Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 14 [12] November 2025. 24-34
©2025 Academy for Environment and Life Sciences, India
Online ISSN 2277-1808
Journal's URL: http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Assessment of Biosorption potential of *Lactococcus lactis* isolated from Industrial effluent-contaminated soil

Gayatri V, Anushree S
Affiliation: Department of Microbiology, St.Francis College for Women, Begumpet, Hyderabad-500016, Telangana , India.
Corresponding Author: Name: Gayatri V
Email :gayathri@sfc.ac.in
Anushree S: ORCID iD: 0009-0009-0522-0882

ABSTRACT
Heavy metal pollution in soil is a global environmental issue, presenting serious risks to ecosystems, agriculture, and human health. In recent years, innovative and efficient remediation strategies for heavy metal-contaminated soils have been rapidly advancing. The present study focuses on an eco-friendly approach to reduce the metal toxicity in metal contaminated soils. The soil sample was collected from iindustrial effluent area near Medchal, Hyderabad. Six bacterial colonies resistant towards heavy metals like zinc, copper, iron and lead were isolated. The isolate with maximum metal tolerance was identified as Lactococcus lactis through morphological, biochemical, and molecular characterization methods. The metal adsorption in the isolate was mediated through extracellular polysaccharides (EPS).Structural analysis of EPS was carried by scanning electron microscopy and composition was determined by Fourier transformed infrared spectroscopy(FTIR).The ability of the bacterial EPS in metal adsorption was determined by ***Inductively coupled*** *plasma mass spectrometric* ***(****ICP-MS)analysis and it was observed that EPS has more adsorption towards Lead, Iron and Zinc than copper metal. So Lactococcus lactis can be an effective biosorbent in reducing the metal toxicity in contaminated soils.*
Keywords: Industrial effluents, Heavy metals, Biosorption, Extracellular polysaccharides

Received 18.09.2025 Revised 28.10.2025 Accepted 26.11.2025

INTRODUCTION

An alarming increase in environmental pollution from hazardous wastes containing heavy metals has occurred with the rapid development of industries like mining, metallurgy, and metal surface treatment. Because toxic metals cannot completely break down chemically or biologically. The health of individuals is seriously threatened by elevated concentrations of hazardous heavy metals because they create harmful compounds that negatively impact biological processes [1]. The use of microbial bioremediation has recently gained attention as a unique technology, as it is a substitute for widely used physico-chemical methods like electrochemical treatment and chemical precipitation. Therefore, an inexpensive and effective biological approach for removing metals from industrial effluent soil is required. Since biosorption is non-polluting, highly specific, more efficient, and cost-effective for treating huge quantities of effluents with low metal concentrations, it is now utilized to remove hazardous metals from soil [2]. The first step in biosorption is the diffusion of metal ions to the surface of microbial biomass, which combines active and passive transport processes [3]. Biosorption can be Extra cellular accumulation and Intracellular accumulation [4]. EPS produced by algae, bacteria, fungi, and yeast is getting attention due to the need for low-cost, and ecofriendly efficient techniques with the objective of extracting heavy metals from industrial soil. EPS plays a major role in the metal biosorption process. Heavy metal adsorption by EPS is energy independent, non-metabolic, and produced by the interaction of the metal cations and The functional groups of extracellular polysaccharides have negative charges[5].With abundant binding sites extracellular polymeric substances(EPS),can be used to decrease heavy metal ions concentration in aqueous solution from ppm to ppb level within hours through adsorption[6].Lactic acid bacteria (LAB) have been used for their probiotic properties and in the food and medical researches. In particular, the use of LAB for the bioremediation of industrial effluents polluted with metals is not widely proven scientifically [7].LAB can be effective biosorbents for harmful metal ions such as copper, iron, lead, and zinc because of

their thick cell wall, metabolic properties, and the ability to produce extracellular polymeric substances(EPS).The objective of this study is to investigate the biosorption ability of *Lactococcus lactis* which was isolated from industrial effluent contaminated soil.

MATERIAL AND METHODS

Collection of industrial effluent soil sample

The soil sample was collected from Medchal industrial area contaminated with industrial effluents, Hyderabad. Soil was transported in sterile polythene bags to the Microbiology Laboratory, St.Francis College for Women, Hyderabad.

Isolation of bacterial isolates from industrial effluent soil

A stock solution was prepared by suspending 1g of soil in 100 mL of sterile distilled water. The sample was diluted 10-fold upto 10^{-6}.0.1mL of 10^{-6} dilution was inoculated onto sterile nutrient agar plates. The agar plates were incubated at 37^0C for 24 hr for the growth of microorganisms [8].

Screening for Heavy metal tolerant isolates

The bacterial isolates from the agar plate were screened for heavy metal tolerance towards metals like copper, iron, lead, and zinc by Agar well diffusion method [9]. Different concentrations of metals i.e.,10,25,50 and100ppm were prepared and suspended into the wells bored in the minimal agar plates seeded with bacterial isolates and incubated for 48 hours at 37^oC.The plates were checked for growth after the incubation period [1].

Morphological Identification of metal tolerant isolates

The metal tolerant bacterial isolates were identified by Gram staining method according to Bergey's Manual of Determinative Bacteriology [10].

Biochemical characterization of metal resistant isolates

In accordance with Bergey's Manual of Determinative Bacteriology, metal resistant isolates were further identified using the IMViC test series, as well as the Oxidase and Catalase test used to identify organisms [11].

Indole Test: The test was carried out in tryptophan broth or Sulfide-motility (SIM) medium. When tryptophanase enzyme is present, it shows that bacterial isolates can break down the amino acid tryptophan to indole. The 24-hour-old cultures was inoculated with tryptone broth and incubated for 24 hours at 37°C. After the incubation period Kovac's reagent is added, the tubes were examined, and the results were noted.

Methyl Red (MR) Test: To determine the production of stable acid end-products from glucose fermentation, MR-VP broth tubes were inoculated with the bacterial cultures and incubated at 37°C for 24 hours. After incubation, methyl red indicator was added.

Citrate Utilization Test: The test was performed using Simmon citrate agar. The purpose of the test was to identify the bacterial isolates which produce the enzyme citrase. The agar surface was streaked with the bacterial inoculum, and incubated for 24 hours at 37°C.

Catalase Test (Slide Method): To identify the presence of the catalase enzyme, A loopfull of a 24-hour-old isolated cultures was placed on the slide along with 3% hydrogen peroxide. The formation of oxygen bubbles indicates a positive result.

Oxidase Test: The test was conducted to determine the presence of the enzyme cytochrome c oxidase. A loopful of a 24-hour-old bacterial culture was applied onto an oxidase test paper disc. A positive result was indicated by the purple color developing within 10 seconds [12].

Molecular characterization of metal resistant isolate

The metal resistant bacterial isolate was subjected to buffer-based lysis, column purification, and elution in order to extract its genomic DNA using the Xploregen Universal gDNA Extraction Kit. The next phase was 16S rDNA typing at Averin biotech Laboratories, Hyderabad. Using the Forward (5′–GGATGAGCCCGCGGCCTA) and Reverse (5′–CGGTGTGTACAAGGCCCGG) primers, 181 ng of the 16S rRNA gene were amplified following spectrophotometric measurement for the quantify the DNA. High-fidelity polymerase, dNTPs, $MgCl_2$, assay buffer, and primers were used in the 50 μL reaction volume for the PCR. The thermal cycling conditions were as follows: 30 cycles of denaturation (94°C, 1 min), annealing (50°C, 1 min), extension (72°C, 2 min), and a final extension at 72°C for 7 min.The PCR results were confirmed by agarose gel electrophoresis [13]. An ABI 3130xl Genetic Analyzer was used to conduct bi-directional sequencing utilizing Big Dye Terminator v3.1[14]. The molecular characterization of metal resistant bacterial isolate BLASTn (Basic Local Alignment Search Tool) program was used, which is accessible via the National Center for Biotechnology Information (NCBI) database, the resultant DNA sequence was examined and multiple sequence alignment was performed using Clustal W software [15][16]. Weighbor method with the Jukes-Cantor model was used to identify phylogenetic relationships.

Determination of metal adsorption by bacterial isolate by UV VIS analysis

UV-VIS Spectrophotometer (Systronsis 2100) was used to determine the adsorption of the metal by bacterial isolate.1000ppm of heavy metals copper, iron, lead, and zinc were added to 100 ml of nutrient broth flasks inoculated with 5 ml of bacterial isolate respectively. The flasks were incubated in orbital shaker at 150rpm, 37^0C for 72hr.After the incubation period, the absorbance maxima were determined at 600nm [17].

Extracellular polysaccharides production in metal resistant bacteria

Screening for EPS production

The ability of bacterial isolates towards metal adsorption is mediated through extracellular polysaccharide production. The bacterial isolate was screened for extracellular polysaccharides by Disc method [18]. 3 sterile De Man, Rogosa and Sharpe Agar (MRS Agar) plates were incorporated with 8% sucrose, lactose and fructose respectively. To the plates, sterile filter paper discs (5 mm) embedded with 3 µL of bacterial isolate was placed and plates were incubated for 48 hour at 37^0C.The plates were checked for mucoid colonies around the disc, for extracellular polysaccharide production [19].

Extraction and Purification of EPS

A 5 mL aliquot of bacterial inoculum was aseptically transferred into 500 mL of De Man, Rogosa and Sharpe (MRS) broth to facilitate the proliferation of lactic acid bacteria. The culture was incubated at 37°C for 24 hours under aerobic conditions. Following incubation, trichloroacetic acid (TCA) was added to achieve a final concentration of 14% (v/v) to precipitate extracellular proteins. The mixture was subjected to agitation at 90 rpm in an orbital shaker at 37°C for 30 to 40 minutes for protein denaturation. Subsequently, the culture was centrifuged at 8,000 rpm for 20 minutes to separate the precipitated proteins and cellular debris. The resulting supernatant was carefully decanted, and cold absolute ethanol was added in a 1:2 ratio (supernatant: ethanol,v/v) to precipitate extracellular polysaccharides. This mixture was incubated at 4°C for 48 hours to facilitate extracellular polysaccharide precipitation [20]. The precipitate has been collected and centrifuged, dissolved in 10 mL of deionized water following the incubation period. After dissolving in water the mixture was dialyzed at 4°C for 48hrs.Then the precipitate was lyophilised at -80°C[21]. The purified EPS was further analysed.

Structural characterization of EPS producing bacterial isolate

The EPS production in the metal resistant bacterial isolate was identified using Scanning electron microscope,was carried out at Averin Biotech Laboratory,Hyderabad.EPS surface morphology and elemental composition was determined.

Determination of Extracellular polysaccharide composition using FTIR

Functional groups of purified extracellular polysaccharides were identified using Fourier Transform Infrared (FTIR) spectrophotometer (Averin Biotech Laboratory, Hyderabad, India).

Determination of EPS mediated metal adsorption by inductively coupled plasma mass spectrometric (ICP- MS) analysis

Metal stock solution(0.01g/10mL) for copper, iron, lead, and zinc were prepared.0.001g EPS was inoculated into the metal solutions respectively and was incubated for 37^oC for 24 hrs. Metal solutions without EPS was used as control. After the incubation period the metal adsorption by EPS was determined by ICP-MS analysis (Averin Biotech Laboratory, Hyderabad, India). Removal efficiency was determined by comparing the amount of adsorbed heavy metals (C_f)to their initial concentration (C_i)[22].

The equation used for calculating biosorption efficiency:

$$\text{Biosorption efficiency (\%)} = \frac{C_i - C_f}{C_i} \times 100$$

RESULTS AND DISCUSSION

Isolation of bacterial isolates from industrial effluent soil

Soil sample collected from industrial effluent soil, medchal was used for the isolation of metal resistant bacterial isolates [23]. The collected samples were serially diluted and inoculated onto nutrient agar plates using the spread plate method. After the incubation period at 37°C for 24 hours, growth of microorganisms were observed. Six bacterial isolates were obtained and the culture labelled as 1A,1B,1C,1D,1E,1F. Each isolate was maintained by subculturing on nutrient agar media and stored at 4°C.

Screening for metal resistant bacterial isolates

The bacterial isolates from nutrient agar plates were screened by Agar well diffusion method [24].Minimal agar plates were prepared and inoculated with 1A,1B,1C,1D,1E,1F .Different concentrations (10,25,50,100 ppm) of metal solutions such as copper, iron, lead, and zinc were suspended onto the wells.After the incubation period at 37°C for 24 hours, zone of inhibition were not observed around the wells.Bacterial growth was observed on the agar plates containing different heavy metals [10]. The minimal agar plates with different concentrations of lead exhibited higher tolerance to Isolates 1A,1B,1C (Fig1 a,b,f) and

inhibition zones were not observed.1E showed comparatively reduced growth, indicating lower tolerance to the metal copper (Fig 1e). Isolate 1D showed tolerance to zinc metal (Fig 1d) and 1F exhibited tolerance to iron metal (Fig 1c).

Characterization of metal resistant bacterial isolates

The colony morphology of the metal resistant bacterial isolates.**1A**, **1B**, 1C,**1D**, **1E**, and **1F** was studied. The colonies(**1A**, **1B**, 1C,**1D**, **1E**,1F)were medium-sized, irregular, raised colonies with smooth, opaque, flat,shiny surfaces in beige to off-white and cream coloured colonies[25].The colony of 1C was small, circular,white with entire margin (Table 1) as indicated by Rahman H U *et al.*, [26].Morphological identification by gram staining indicated the isolates to be Gram positive bacilli (1A, 1B, 1D, 1E, and **1F) and cocci(1C).The isolates** were not produced the enzyme tryptophanase for Indole test. Methyl red test confirmed the production of stable acid end products by 1C isolate with a colour change from yellow to red colour in MR-VP broth tubes inoculated with isolates.Isolates 1A ,1B,1D,1E,1F tested positive for citrate utilization, indicating their ability to use citrate as the sole carbon source. Isolates 1A ,1B,1D,1E,1F were positive for the catalase test,indicated with production of gas bubbles.All isolates except 1C showed oxidase activity indicated by the colour change from colourless to purple on the oxidase disc with the culture.The biochemical tests confirmed the isolates to be *Bacillus subtilis*(1A,1B,1E,1D,1F) and *Lactococcus lactis* (1C)**(Table 2).*Lactococcus lactis*** is primarily known for its probiotic benefits, this study explores its potential for heavy metal biosorption processes. The isolate,1C was further characterized by 16S rDNA typing(Averin Biotech Laboratory, Hyderabad)and was identified to be *Lactococcus lactis* MF429197.1.Based on the BLAST analysis**(Table 3)** and sequence similarity using the System Software aligner and conserved alignment model positions the phylogenetic tree was constructed**(Fig 2)**.Zammouri, A *et al*.,2024 reported that the majority of LAB isolates share a common evolutionary ancestor[27].

Determination of metal adsorption by *Lactococcus lactis*

The ability of ***Lactococcus lactis*** to adsorb the heavy metals was primarily determined by UV-VIS absorption spectrophotometer .The absorbance of *L. lactis* cultures was measured at 600 nm for monitoring bacterial growth, as it provides a reliable estimation of cell density and does not harm the cultures.Metal solutions(1000 ppm of Cu^{2+}, Pb^{2+}, Zn^{2+}, and Fe^{2+})inoculated with ***L. lactis*** culture(5mL)and incubated at 37 °C for 96 hoursThe metal absorbance of the isolate towards Lead, Zinc, and Iron was 0.205, 0.205,0.204 respectively. For Copper, lowest absorbance of 0.201 was observed **(Fig 3)**. The study by Xu, Y *et al*., [28] revealed that lactic acid bacteria showed good adsorbance towards lead metal at higher concentrations.

3.8 Extracellular polysaccharide production in metal resistant *Lactococcus lactis*

Microbial polysaccharides are macromolecules that are released by bacteria and consist of glycosidic bonds between several monosaccharides. These polymeric materials can be found in the cell extracellularly or intracellularly as glycogen [9]. The extracellular polysaccharide was screened by disc method. The bacterial isolate inoculated onto MRS agar media supplemented with 8% Sucrose, fructose and dextrose respectively. The agar plates were incubated at 37°C for 24 hours. Mucoid colonies were observed in MRS agar plates supplemented with 8% Sucrose **(Fig 4).** The most effective carbon source for EPS synthesis was determined to be sucrose, which is consistent with the results of Angelov *et al*., (2023), reported that *Lactiplantibacillus plantarum* ZE2 produced a maximum yield of 211.53 mg/L when sucrose was utilized [29]. EPS was purified using ethanol precipitation method **(Fig 5).** The dry weight of EPS produced by *L.lactis* was found to be 20mg.Similar yield ranged between 25.45–257.362 mg/L was observed in *L. plantarum* YM-2[30]. The dry yield of EPS is dependent on medium composition, climatic conditions, and the selection of superior strains [20]. By optimizing the conditions for biosynthesis of EPS, production can be increased.

Structural characterization of EPS producing *Lactococcus lactis*

The structural analysis of the EPS produced by *Lactococcus lactis* was performed using scanning electron microscopy (SEM). The SEM images provided detailed information about the surface morphology of the extracted EPS.At higher magnification, the SEM images clearly showed a scaly, layered structure, characteristic of microbial EPS. The EPS appeared as tightly, irregular sheets, forming a dense matrix with various thicknesses across the surface. These scale-like features are likely due to the entanglement and aggregation of polysaccharide molecules, which form a protective and stable structure around the bacterial cells**(Fig 6)**.The structural microscopy images of *L. plantarum* were showing similar irregular structure like *L.lactis* with a rough surface and porous structure[31].The EPS surface exhibited porous regions between the scales, indicative of the ability to bind to heavy metals or other substrates, highlighting its potential in metal biosorption .

Determination of extracellular polysaccharide composition using FTIR

The functional composition of the extracted extracellular polysaccharide produced by ***Lactococcus lactis*** was determined using Fourier Transform Infrared Spectroscopy (FTIR), which was performed at Averin

Laboratories, Hyderabad [32]. The FTIR spectrum of the EPS sample **(Fig 7)** revealed a broad absorption band at 3309.96 cm^{-1}, indicating the presence of O–H stretching vibrations associated with hydroxyl groups, a typical feature of carbohydrate structures.Peaks observed between 2858.6–2928.04 cm^{-1} were attributed to C–H stretching vibrations of methyl and methylene groups, commonly found in monosaccharides such as glucose, galactose, rhamnose, and fucose. The carbonyl group's (C=O) stretching vibration was the origin of the absorptions in the 1741.78cm^{-1}.Stretching bands around the 1745.64–1988.68 cm^{-1}region were C=O stretching vibration, and absorptions around the 1224.84–1415.8cm^{-1}region were carboxyl group (C=O) stretching vibration.The fingerprint region between 1053.17–1060.88 cm^{-1} confirmed the presence of polysaccharides.Sugar monomers were identified by a peak near 1244.13 cm^{-1}, with a vibration band at 968.3 cm^{-1} indicated the C–O–C linkage of glycosidic bonds.A specific peak at 860.28 cm^{-1} represented α-D-glucan structures.The presence of functional groups such as hydroxyl, carboxyl, carbonyl, and amino groups,contribute to the biological activity of EPS, including metal-binding mechanism.Similar peaks were reported in EPS derived from *Lactobacillus plantarum* C7 and *Lactococcus lactis* SLT10, confirming the carbohydrate nature and biosorption potential of the polymers[33][34].

Determination of EPS mediated metal adsorption by inductively coupled plasma mass spectrometric (ICP- MS) analysis

The efficiency of the extracted extracellular polysaccharide (EPS) from ***Lactococcus lactis*** in removing heavy metals was evaluated using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for the quantitative determination of trace metals and elemental composition in samples. For this analysis, liquid samples were prepared by adding 0.1% (w/v) metal solutions of zinc, iron, lead, and copper to 10 mL of sterile MRS broth, resulting in a final metal concentration of 1000 ppm, along with 0.01 g of EPS extracted from ***L. lactis***.After treating EPS with heavy metals ,shown reduction in metal concentrations and the biosorption efficiency was determined using the mass balance equation[35].The initial iron (Fe) concentration of 964.8 ppm was reduced to 85.1 ppm, corresponding to a removal efficiency of 91.18%. Similarly, copper, zinc,lead with initial concentrations of 1127.2 ppm,1696 ppm, 766.8 ppm were reduced to 123.2 ppm,63.5 ppm,and 0.1 ppm having removal efficiencies of 89.07% , 96.26% and 99.98% respectively. Copper and iron were also adsorbed by the EPS of the isolate, but their biosorption efficiencies were lower as compared to lead and zinc. Previous studies reported that EPS produced by *Bacillus cereus* NSPA8 showed lead biosorption of approximately 90% and also showed affinity for cadmium and copper, as determined by atomic absorption spectroscopy[36][37]. The biosorption ability of ***L. lactis*** EPS for these heavy metals is shown in Figure 8.The results indicate that ***Lactococcus lactis***, isolated from industrial effluent-contaminated soil, exhibited effective biosorption of heavy metals, with the highest removal efficiency was observed for lead metal.

TABLE 1: Morphological characterization of metal resistant bacterial isolates

ISOLATE	GRAM NATURE	COLONY MORPHOLOGY
1A	Gram-positive rods	Medium, irregular, undulate,raised, smooth,beige to off- white, opaque, shiny
1B	Gram-positive rods	Medium, irregular, undulate, raised, opaque, flat, shiny, white colour colonies.
1E	Gram-positive rods	Medium, irregular, undulate, raised, smooth, beige to off- white, opaque, shiny
1D	Gram-positive rods	Medium, irregular, entire, raised, smooth, white, opaque, shiny cream colour colonies.
1C	Gram-positive cocci	Small, circular, entire, raised, smooth, beige, opaque, shiny, white colour colonies.
1F	Gram-positive rods	Medium, irregular, entire,raised, smooth,white, opaque, shiny cream colour colonies.

TABLE 2: Biochemical characterization of metal resistant bacterial isolates

Identification	Indole	Methyl red	Citrate	Catalase	Oxidase
1A	-	-	+	+	+
1B	-	-	+	+	+
1C	-	+	-	-	-
1D	-	-	+	+	+
1E	-	-	+	+	+
1F	-	-	+	+	+

Table 3: BLAST data analysis of the *Lactococcus lactis sp.*

Sl. No.	Organism Name	Accession No.	% Match
1	***Lactococcus lactis*** subsp. ***lactis*** strain CAU2082 16S ribosomal RNA gene	MF429197.1	98.17%
2	***Lactococcus lactis*** subsp. ***lactis*** strain IMAU11523 (BM41-5) 16S ribosomal RNA gene	KP763979.1	98.17%
3	***Lactococcus lactis*** DCR3-2 gene for 16S rRNA	LC819332.1	98.17%
4	***Lactococcus*** sp. strain M81 16S ribosomal RNA gene	OQ775374.1	98.17%
5	***Lactococcus*** sp. strain FP2GJF02c 16S ribosomal RNA gene	OR373509.1	98.17%

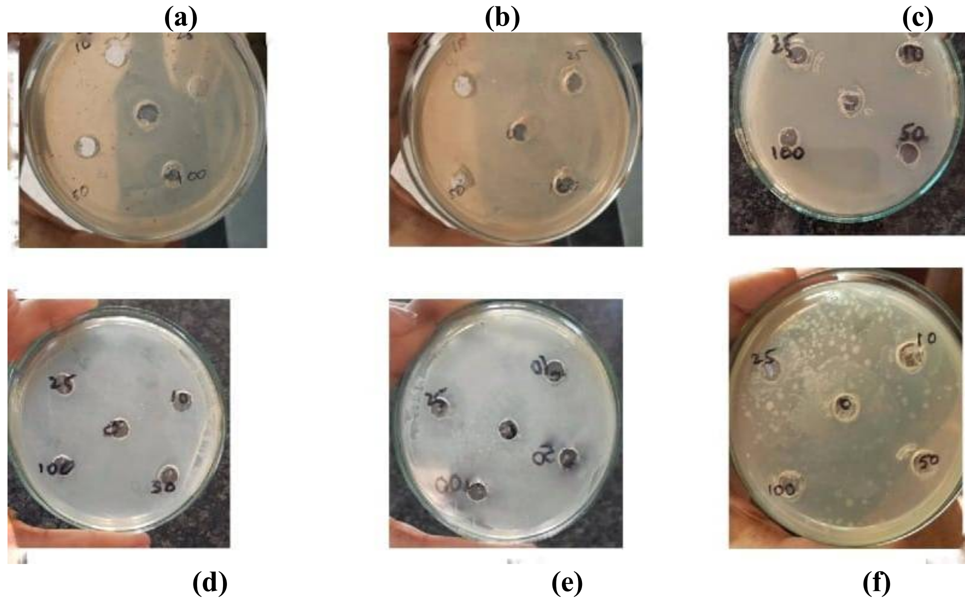


Figure 1: Screening for heavy metal tolerant isolates using agar well diffusion method(a)Minimal agar plates inoculated with culture 1A with metal lead(b)plates inoculated with culture 1B in presence of metal lead(c)plates inoculated with culture 1F in presence of metal iron(d)plates inoculated with culture 1D in presence of metal zinc(e)plates inoculated with culture 1E in presence of metal copper(f)plates inoculated with culture 1C in presence of metal lead.

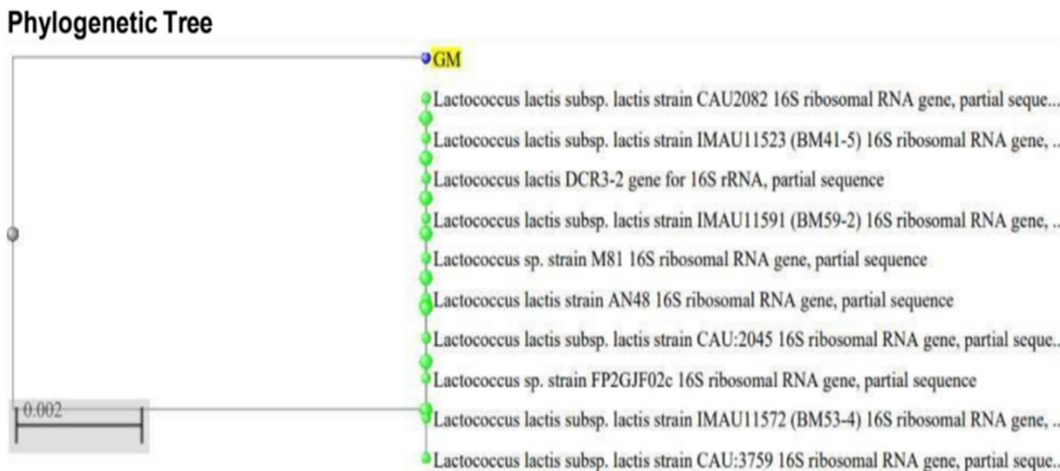


Fig 2. The phylogenetic tree made by using weigh or algorithm method

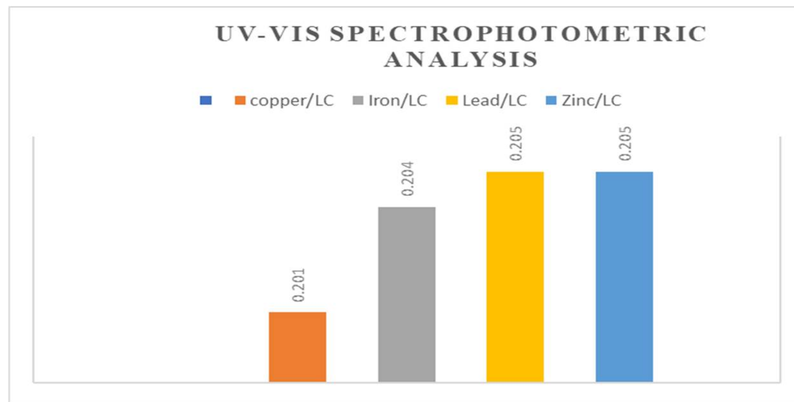


Fig 3: Absorbance of ***Lactococcus lactis towards*** copper (Cu), lead (Pb), zinc (Zn), and iron (Fe) measured at 600 nm using UV-VIS absorption spectrophotometer.

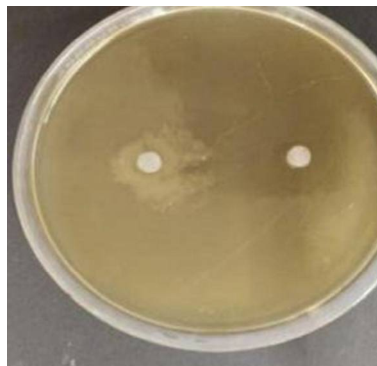
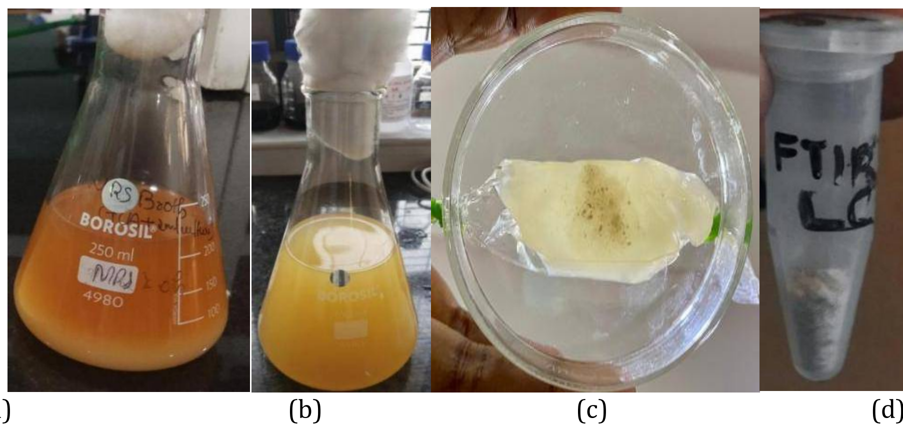


Fig 4. Mucoid colonies were observed in MRS agar plates supplemented with 8% Sucrose



(a) (b) (c) (d)

Fig 5. Purified EPS obtained from ***Lactococcus lactis*** using ethanol precipitation method(a)MRS modified broth inoculate with *L.lactis* which is amended with 8% sucrose including trichloroacetic acid(b)Addition of double volume of ethanol to supernatant broth(c)Dialysed precipitate (d)Purified EPS

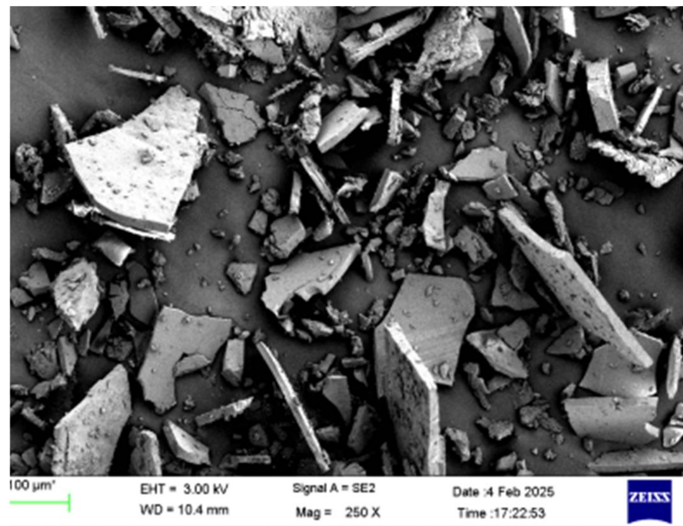


Fig 6 . Scanning electron microscope images of the extracellular polysaccharides produced by *Lactococcus lactis*

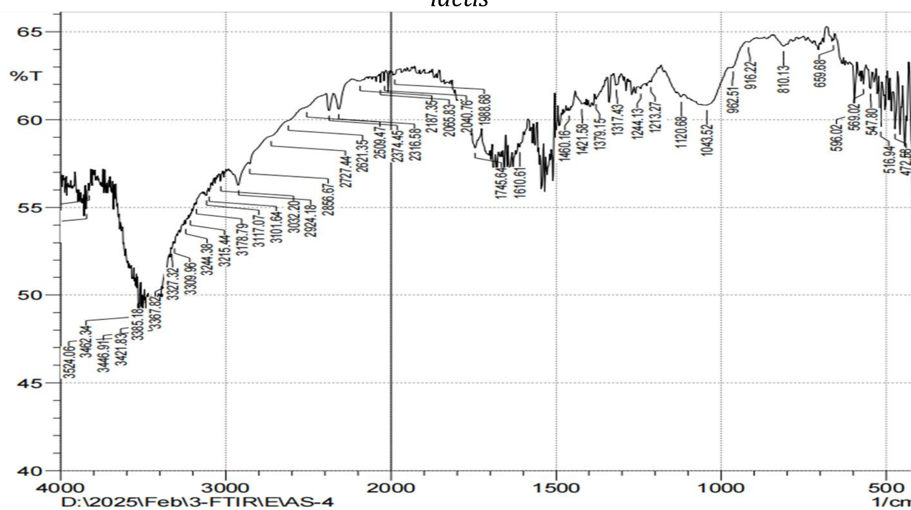


Fig 7. The FTIR Spectrum of extracellular polysaccharide produced by *Lactococcus lactis*

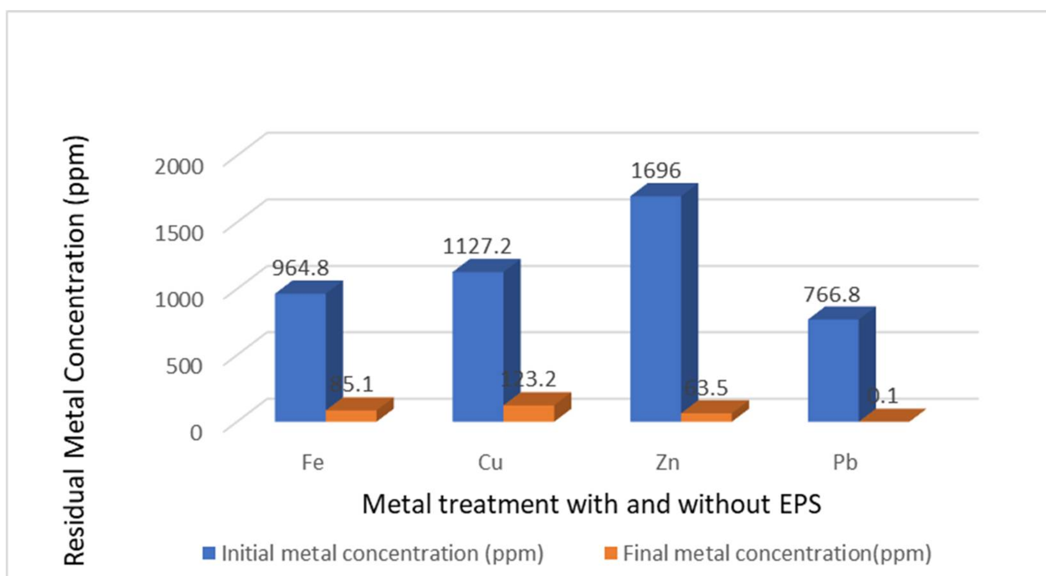


Fig 8. Biosorption of Heavy metals by EPS produced from *Lactococcus lactis*

CONCLUSION

The present study concludes the application of extracellular polysaccharides by *Lactococcus lactis* in removal of heavy metals from soil thus providing a sustainable approach of bioremediation.

ABBREVIATIONS

Cu-Copper; Pb-Lead; Fe-Iron; Zn-Zinc; EPS-Extracellular polysaccharides; Ci-Initial metal concentration; Cf-Final metal concentration; LAB-Lactic acid bacteria; mL-Milliliter; ppm-parts per million; nm-Nanometre; rpm-revolutions per minute

Acknowledgements

The authors thank St. Francis College for Women, Hyderabad,for their support and guidance during this research.

Funding

The work was supported by the management of St. Francis College for women.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work included in this study.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Gayatri V and Anushree S . The first draft of the manuscript was written by all authors and required corrections were made in the manuscript. The final manuscript was read and approved the all authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available and exist in this manuscript.

Ethics approval

The authors did not conduct any studies on humans or animals for this publication.

Consent to participate

Not applicable

Consent to publish

Not applicable

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1. Velusamy, P., Awad, Y. M., Abd El-Azeem, S. A. M., & Ok, Y. S. (2011). Screening of heavy metal resistant bacteria isolated from hydrocarbon contaminated soil in Korea. J Agric Life Environ Sci, 23(1), 40-43.
2. Gayatri, Y., Shailaja, R. M., & Vijayalakshmi, B. (2017). Biosorption of lead by *Bacillus licheniformis* isolated from E-waste landfill, Hyderabad, Telangana, India. International Journal of Bioassays, 6(2), 5240-5244.
3. Zouboulis, A. I., Loukidou, M. X., & Matis, K. A. (2004). Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metal-polluted soils. Process biochemistry, 39(8), 909-916.
4. Nanda, M., Sharma, D., & Kumar, A. (2011). Removal of heavy metals from industrial effluent using bacteria. International journal of environmental sciences, 2(2), 765-780.
5. Gawali Ashruta, A., Nanoty, V., & Bhalekar, U. (2014). Biosorption of heavy metals from aqueous solution using bacterial EPS. Int. J. Life Sci, 2, 373-377.
6. LLi, W. W., & Yu, H. Q. (2014). Insight into the roles of microbial extracellular polymer substances in metal biosorption. Bioresource technology, 160, 15-23.
7. Ameen, F. A., Hamdan, A. M., & El-Naggar, M. Y. (2020). Assessment of the heavy metal bioremediation efficiency of the novel marine lactic acid bacterium, Lactobacillus plantarum MF042018. Scientific reports, 10(1), 314.
8. Nwosu, I. G., Abu, G. O., & Agwa, K. O. (2019). Isolation, screening and characterization of exopolysaccharide producing bacteria. Microbiol. Res. J. Int, 29, 1-9.

9. Hassan, S. H. A., Abskharon, R. N. N., Gad El-Rab, S. M. F., & Shoreit, A. A. M. (2008). Isolation, characterization of heavy metal resistant strain of *Pseudomonas aeruginosa* isolated from polluted sites in Assiut city, Egypt. Journal of Basic Microbiology, 48(3), 168-176..
10. Garrity, G. M., Brenner, D. J., Krieg, N. R., Staley, J. R., & Manual, B. S. (2005). Systematic bacteriology. The Proteobacteria, Part C: The Alpha-, Beta-, Delta-, and Epsilonproteobacteria, Bergey's Manual Trust, Department of Microbiology and Molecular Genetics, 2.
11. Krieg, N. R., & Manual, H. J. C. B. (1984). Systematic bacteriology. Williams Baltimore, 1(161), 172.
12. Sree, B. S., & Gayatri, M. V. (2021). A Study on Isolation and Identification of Exopolysaccharide (EPS) producing Bacteria from Soil. International Journal for Research in Applied Science & Engineering Technology, 9, 650-671.
13. Stephen, J. M., & Saleh, A. M. (2023). Homofermentative Lactobacilli isolated from organic sources exhibit potential ability of lactic acid production. Frontiers in Microbiology, 14, 1297036.
14. Turrina, S., Caratti, S., & De Leo, D. (2013). Powerplex® Y 23 System: Molecular characterization of a null allele at locus DYS549. Forensic Science International: Genetics Supplement Series, 4(1), e117-e118.
15. Liu, D. (Ed.). (2011). Molecular detection of human bacterial pathogens. CRC press.
16. Manjul, A. S., & Shirkot, P. (2018). 16S rRNA gene sequencing for bacterial identification of pullulanase synthesizing thermophilic bacteria contributing to big data. IJCS, 6(2), 2769-2773.
17. Kinoshita, H., Sohma, Y., Ohtake, F., Ishida, M., Kawai, Y., Kitazawa, H., ... & Kimura, K. (2013). Biosorption of heavy metals by lactic acid bacteria and identification of mercury binding protein. Research in microbiology, 164(7), 701-709.
18. GUIMARÃES, D. P., Costa, F. A. A., RODRIGUES, M. I., & Maugeri, F. (1999). Optimization of dextran synthesis and acidic hydrolisis by surface response analysis. Brazilian Journal of Chemical Engineering, 16(2), 129-139.
19. Paulo, E. M., Vasconcelos, M. P., Oliveira, I. S., Affe, H. M. D. J., Nascimento, R., Melo, I. S. D., ... & Assis, S. A. D. (2012). An alternative method for screening lactic acid bacteria for the production of exopolysaccharides with rapid confirmation. Food Science and Technology, 32, 710-714.
20. Kusmiati, K., Nurkanto, A., Fanani, A., Nurcahyanto, D. A., Mamangkey, J., Marissa, S., ... & Gangga, E. (2025). Anti-hypercholesterolemia properties of exopolysaccharide from *Lactiplantibacillus plantarum* MI01: Computational and in vivo approaches. Case Studies in Chemical and Environmental Engineering, 11, 101146.
21. Bajpai, V. K., Majumder, R., Rather, I. A., & Kim, K. (2016). Extraction, isolation and purification of exopolysaccharide from lactic acid bacteria using ethanol precipitation method. Bangladesh Journal of Pharmacology, 11(3), 573-576.
22. Abd-Elnaby, H., Abou-Elela, G. M., & El-Sersy, N. A. (2011). Cadmium resisting bacteria in Alexandria Eastern Harbor (Egypt) and optimization of cadmium bioaccumulation by *Vibrio harveyi*. African Journal of biotechnology, 10(17), 3412-3423.
23. Ayangbenro, A. S. (2019). Bioremediation of heavy metals polluted soil of active gold mines using bacteria biopolymers (Doctoral dissertation, North-West University (South Africa)).
24. Hassan, A., Rahman, S., Deeba, F., & Mahmud, S. (2009). Antimicrobial activity of some plant extracts having hepatoprotective effects. Journal of medicinal plants research, 3(1), 020-023.
25. Kumari, N., Chandrasekharan, N. V., & Wijayarathna, C. D. (2021). Characterization of a multi-metal resistant Bacillus cereus strain TWSL_5 and it's bioremediation potential for Cr, Pb, Zn and Fe. Am J Environ Sci, 17, 49-63.
26. Rahman, H. U., Ashraf, M., Akram, A., Qureshi, M. H. F., Saeed, M. I., Saleem, M., ... & Arshad, M. (2023). in-vitro evaluation for cholesterol reduction potential of lactococcus species isolated from garlic and ginger. FUUAST Journal of Biology, 13(1), 1-6.
27. Zammouri, A., Ziadi, M., Gharsallaoui, A., Fguiri, I., Sbissi, I., Hammadi, M., & Khorchani, T. (2024). Characterization of Novel Exopolysaccharides from *Weissella cibaria* and *Lactococcus lactis* Strains and Their Potential Application as Bio-Hydrocolloid Agents in Emulsion Stability. Fermentation, 10(10), 532.
28. Xu, Y., Shu, G., Liu, Z., Wang, Z., Lei, H., Zheng, Q., ... & Chen, L. (2024). Preliminary study on screening and genetic characterization of lactic acid bacteria strains with cadmium, lead, and chromium removal potentials. Fermentation, 10(1), 41.
29. Angelov, A., Georgieva, A., Petkova, M., Bartkiene, E., Rocha, J. M., Ognyanov, M., & Gotcheva, V. (2023). On the molecular selection of exopolysaccharide-producing lactic acid bacteria from Indigenous fermented plant-based foods and further fine chemical characterization. Foods, 12(18), 3346.
30. Zhang, L., Zhao, B., Liu, C. J., & Yang, E. (2020). Optimization of biosynthesis conditions for the production of exopolysaccharides by Lactobacillus plantarum SP8 and the exopolysaccharides antioxidant activity test. Indian journal of microbiology, 60(3), 334-345.
31. Xu, Y., Cui, Y., Wang, X., Yue, F., Shan, Y., Liu, B., ... & Lü, X. (2019). Purification, characterization and bioactivity of exopolysaccharides produced by Lactobacillus plantarum KX041. International journal of biological macromolecules, 128, 480-492.
32. Ajao, V., Nam, K., Chatzopoulos, P., Spruijt, E., Bruning, H., Rijnaarts, H., & Temmink, H. (2020). Regeneration and reuse of microbial extracellular polymers immobilised on a bed column for heavy metal recovery. Water Research, 171, 115472.
33. Ziadi, M., Bouzaiene, T., M'Hir, S., Zaafouri, K., Mokhtar, F., Hamdi, M., & Boisset-Helbert, C. (2018). Evaluation of the efficiency of ethanol precipitation and ultrafiltration on the purification and characteristics of exopolysaccharides produced by three lactic acid bacteria. BioMed Research International, 2018(1), 1896240.

34. Sirin, S., & Aslim, B. (2020). Characterization of lactic acid bacteria derived exopolysaccharides for use as a defined neuroprotective agent against amyloid beta1–42-induced apoptosis in SH-SY5Y cells. Scientific Reports, 10(1), 8124.
35. Guha, S., Debnath, S., & Gayen, S. (2021). Biosorption of hexavalent chromium (VI) by *Aspergillus nomius* biomass and optimization of biosorption parameters. International Journal of Ecology and Environmental Sciences, 3(1), 461-467.
36. 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.
37. Loaëc, M., Olier, R., & Guezennec, J. (1997). Uptake of lead, cadmium and zinc by a novel bacterial exopolysaccharide. Water Research, 31(5), 1171-1179.

CITATION OF THIS ARTICLE

Gayatri V, Anushree S. Assessment of Biosorption potential of ***Lactococcus lactis*** isolated from Industrial effluent-contaminated soil. Bull. Env. Pharmacol. Life Sci., Vol 14 [12] November 2025: 24-34