waste contaminated localities of Delhi and nearby areas of NCR (National Capital Region) were selected. The study site I was Mandoli Industrial Area, situated in the northeast zone of Delhi, India. For more than a decade this area has been prominently used for informal or unauthorized recycling of E-waste. Site II was the dump yard near Jhilmil Industrial Area, which is at the eastern border of Delhi, NCR. This is the well-known center for the manufacture of automobiles and electronic products. Site III was the area around Loni Industrial Area, Ghaziabad, Uttar Pradesh.

Heavy metal selection

The heavy metals selected for study were identified through soil sampling at targeted sites. Soil samples were analyzed for heavy metal concentrations using ICP-Ms. These concentrations were then compared against permissible limits established by WHO (World Health Organisation) and other relevant organisations (Table 1) (Al-Boghdady, 2019 and Ibrahim, 2018).

Table 1. Concentration of Heavy metals (ppm) in soil samples of selected site and permissible limit of metals from WHO.

Heavy metal (ppm)	WHO	Mandoli Industrial Area	Jhilmil Industrial Area	Loni Industrial Area
Pb	100	2620.31	116	1506.31
Ni	80	125.45	375.50	256.2
Cu	30	129.61	8.67	94
Cd	3	6.7	47.60	96.1
Cr	100	115.50	48.8	56.5
Zn	300	76.84	81.6	115

Isolation of heavy metal tolerant bacterial strains

Soil samples were diluted in 50ml of nutrient broth for overnight at 37℃ and 120 rpm. Subsequently, dilutions (10^{-6} to 10^{-10}) were spread over nutrient agar plate, spiked with Pb $(NO_3)_2$ (100 mg/kg), $CdCl_2.H_2O$ (100 mg/kg) & Ni $(NO_3)_2.6H_2O$ (100 mg/kg), separately and incubated at 37℃ for 48 hours. Morphologically different and healthy colonies were purified by replating on NB agar. Then MIC was determined by spreading pure isolates on increasing concentration of Pb (500ppm-1000ppm), Cd (100ppm-750ppm) & Ni (100ppm-400ppm). On the basis of MIC results, isolates mentioned in Table 2,3,4 were able to tolerate heavy concentration of Pb, Cd & Ni. Only two bacterial strain which were named as 2L and 5J were found to tolerate up to 1000ppm of lead, 750 ppm of Cd and 200ppm of Ni. These two strains were considered to be the potential bacterial strains for further studies over lead, cadmium and nickel remediation. (For further studies isolates 2L and 5J were named as PGRG2 and PGRG5) (Alfarras and Hamid (2022)).

Performance assessment of bacterial strains for bioremediation of selected heavy metals

For remediation studies, 50ml of nutrient broth initially mixed with 1000 ppm of Pb $(NO_3)_2$, 750ppm of $CdCl_2.H_2O$ and 200ppm of Ni $(NO_3)_2.6H_2O$. This medium was further inoculated with 1% of overnight grown culture PGRG2 (7.6×108 CFU/ml) and PGRG5 (5.24×108 CFU/ml), separately. Medium without culture were taken as control and all the flasks were incubated at 37℃, 120 rpm for 72 hours. Samples were retrieved after 24,48 and 72 hours and centrifuged at 7000×g, 20 min, 4℃. In this study, the supernatant was digested and concentration of heavy metals remained in the media was analyzed using Inductively Coupled Plasma Mass Spectrometry (Agilent ICP-Ms 7850). For digestion, samples were digested by the microwave assisted digestion system; 8:1 ratio of nitric acid and hydrogen peroxide were subjected to microwave assisted digestion. After digestion, samples were transferred into the clean vial and diluted to the desired volume with milli-q water and filtered before ICP-Ms analysis (Cerveira et al., 2020). Remediation of metals was further calculated using Equation 1 (Bestawy et al., 2013).

$$\%Remediation\ of\ metals = \frac{Initial\ Conc.of\ metal - Final\ Conc.of\ metal}{Initial\ Conc.of\ metal} \times 100 \quad \text{Equation (1)}$$

Table 2. Screening of isolates for tolerance capacity on varying concentration of Pb.

Name of Isolate	Concentration of Lead (ppm)						
	500	650	700	800	900	950	1000
I3M	+++	-	-	-	-	-	-
I4M	+++	+++	+++	-	-	-	-
I5M	+++	+++	+	-	-	-	-
I6M	+++	+++	+++	-	-	-	-
I7M	+++	+++	+	-	-	-	-
I8M	+++	+++	-	-	-	-	-
I9M	+++	-	-	-	-	-	-
I10M	+++	-	-	-	-	-	-
I11M	+++	+++	+++	-	-	-	-
I1J	+++	+++	++	++	++	-	-
I3J	+++	+++	+++	++	++	-	-
I5J	+++	+++	+++	+++	+++	+++	++
I1L	+++	+++	+++	+++	+++	+++	-
I2L	+++	+++	+++	+++	+++	+++	+++

(+++ indicates good growth, ++ indicates moderate growth, + indicates very less growth, - indicates no growth).

Table 3. Screening of isolates for tolerance capacity on varying concentration of Cd

Name of Isolate	Concentration of Cadmium (ppm)								
	100	150	200	300	400	500	600	700	750
I3M	-	-	-	-	-	-	-	-	-
I4M	-	-	-	-	-	-	-	-	-
I5M	-	-	-	-	-	-	-	-	-
I6M	-	-	-	-	-	-	-	-	-
I7M	-	-	-	-	-	-	-	-	-
I8M	-	-	-	-	-	-	-	-	-
I9M	-	-	-	-	-	-	-	-	-
I10M	+++	-	-	-	-	-	-	-	-
I11M	-	-	-	-	-	-	-	-	-
I1J	+++	+++	+++	+++	+++	-	-	-	-
I3J	+++	+++	+++	++	+++	+++	-	-	-
I5J	+++	+++	+++	+++	+++	+++	+++	++	++
I1L	+++	+++	+++	+++	+++	+++	-	-	-
I2L	+++	+++	+++	+++	+++	+++	+++	++	++

(+++ indicates good growth, ++ indicates moderate growth, + indicates very less growth, - indicates no growth).

Table 4. Screening of isolates for tolerance capacity on varying concentration of Ni.

Name of Isolate	Concentration of Nickel (ppm)				
	50	100	150	200	250
I3M	-	-	-	-	-
I4M	-	-	-	-	-
I5M	-	-	-	-	-
I6M	-	-	-	-	-
I7M	-	-	-	-	-
I8M	-	-	-	-	-
I9M	-	-	-	-	-
I10M	+++	-	-	-	-
I11M	-	-	-	-	-
I1J	+++	+++	-	-	-
I3J	+++	+++	-	-	-
I5J	+++	+++	+++	+++	+
I1L	+++	+++	+++	+++	-
I2L	+++	+++	+++	+++	-

(+++ indicates good growth, ++ indicates moderate growth, + indicates very less growth, - indicates no growth).

Sem analysis

In order to further determine the bio-adsorption mechanism, the normal and stressed cells were analyzed using SEM. For SEM analysis, bacterial cells cultured with stress and without stress were fixed using glutaraldehyde (2.5% v/v) for 3 hours. This was followed by washing of cells with 0.1M phosphate buffer thrice (each washing for 15 minutes). Cells were then fixed with 1ml of 1% (w/v) tannic acid and glutaraldehyde for 8 hours at room temperature. The samples were subsequently washed twice with PBS and dehydrated using 100% ethanol. The dried pellets underwent analysis using FE-SEM (FEI Nova Nano FESEM 450) to observe morphology and surface topography changes in both metal-treated and control bacterial cells (Zhang, 2019 and Shen, 2021).

Identification of potential bacterial strains

Molecular analysis for PGRG2 and PGRG5 was conducted at Barcode Bioscience, Bangalore. DNA was extracted from bacterial strains cultured on nutrient agar. The isolated segment of the 16S rRNA gene underwent amplification using the BDT v3.1 Cycle sequencing kit, followed by analysis using the ABI 4730xl Genetic Analyzer. After amplification with 16S rRNA-F and 16S rRNA-R primers, a 1500-bp amplicon was observed in the positive control, while no amplicon was visible in the negative (no DNA) control. The test amplicon was purified to eliminate contaminants. Following purification, the amplified product underwent Sanger's DNA sequencing method. Subsequently, the sequence data were compiled and aligned with publicly available sequences, followed by an identity analysis using the NCBI Database (Thakur and Gauba (2021)).

Whole genome analysis

Whole-genome analysis of the bacterial strains was conducted at Eurofins Genomics India Pvt. Ltd., Bengaluru, India. Genomic DNA from both strains was extracted using the Quick-DNA Miniprep Kit (Zymo Research). DNA quality and quantity were assessed using NanoDrop to determine the A260/280 ratio, followed by agarose gel electrophoresis for confirmation. Molecular identification of the extracted DNA samples targeted the bacterial 16S region using the Sanger sequencing technique. PCR amplification of the bacterial 16S region produced a single discrete amplicon band on agarose gel. Illumina TruSeq Nano DNA Library Prep Kit was used to prepare the paired-end sequencing library of the QC passed genomic DNA samples. Initial quality control confirmed the suitability of all prepared libraries for sequencing on the Illumina Novaseq6000 platform, generating 2x150 bp reads (Thakur and Gauba (2022)). Raw sequence data underwent processing with Trimmomatic v0.28 to remove adapter sequences, ambiguous reads, and low-quality sequences (reads with less than 25 phred score) (Muñoz-García et al., 2022). High-quality reads from both samples were aligned to the reference genome utilizing BWA MEM (version 0.7.17), and consensus sequences were derived using Samtools mpileup. Samtools (v 0.1.18) mpileup utility was used to identify SNPs and InDels from the sorted BAM files of the mapping process, applying a minimum read depth of 15 and a quality threshold of 25 for variant filtering. Identified variants were annotated using bedtools intersect tool (Elarabi et al., 2023). Annotated sequences of both strains were submitted to NCBI GenBank.

Genome Functional Annotation

Functional annotation of genes was conducted using tools and databases including the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) and the BV-BRC tool (PATRIC). The Rapid Annotations using Subsystems Technology toolkit (RASTtk) within PATRIC version 3.6.9 facilitated further annotation (Thakur and Gauba (2023)). The PATRIC tool version 3.6.9 was employed to generate the circular genome representation. Default parameters were applied for genome functional annotation.

Genome-wide prediction and screening of heavy metal resistance genes in PGRG2

This study assesses about the heavy metal resistant genes present on the genome of PGRG2. The search for genes involved in heavy metal remediation within the genome of PGRG2 began with an extensive survey of the literature. The confirmation of the presence of these genes were done using the results obtained from BV-BRC tool (Patric) (Thakur and Gauba (2023)) and NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Zaghloul et al., 2024). Further, BacMet-Scan command line tool was used to check total number of confirmed genes available on the genome of PGRG2 which are involved in heavy metal remediation. For this, all the translated proteins of PGRG2 were compare to the protein sequence of the metal resistance genes available at BacMet database with the help of “Protein-protein BLAST (blastp)”. For blast, only experimentally confirmed genes present on the BacMet-Scan (version 2.0) software was used. Rather than relying solely on predicted resistance genes databases, this study utilized the experimentally confirmed database from BacMet (Tseng, 2023 and Lan, 2023). This approach facilitated comparisons between current findings and previous literature, ensuring robustness and reliability in this investigation. There are total 753 experimentally confirmed resistance genes out of which 420 genes are responsible for heavy metal resistance in the BacMet database (version 2.0).

Results and Discussion

Investigation of Pb and Cd tolerant bacteria

Samples from sites I, II and III were subjected to agar plates which were separately mixed with Pb (100 ppm), Cd (100 ppm) & Ni (100 ppm). From three soil samples total 22 colonies were found to grow on heavy metals subjected plates. To obtain pure bacterial strains, these distinctive isolates were streaked again. Out of 22 isolates 14 isolates were seem to be pure and appeared different from each other. The gram-staining study clearly revealed that all the isolates were gram negative and are different from one another. These 14 colonies were further, subjected to higher concentration of selected metals and tested for minimum inhibitory concentration (MIC) of selected metals. Table-2,3 & 4 shows the growth of isolates from site I, II & III on increasing concentration of Pb (500-1000 ppm), Cd (100-750 ppm) & Ni (100-400 ppm), respectively. As shown in Table-2, 3 &4, 5J obtained from Jhilmil industrial area and 2L from Loni industrial area were able to grow on 950ppm-1000ppm of Pb, 750ppm of Cd & 200 ppm of Ni. The screening results thus confirmed that isolates PGRG5 (5J) and PGRG2 (2L) are capable of tolerating high concentration of Pb, Cd & Ni.

Bioremediation of Pb & Cd by selected strains

The two bacterial strains which showed tolerance against higher concentration of selected metals were selected to further check their metal remediation potential. 1% inoculum of each isolate was inoculated in 50ml nutrient broth separately spiked with lead, cadmium and nickel salts and incubated at 37°C for 72hrs and 120 rpm. After incubation, samples were digested and the concentration of each metal left in the sample was measured using ICP-Ms along control. The remediation potential of each strain was calculated using equation 1. PGRG2 and PGRG5 showed remediation of 62.86% and approximately 55%, respectively under lead stress. While PGRG2 and PGRG5 showed remediation of 29.97% and 30.06%, respectively with cadmium stress. In case of nickel both the strains showed ~45 % of remediation in 24hour, at neutral pH (Figure 1& 2).

The growth of bacterial strains along with heavy metals may depend upon pH. The role of pH is crucial in bacterial activities, influencing both bacterial growth and its capacity for heavy metal remediation (Oziegbe et al., 2021). As a result, when the revalidation study was carried out using

PGRG5 and PGRG2 at different level of pH it was observed that pH level also affects the remediation potential of bacterial strains in some cases. As shown in Figure 3, in case of Pb it was observed that there existed no significant disparity in the remediation potential of PGRG5 at different pH conditions. It approximately showed 58% reduction at pH 5,7 and 9 but in case of Cd it was observed that PGRG5 is able to remediate better at pH 9 as compared to lower pH levels (Figure 4). In case of PGRG2 there was 52.25% reduction of Pb in 72hrs at pH 5, while at pH 7and 9 there was approximately a reduction of 60% in 24hrs. (Figure 3), which indicates that remediation potential of PGRG2 is better at neutral and alkaline pH. But in case of Cd PGRG2 showing better remediation potential at pH 9 (Figure 4). PGRG5 showed 53% of remediation at pH5 in presence of nickel stress, which indicates that PGRG5 can remediate nickel more efficiently at pH 5.

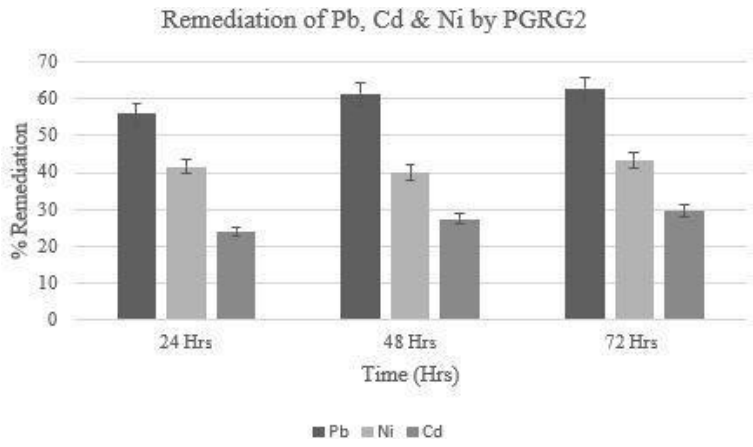


Figure 1. Remediation potential of PGRG2 for Pb, Cd & Ni.

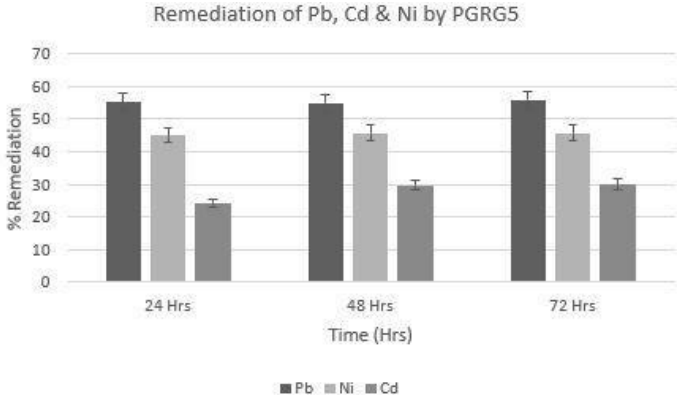


Figure 2. Remediation potential of PGRG5 for Pb, Cd & Ni.

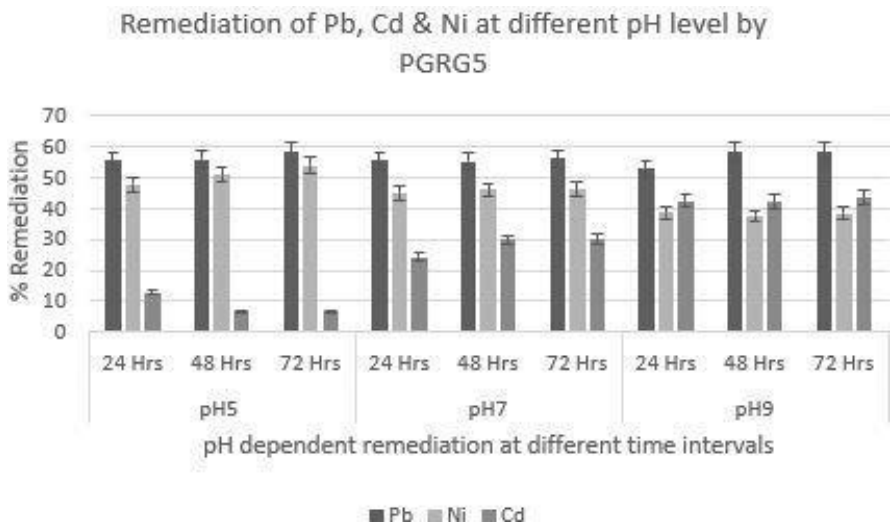


Figure 3. Remediation potential of PGRG5 for Pb, Cd and Ni at different pH.

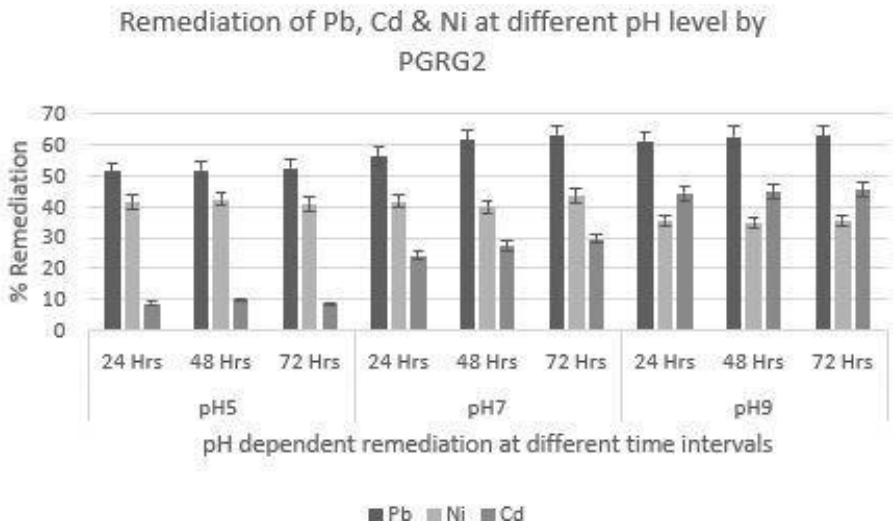


Figure 4. Remediation potential of PGRG2 for Pb, Cd and Ni at different pH.

SEM results

It was seen that due to heavy metal exposure morphology of bacterial cells change. By reducing the attachable cell surface relative to the entire cell plane, it may be possible to mitigate the negative effects of heavy metals through a decrease in the cell surface to cell volume fraction. In an unfavorable condition like heavy metal exposure, the cells sustain with higher cell volume than cell surface (Igiri et al., 2018).

SEM analysis of heavy metal treated cell and non-treated cells were compared and it was observed that surface of treated cells was rough which indicate the presence of exopolysaccharide (EPS) matrix over the cell surface. It was observed that each metal induced different cell morphology. Figure 5 show the SEM images of PGRG2 bacterial cell without treatment. While, figure 6 shows the presence of EPS in the form of spherical microcolonies and fibrous network-like structure in the presence of Ni and Pb stress. While in case of Cd stress substantial amount of EPS production was observed due to which a matrix or web-like structure that obscures the underlying bacterial cells. In case of no stress, a rod shape smooth surface SEM image of PGRG5 was obtained (figure 7) while in case of Pb stress a very rough and fibrous network like image of bacterial cell was obtained which confirms the presence of EPS over the cell. In case of Cd stress, a very irregular and rough surface over the cell was observed. A very hazy appearance over a cell surface and spherical microcolonies was observed over PGRG5 with Ni stress (figure 8).

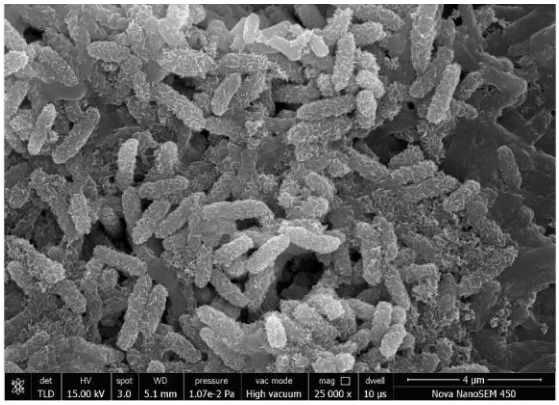


Figure 5. SEM image of PGRG2 without any stress

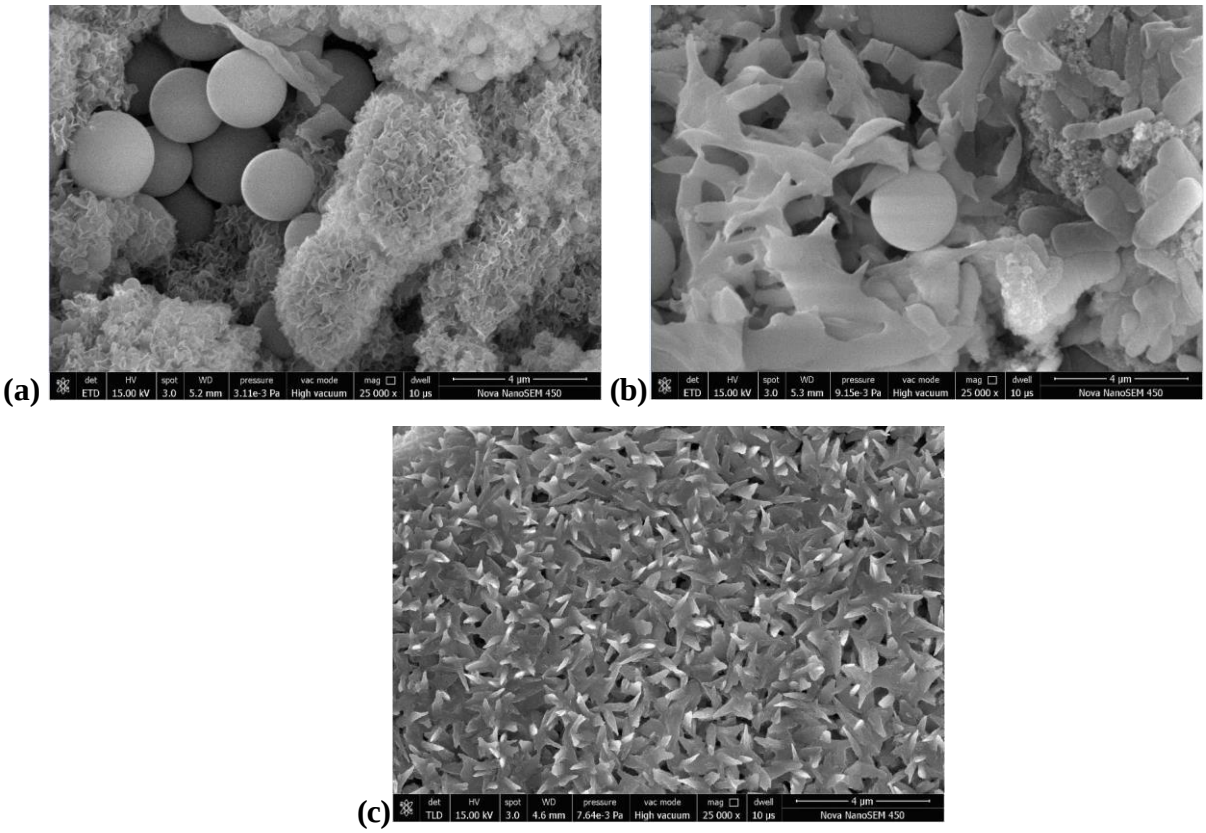


Figure 6. SEM image of PGRG2 with (a) Ni (b) Pb and (c) Cd

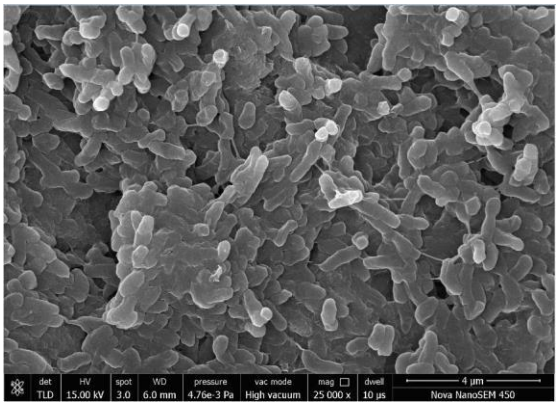


Figure 7. SEM image of PGRG5 without any stress

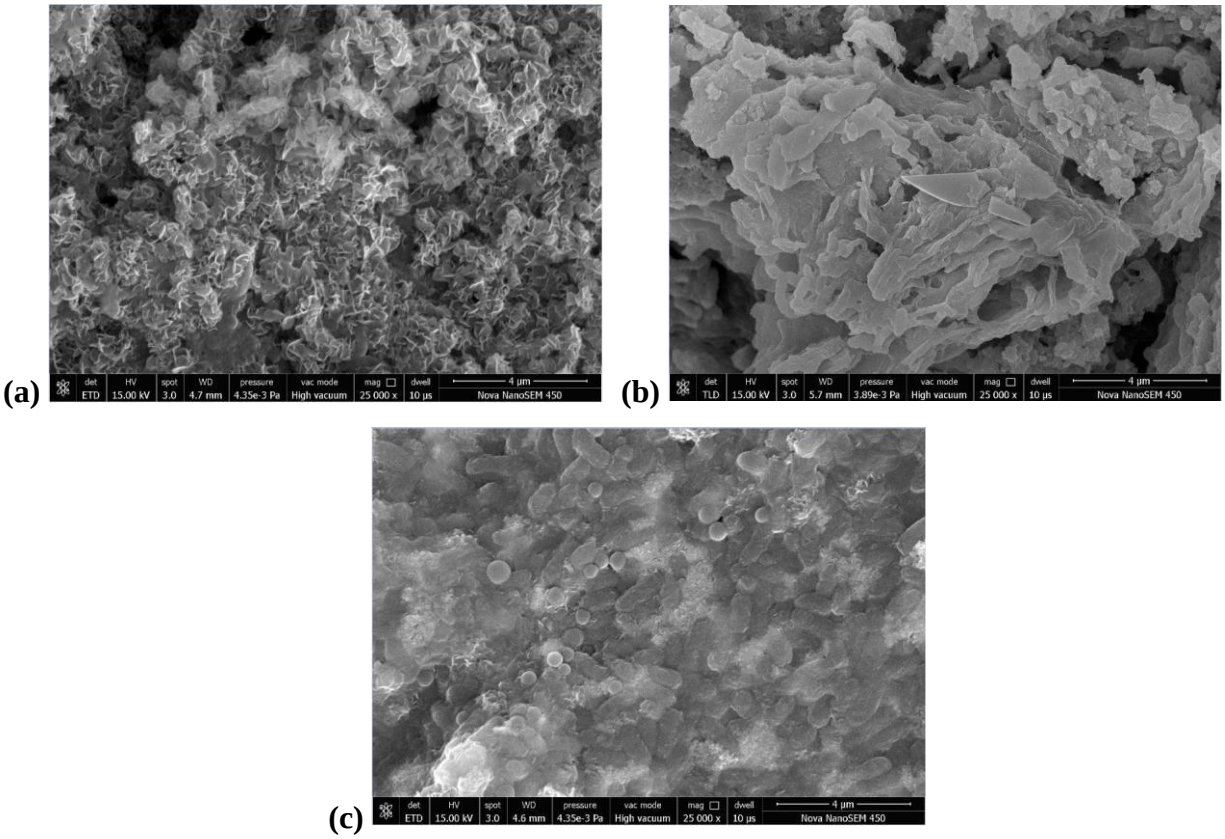


Figure 8. SEM image of PGRG5 with (a) Ni (b) Pb and (c) Cd

Molecular characterization of PGRG5 and PGRG2.

The results of the molecular identification indicated a genetic similarity of 99.73% between PGRG5 and *Achromobacter insolitus* LMG 6003. Furthermore, PGRG2 exhibited a 99.62% homology with the *Enterobacter sp*. SA187 (a novel specie within the family Enterobacteriaceae), as observed through alignment to their partial 16S rDNA gene sequences by aligning with sequences available in the NCBI database through the utilization of the Mega 11 software. Hence based on molecular characterization, PGRG 5 was named as *Achromobacter insolitus* PGRG5 and PGRG2 was named as *Enterobacter sp.* PGRG2. Figure 1 & 2 depicts the phylogenetic distance of *Achromobacter insolitus* PGRG5 and *Enterobacter sp*. PGRG2 from other reported species of genus *Achromobacter* and *Enterobacter*. Figure 9 &10 depict the phylogenetic tree that was derived from 16S rRNA sequencing data using a neighbour- joining tree.

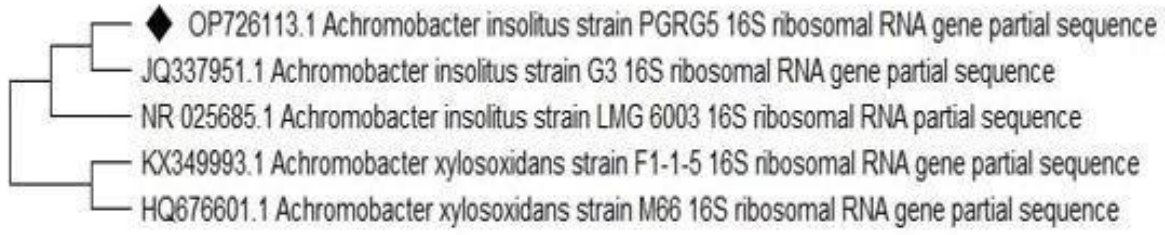


Figure 9. Phylogenetic tree of *Achromobacter insolitus* PGRG5 constructed using MEGA11

Figure 10. Phylogenetic tree of *Enterobacter sp.* PGRG2 constructed using MEGA11

General genomic feature of PGRG5 & PGRG2

Whole genome analyses of both strains were conducted to characterize genes involved in heavy metal remediation. The genome of *Achromobacter insolitus* PGRG5 was assembled into 186 contigs totaling 6,428,890 bp, with an average G+C content of 65.5%. Annotation revealed 5,762 genes, including 5,689 protein-coding sequences (CDSs), as well as 13 rRNA and 56 tRNA genes. Similarly, *Enterobacter sp.* PGRG2 genome assembly resulted in 229 contigs totaling 4,411,285 bp, with an average G+C content of 56.5%. Annotation identified 3,913 genes, including 3,801 CDSs, along with 22 rRNA and 84 tRNA genes. Detailed genome characteristics of strains PGRG5 and PGRG2 are summarized in Table 5. The circular genome representations are depicted in Figures 11 and 12.

Table 5. General genomic features of PGRG2 & PGRG5 strain

Feature	PGRG2	PGRG5
Size of Genome (bp)	4,411,285	6,428,890
Contigs	229	186
N_{50} contigs size (bp)	48800	136100
G+C content (%)	56.5	65.5
RNA genes	112	73
No. of rRNAs	22	13
No. of tRNAs	84	56
Total genes	3,913	5,762
CDSs (total)	3,801	5689
Coding genes	3,772	5,670
Pseudo genes	29	19
Accession number	CP127837	CP129898
Bio Project	PRJNA981674	PRJNA979826
Bio Sample	SAMN35682530	SAMN35579937

Figure 11. Annotations of the PGRG2 strain's genome are presented in a circular plot. Gene cluster classifications are color-coded across the sequenced genome, with each circle description progressing from the outermost to the inner most circle.

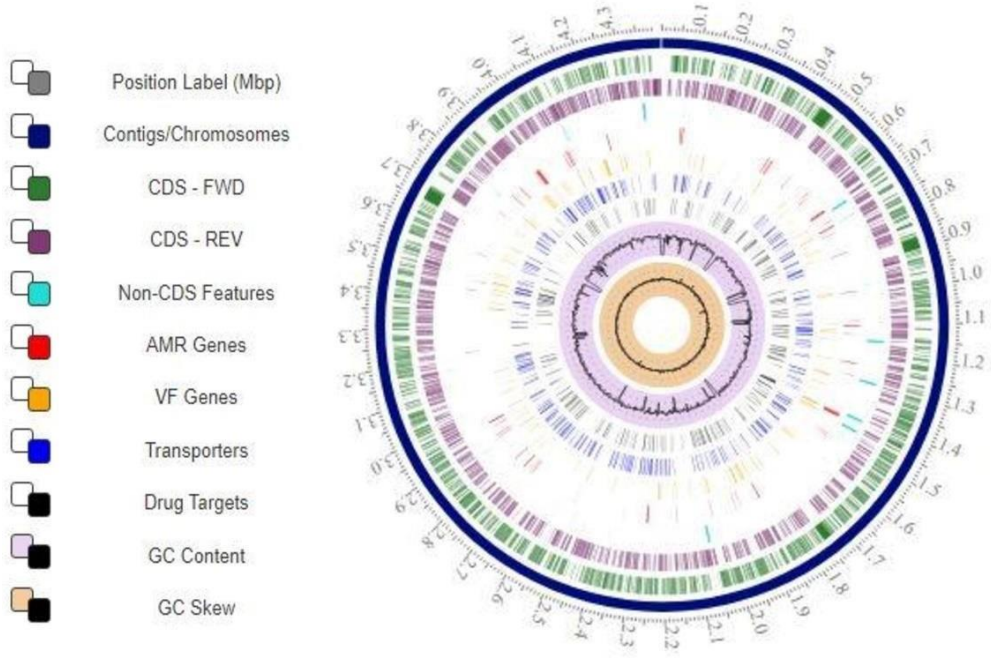
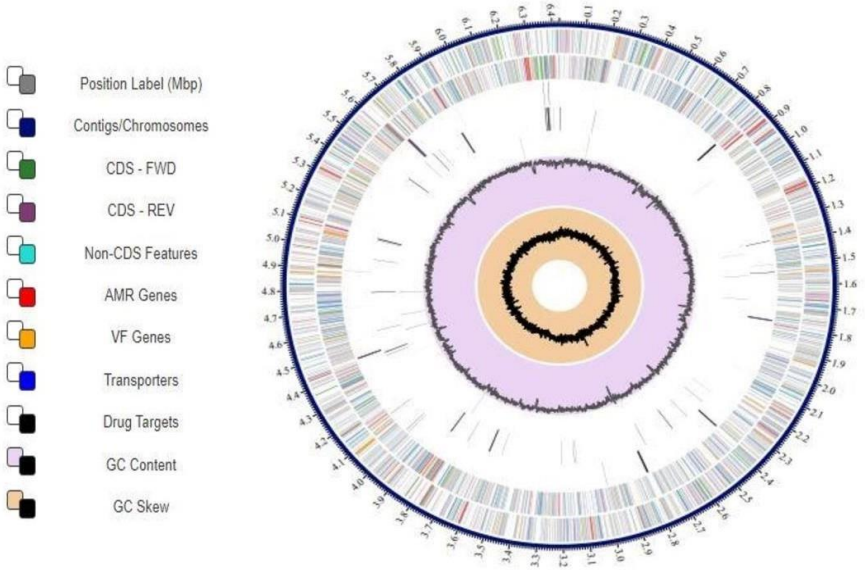


Figure 12. Annotations of the PGRG5 strain's genome are presented in a circular plot. Gene cluster classifications are color-coded across the sequenced genome, with each circle description progressing from the outermost to the innermost circle



Heavy metal resistance genes present on the genome of PGRG2

Microorganisms in contaminated environments frequently become resistant to heavy metals, making them potential candidates for engineering bacteria (Pande et al., 2022). Three major resistance mechanisms used by bacteria are intra- and extracellular sequestration, enzymatic conversion to a less toxic form, and transportation through the efflux pump system (Thai et al. 2023). Along with these mechanisms, bacterial strains also synthesise exoploysaccrides (EPS) in their defence from harsh conditions like exposure to heavy metals (Gupta and Diwan (2016)). Genomic analysis of PGRG2 strain revealed that PGRG2 contains genes that encode resistance proteins for Pb, Cd, Ni, Cu, Zn, As, Cr, Hg and Co. Furthermore, the resistance of the tested strains to Pb, Cd & Ni was confirmed in MIC tests mentioned above. Using the Bacmet database (version 2.0), (Tseng et al., 2023), a large collection of genes associated with metal, antibiotic and biocide resistance were identified on the genome of PGRG2 (result of blast is in supplementary data). The genome also harbors genes encoding various transporters such as uni-sym and antiporters, cation and ABC transporters, potentially facilitating the transport of heavy metals. In addition, PGRG2 includes a transcriptional regulator of the MerR family that regulates the internal levels of various heavy metals. Exposure to these metals triggers the expression of genes that govern defense mechanisms (Fang and Zhang (2022)).

It is well known and reported that gram negative bacteria have a better resistance mechanism as compare to the gram-positive bacteria. This phenomenon may be attributed to the additional outer membrane layer enveloping gram-negative bacterial cells, which is believed to act as a protective barrier against the ingress of harmful agents (Breijyeh et al., 2020). Members of gram-negative bacteria family generally contains multidrug efflux pumps belonging to five superfamilies, such as ABC (ATP-binding cassette), SMR (small multidrug resistance), MFS (major facilitator superfamily), MATE (multiple antibiotic and toxin extrusion), and RND (Resistance-Nodulation- Division) (Huang et al., 2022). This study confirms that PGRG2 genome carry various genes which belongs to these families and encode for membrane proteins which are mention in table 6. Apart from transporter genes there are some genes which are indirectly or directly involved in the remediation of heavy metals. It was seen that, *acn*A and *acn*B encodes for the aconitase enzymes, which are involve in maintaining the cellular process which got affected by heavy metal exposure and are indirectly involve in the heavy metal remediation (Koyama et al., 2024).

Another gene *nfs*A which encodes for nitroreductase enzyme is also present on PGRG2, this gene helps bacterial strain in the adaptation to heavy metal stress (Sharrock et al., 2021). *Rcn*B gene which encodes for periplasmic protein is also present on this genome, which is very well studied for the intracellular concentration of heavy metals like Ni and Co (Elarabi et al., 2023). Further, Pb, Cd, Ni, Co, Mn and other heavy metal genes present on PGRG2 genome are mentioned in table 7.

EPS supporting genes present on the genome of PGRG2

Exopolysaccharides are biopolymers which are secreted by bacteria in order to tolerate harsh environment. These biopolymers perform fundamental tasks like preserving the bacterial cell envelope's structural integrity and facilitating communication within and between bacterial communities. It is significant to note that the genome of PGRG2 has *bcs* operons and other genes which are involved in EPS production are mentioned in table 8. In addition to acting as a structural matrix, biofilm extracellular polymer (EPS) may also include genes like *btuB* and *CbiB* which encodes functional elements that facilitate cell surface attachment and biofilm formation (Di 2018). In this study, SEM analysis also confirms the production of EPS by PGRG2. EPS production is one more method followed by PGRG2 in its defence against the high concentration of heavy metals.

Table 6. Multidrug transporter genes present on the genome of PGRG2

Gene	Product	Protein ID
acrAB	MDR efflux pump *acr*AB transcriptional activator *Rob*A	WJD50864
acrAB	MDR efflux pump *acr*AB transcriptional activator MarA	WJD48473
acrA	Multidrug efflux RND transporter periplasmic adaptor subunit *acr*A	WJD47627
acrB	Multidrug efflux RND transporter permease subunit *acr*B	WJD47626
acrD	Multidrug efflux RND transporter permease subunit *acr*D	WJD49272
acrR	*Tet*R/*acr*R family transcriptional regulator	WJD50804, WJD51112, WJD47697, WJD48227, WJD48354, WJD48463, WJD48948, WJD5136
acrR	Multidrug efflux transporter transcriptional repressor *acr*R	WJD47628
actP	Cation/acetate symporter *act*P	WJD50627
mdtA	*mdt*A/*Mux*A family multidrug efflux RND transporter periplasmic adaptor subunit	WJD49048
mdtB	*mdt*B/*Mux*B family multidrug efflux RNA transporter permease subunit	WJD49049
mdtC	Multidrug efflux RND transporter permease subunit *mdt*C	WJD49050
mdtG	Multidrug efflux MFS transporter *mdtG*	WJD48173
mdtH	Multidrug efflux MFS transporter *mdtH*	WJD48185
mdtK	*mdtK* family multidrug efflux MATE transporter	WJD48341
pstA	Phosphate ABC transporter permease *pstA*	WJD50306
pstB	Phosphate ABC transporter ATP-binding protein *pstB*	WJD50305
pstC	Phosphate ABC transporter permease *pstC*	WJD50307
pstS	Phosphate ABC transporter substrate-binding protein *pstS*	WJD50308
emrA	Multidrug efflux MFS transporter periplasmic adaptor subunit *emrA*	WJD49452
emrB	Multidrug efflux MFS transporter permease subunit *emrB*	WJD49453
emrD	Multidrug efflux MFS transporter *emrD*	WJD50278
tolC	Outer membrane channel protein *tolC*	WJD49739
dinF	MATE family efflux transporter *DinF*	WJD50604
yde	Efflux MFS transporter *ydeE*	WJD48471

Table 7. Heavy metal resistance genes present on PGRG2

Gene	Product	Protein ID
Lead		
zntA	Zn (II)/Cd (II)/Pb (II) translocating P-type ATPase *zntA*	WJD50111
zinT	Metal-binding protein *zinT*	WJD48614
merR	MerR family transcriptional regulator	WJD48512, WJD49083
Cadmium		
dsbA	thiol:disulfide interchange protein *dsbA*	WJD50340
dsbB	disulfide bond formation protein *dsbB*	WJD48769
dsbC	bifunctional protein-disulfide isomerase/oxidoreducatase *dsbC*	WJD49645
zntA	Zn (II)/Cd (II)/Pb (II) translocating P-type ATPase *zntA*	WJD50111
zinT	Metal-binding protein *zinT*	WJD48614
bfr	Bacterioferritin	WJD50001
Nickel		
nikA	nickel ABC transporter substrate-binding protein	WJD51225
nikB	nickel ABC transporter permease subunit *nikB*	WJD51226
nikC	nickel ABC transporter permease subunit *nikC*	WJD50631
nikD	nickel import ATP-binding protein *nikD*	WJD50632
nikE	nickel import ATP-binding protein *nikE*	WJD50633
nikR	nickel-responsive transcriptional regulator *nikR*	WJD50634
NiCoTs	Nickel/cobalt transporter	WJD50641, WJD49340
Arsenic		
arsC	glutaredoxin-dependent arsenate reductase	WJD48595
Copper		
copA	copper-exporting P-type ATPase *copA*	WJD47646
copD	copper homeostasis membrane protein *copD*	WJD48812
copD	LysR family transcriptional regualtor	WJD50148
copC	*copC* domin-containing protein *YobA*	WJD51303
corC	HlyC/CorC family transporter	WJD51349
corA	magnesium/cobalt transporter *corA*	WJD50163
corC	CNNM family magnesium/cobalt transport protein *corC*	WJD47804
cueO	multicopper oxidase *cueO*	WJD50991
cutA	divalent cation tolerance protein *cutA*	WJD50653
cutC	copper homeostasis protein *cutC*	WJD48848
ompR	Two-component system response regulator *ompR*	WJD50061
Manganese		
mgtA	magnesium-translocating P-type ATPase	WJD50774
mntR	manganese-bindidng transcriptional regualtor *mntR*	WJD47970
mntP	manganese efflux pump *mntP*	WJD48791
apaG	Co2−/Mg2− efflux protein *apaG*	WJD50906

Table 8. Genes involve in Exopolysaccharides (EPS) production in PGRG2

Gene	Product	Protein ID
bcsA	UDP-forming cellulose synthase catalytic subunit	WJD50495, WJD50505
bcsB	UDP-forming cellulose synthase catalytic subunit	WJD50506, WJD50496
bcsC	Cellulose synthase subunit *bcsC*-related outer membrane protein	WJD50497, WJD50507
blsA	Multiple stress resistance protein *blsA*	WJD51111
pgpA	Phosphatidylglycerophosphatase A	WJD51245
btuB	TonB-dependent vitamine B12 receptor *btuB*	WJD50445
btuF	Vitamin B12 ABC transporter substrate-binding protein *btuF*	WJD51020
btuD	Vitamin B12 ABC transporter ATP-binding protein	WJD48318
CbiB	Adenosylcobinamide-phosphate synthase *CbiB*	WJD48988

Conclusion

This study discusses about the remediation potential of bacterial strains isolated from E-waste contaminated soil. Both the strains showed potential for the remediation of selected heavy metals *i.e.,* lead, cadmium and nickel. Out of two strains one was identified as unclassified new species from *Enterobacter* genus, which was unexplored for its responses in harsh environmental conditions, is explored in this study. Complete genome sequence analysis confirms the presence of various heavy metal resistances genes present on its genome which makes it a potential bioremediation agent of multi-metal-contaminated sites. Additional, tolerance was achieved through production of exopolysaccharide in the presence of different heavy metals which was further confirmed by SEM and whole genome studies. All the results in this research support that the two evaluated strains considered as heavy metal tolerant bacteria have beneficial characteristics for remediating the contaminated soil.

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References

Al-Boghdady, A. A. and Hassanein, K. M. A. (2019). Chemical analysis and environmental impact of heavy metals in soil of Wadi Jazan area, southwest of Saudi Arabia. *Appl. ecol.environ. res.*, 17(3), 7067-7084.

Alengebawy, A., Abdelkhalek, S. T., Qureshi, S. R. and Wang, M. Q. (2021). Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics*, 9(3), 42.

Alfarras, F., and Hamid, A. F. M. (2022). Heavy Metal Resistance Ability of Pseudomonas Species Isolated from Sludge and Sewage in Iraq. *Archives of Razi Institute*, 77(3), 1041.

Ayangbenro, A. S. and Babalola, O. O. (2017). A new strategy for heavy metal polluted environments: a review of microbial biosorbents. *Int J Environ Res Public Health*, 14(1), 94.

Bala, S., Garg, D., Thirumalesh, B. V., Sharma, M., Sridhar, K., Inbaraj, B. S. and Tripathi, M. (2022). Recent strategies for bioremediation of emerging pollutants: a review for a green and sustainable environment. *Toxics*, 10(8), 484.

Bestawy, E. E., Helmy, S., Hussien, H., Fahmy, M. and Amer, R. (2013). Bioremediation of heavy metal-contaminated effluent using optimized activated sludge bacteria. *Applied water science*, 3, 181-192.

Breijyeh, Z., Jubeh, B. and Karaman, R. (2020). Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules*, 25(6), 1340.

Cerveira, C., Hermann, P. R. S., Pereira, J. S. F., Pozebon, D., Mesko, M. F. and Moraes, D. P. (2020). Evaluation of microwave-assisted ultraviolet digestion method for rice and wheat for subsequent spectrometric determination of As, Cd, Hg and Pb. *J.Food Compos. Anal*, 92, 103585.

Collin, S., Baskar, A., Geevarghese, D. M., Ali, M. N. V. S., Bahubali, P., Choudhary, R., and Swamiappan, S. (2022). Bioaccumulation of lead (Pb) and its effects in plants: A review. *JHM*

Letters, 3, 100064.

Di Martino, Patrick. (2018). Extracellular polymeric substances, a key element in understanding biofilm phenotype. *AIMS microbiology* 4 (2), 274-288.

Elarabi, N. I., Halema, A. A., Abdelhadi, A. A., Henawy, A. R., Samir, O. and Abdelhaleem, H. A. (2023). Draft genome of Raoultella planticola, a high lead resistance bacterium from industrial wastewater. *AMB Express*, 13(1), 14.

Elarabi, N. I., Halema, A. A., Abdelhadi, A. A., Henawy, A. R., Samir, O. and Abdelhaleem, H. A. (2023). Draft genome of *Raoultella planticola*, a high lead resistance bacterium from industrial wastewater. *AMB Express*, 13(1), 14.

Fang, C. and Zhang, Y. (2022). Bacterial MerR family transcription regulators: activationby distortion: The mechanism of transcription regulation by MerR. *Acta Biochimica et Biophysica Sinica*, 54(1), 25-36.

Ferronato, N. and Torretta, V. (2019). Waste mismanagement in developing countries: A review of global issues. *Int J Environ Res Public Health*, 16(6), 1060.

Genchi, G., Carocci, A., Lauria, G., Sinicropi, M. S. and Catalano, A. (2020). Nickel: Human health and environmental toxicology. *Int J Environ Res Public Health*, 17(3), 679.

Gupta, P. and Diwan, B. (2017). Bacterial exopolysaccharide mediated heavy metal removal: a review on biosynthesis, mechanism and remediation strategies. *Biotechnol Rep (Amst)*., 13, 58-71.

Hao, Y., Zheng, S., Zhao, M., Wu, H., Guo, Y. and Li, Y. (2020). Reexamining the relationships among urbanization, industrial structure, and environmental pollution in China—New evidence using the dynamic threshold panel model. *Energy Reports*, 6, 28-39.

Huang, L., Wu, C., Gao, H., Xu, C., Dai, M., Huang, L. and Cheng, G. (2022). Bacterial multidrug efflux pumps at the frontline of antimicrobial resistance: an overview. *Antibiotics*, 11(4), 520.

Ibrahim, U., Kana, A. A., AbdullahiAuta, A., Mustapha, I. M. and Mundi, A. A. Health Risk Assessment of Heavy Metals in Swampy Agricultural Soils in Nasarawa West, Nigeria. *DUJOPAS*. 4, 2476-8316.

Igiri, B. E., Okoduwa, S. I., Idoko, G. O., Akabuogu, E. P., Adeyi, A. O. and Ejiogu, I. K. (2018). Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: a review. *J Toxicol*., 2018(1), 2568038.

Kalita, D. and Joshi, S. R. (2017). Study on bioremediation of Lead by exopolysaccharide producing metallophilic bacterium isolated from extreme habitat. *Biotechnol Rep (Amst)*, 16, 48-57.

Koyama, H., Kamogashira, T. and Yamasoba, T. (2024). Heavy metal exposure: molecular pathways, clinical implications, and protective strategies. *Antioxidants*, 13(1), 76.

Lan, Y., Liu, M., Song, Y., Cao, Y., Li, F., Luo, D. and Qiao, D. (2023). Distribution, characterization, and evolution of heavy metal resistance genes and Tn7-like associated heavy metal resistance Gene Island of *Burkholderia*. *Front. Microbiol*., 14, 1252127.

Martis, S., Mohan, A. K., Chiplunkar, S., Kamath, S., Goveas, L. C. and Rao, C. V. (2021). Bacterium isolated from coffee waste pulp biosorps lead: Investigation of EPS mediated mechanism. *Curr Res Microb Sci.*, 2, 100029.

Mathivanan, K., Chandirika, J. U., Vinothkanna, A., Yin, H., Liu, X., & Meng, D. (2021). Bacterial adaptive strategies to cope with metal toxicity in the contaminated environment–A review. *Ecotoxicol Environ Saf.*, 226, 112863.

Michael-Igolima, U., Abbey, S. J. and Ifelebuegu, A. O. (2022). A systematic review on the effectiveness of remediation methods for oil contaminated soils. *Environmental Advances*, 9, 100319.

Mitra, S., Chakraborty, A. J., Tareq, A. M., Emran, T. B., Nainu, F., Khusro, A. and Simal-Gandara, J. (2022). Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. *J. King Saud Univ. Sci.*, 34(3), 101865.

Muñoz-García, A., Arbeli, Z., Boyacá-Vásquez, V. and Vanegas, J. (2022). Metagenomic and genomic characterization of heavy metal tolerance and resistance genes in the rhizosphere microbiome of *Avicennia germinans* in a semi-arid mangrove forest in the tropics. *Mar. Pollut. Bull*, 184, 114204.

Naidu, R., Biswas, B., Willett, I. R., Cribb, J., Singh, B. K., Nathanail, C. P. and Aitken, R. J. (2021). Chemical pollution: A growing peril and potential catastrophic risk to humanity. *Environment International*, 156, 106616.

Olufemi, A. C., Mji, A. and Mukhola, M. S. (2022). Potential health risks of lead exposure from early life through later life: implications for public health education. *Int J Environ Res Public Health*, 19(23), 16006.

Oziegbe, O., Oluduro, A. O., Oziegbe, E. J., Ahuekwe, E. F. and Olorunsola, S. J. (2021). Assessment of heavy metal bioremediation potential of bacterial isolates from landfill soils. *Saudi J Biol Sci*, 28(7), 3948-3956.

Pande, V., Pandey, S. C., Sati, D., Bhatt, P. and Samant, M. (2022). Microbial interventions in bioremediation of heavy metal contaminants in agroecosystem. *Front Microbiol,* 13, 824084.

Priya, A. K., Muruganandam, M., Ali, S. S. and Kornaros, M. (2023). Clean-up of heavy metals from contaminated soil by phytoremediation: A multidisciplinary and eco-friendly approach. *Toxics*, 11(5), 422.

Romantschuk, M., Lahti-Leikas, K., Kontro, M., Galitskaya, P., Talvenmäki, H., Simpanen, S. and Sinkkonen, A. (2023). Bioremediation of contaminated soil and groundwater by in situ biostimulation. *Front Microbiol*, 14, 1258148.

Shameer, S. (2016). Biosorption of lead, copper and cadmium using the extracellular polysaccharides (EPS) of Bacillus sp., from solar salterns. *3 Biotech*, 6(2), 194.

Sharrock, A. V., McManaway, S. P., Rich, M. H., Mumm, J. S., Hermans, I. F., Tercel, M. and Ackerley, D. F. (2021). Engineering the Escherichia coli Nitroreductase NfsA to create a flexible enzyme-prodrug activation system. *Front Pharmacol*, 12, 701456.

Shen, L., Chen, R., Wang, J., Fan, L., Cui, L., Zhang, Y. and Zeng, W. (2021). Biosorption behavior and mechanism of cadmium from aqueous solutions by *Synechocystis sp*. PCC6803. *RSC advances*, 11(30), 18637-18650.

Siddiqua, A., Hahladakis, J. N. and Al-Attiya, W. A. K. (2022). An overview of the environmental pollution and health effects associated with waste landfilling and open dumping. *Environ Sci Pollut Res*., 29(39), 58514-58536.

Thai, T. D., Lim, W., & Na, D. (2023). Synthetic bacteria for the detection and bioremediation of heavy metals. *Front Bioeng Biotechnol*, 11, 1178680.

Thakur, P. and Gauba, P. (2021). Tolerance and remediation potential of water microbes against nitrate. *Int J Curr Res Rev*, 13, 58-64.

Thakur, P. and Gauba, P. (2022). Genomic characterization of *Lelliottia amnigena* PTJIIT1005, a nitrate tolerant strain isolated from water sample of Yamuna River, Delhi, India. *Microbiol. Resour. Announc.*, 11(6), e00229-22.

Thakur, P. and Gauba, P. (2023). Identification and examination of nitrogen metabolic genes in *Lelliottia amnigena* PTJIIT1005 for their ability to perform nitrate remediation. *BMC genomics*, 24(1), 104.

Tseng, A. S., Roberts, M. C., Weissman, S. J. and Rabinowitz, P. M. (2023). Study of heavy metal resistance genes in Escherichia coli isolates from a marine ecosystem with a history of environmental pollution (arsenic, cadmium, copper, and mercury). *Plos one*, 18(11), e0294565.

Wang, G., Ren, Y., Bai, X., Su, Y. and Han, J. (2022). Contributions of beneficial microorganisms in soil remediation and quality improvement of medicinal plants. *Plants*, 11(23), 3200.

Zaghloul, E. H. and Halfawy, N. M. E. (2024). Marine *Pediococcus pentosaceus* E3 Probiotic Properties, Whole-Genome Sequence Analysis, and Safety Assessment. *PROBIOTICS ANTIMICRO.*, 1-12.

Zhang, J., Li, Q., Zeng, Y., Zhang, J., Lu, G., Dang, Z. and Guo, C. (2019). Bioaccumulation and distribution of cadmium by *Burkholderia cepacia* GYP1 under oligotrophic condition and mechanism analysis at proteome level. *Ecotoxicol. Environ. Saf.*, 176, 162-169.