https://doi.org/10.48047/AFJBS.6.14.2024.11590-11604

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Characterization, Identification, and Cadmium Biosorption Capacity of Indigenous *Bacillus cereus* Strain Isolated from Industrial Contaminated Soil

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Volume 6, Issue 14, Aug 2024

Received: 15 June 2024

Accepted: 25 July 2024

Published: 15 Aug 2024

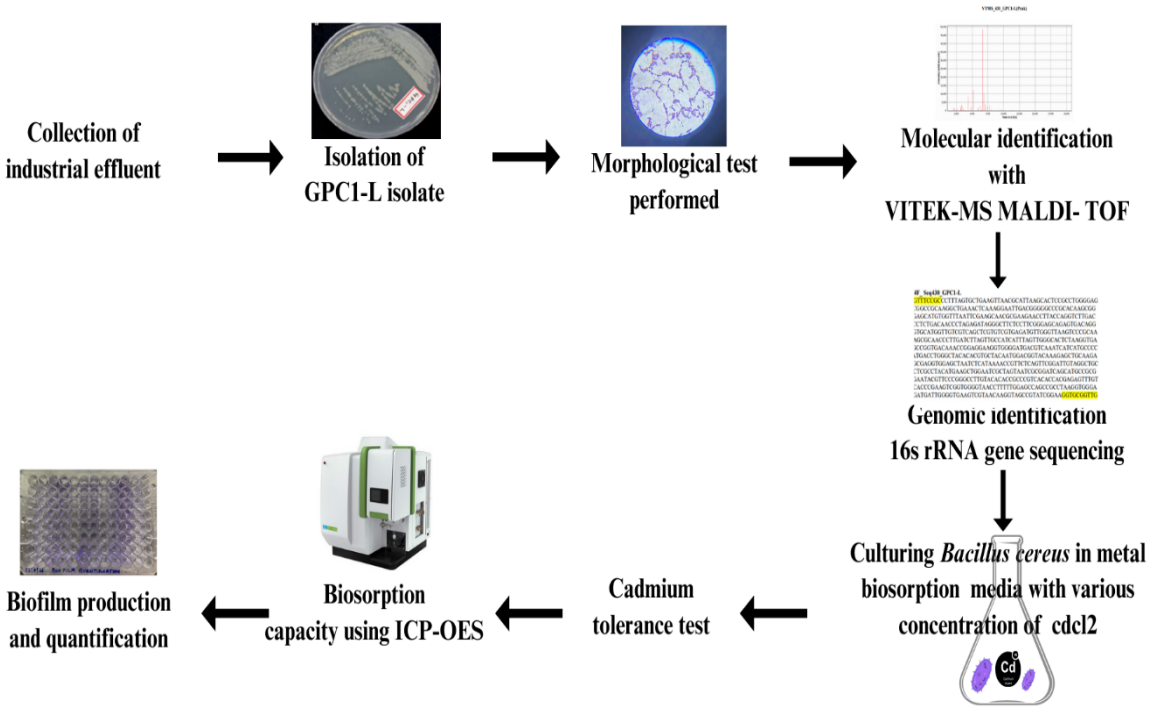
doi: 10.48047/AFJBS.6.14.2024.11590-11604

ABSTRACT

The current research on identification and characterization of cadmium-resistant Gram-positive *Bacillus cereus* (GPC1L), emphasizing its biosorption capabilities towards cadmium. Consequently, quantifying and characterizing the Exopolysaccharides (EPS) in biofilm production of the isolate GPC1 L. GPC1L was obtained from industrially contaminated soil based on high level of cadmium via enrichment culture. Subsequently, Morphological, Identification techniques of the strain GPC1L were performed using Gram staining, VITEK-MS Matrix-Assisted Laser Desorption Ionization Time-OF-Flight (MALDI-TOF) spectral analysis and 16S rRNA gene sequencing which was found to be *Bacillus cereus* in this study, Furthermore, Cadmium tolerance was evaluated which displayed peak cadmium tolerance at 6mgL $^{-1}$ over 80 hrs. Biosorption capacity of GPC1L were gauged using inductively coupled plasma optical emission spectroscopy (ICP-OES) with the highest biosorption capacity recorded at 2mgL^{-1}. Remarkably, GPC1 L exhibited significant EPS production along with biofilm with a bacterial growth of 0.24 OD (660nm). The results propose that GPC1 L, identified as *Bacillus cereus* is a potential candidate for cadmium bioremediation providing insights for an effective strategy for sustainable bioremediation of cadmium from the ecosystem.

Keywords: Biosorption, *Bacillus cereus (GPC1L)*, Cadmium, industrial contaminated soil, Exopolysaccharides

Graphical abstract:



INTRODUCTION:

In the wake of unrestrained industrialization and technological progression, driven by escalating human greed, substantial environmental degradation has emerged, leading to a marked decline in ecological quality and pervasive pollution. These industrial entities subsequently release deleterious substances through industrial effluents, encompassing toxic heavy metals into the environment (Elahi *et al.*, 2022). These pollutants contaminate soil and water systems, posing significant risks to ecosystems and human health (PUBLIC HEALTH STATEMENT, 2012). The well-recognized heavy metals with substantial health implications include cadmium, lead, zinc, copper and chromium. Non-biodegradability and extended environmental half-lives make heavy metals a major environmental problem (Siddique et al., 2024). Even in minimal concentrations, these heavy metals pose inherent risks without fulfilling any evident biological purpose (Gupta *et al.*, 2012). Bioaccumulation of heavy metals leads to disruptive cellular metabolism, inducing oxidative stress, and genomic instability. Over time, it results in carcinogenicity (Balali-Mood *et al.*, 2021).

Cadmium, one of the heavy metals chemically similar to zinc, is a significant environmental concern. Naturally occurring at 0.1 ppm in the Earth's crust, it is also a byproduct of industrial processes such as lead and zinc smelting [Bernhoft *et al.*, 2013]. Cadmium contamination in the environment predominantly arises from natural resources like erosion of rocks and soils, volcanic eruption, forest fires etc. However, major disaster was caused by anthropogenic sources like the combustion of fossil fuels, nickel and copper smelting, use of phosphate fertilizers, non-phosphorous metal smelters and electronic waste recycling, Additionally, industrial application of cadmium as a corrosive agent, PVC stabilizers, pigments and nickel-cadmium batteries further amplifying the cadmium burden (Casado *et al.*, 2008). Cadmium's high solubility in water (PUBLIC HEALTH STATEMENT, 2012) facilitates its uptake by plants and subsequent bioaccumulation in the food chain, posing significant risks to human health. Chronic exposure, often characterized by a latency period of 20-30 years, can lead

to kidney damage, osteoporosis, and neurological effects (Satarug et al., 2003). Furthermore, cadmium exposure has been linked to epigenetic changes, including DNA and histone modifications, contributing to its carcinogenic properties [Genchi *et al.*, 2020]. These factors underscore the critical need for effective cadmium remediation strategies.

Cadmium polluted environment can be remediated using a variety of physiochemical approaches, including chemical precipitation, solidification, vitrification, soil flushing, electro-reclamation (Dada *et al.*, 2015) etc. However, each of these methods requires heavy chemical input which is not only expensive but also has many side effects. Current research suggests that Bioremediation, an innovative technology harnessing living organisms or their byproducts, offers a natural and selective approach in neutralizing toxins within damaged ecosystems. Particularly, biosorption is a well-established bioremediation method that utilizes biological materials, such as dead or living microorganisms (bacteria, fungi, yeast, algae) and their components, plant material (phytoremediation), seaweeds, agricultural wastes and natural residues (Fomina *et al.,* 2014). Contemplating the microorganisms for biosorption is based on their specific characteristics. Due to the minute and ubiquitous nature of microorganisms, they proliferate rapidly in heavy metal-contaminated areas (Nanda *et al.,* 2019). Consequently, they develop tolerance and exhibit remarkable capability in converting those heavy metal pollutants into energy and raw materials. Moreover, indigenous species of microorganisms extracted from polluted environments exhibit strong biodegradation capacity due to their genetic and metabolic adaptation to that extremely harsh environment (Al-Marri *et al.,* 2023). These characteristics likely make microorganisms to be used an excellent choice for a cost-effective and sustainable method of bioremediation (Kumar *et al.,* 2011).

Bacteria being one of the microorganisms display adsorption of heavy metals because of the peptidoglycan layer they possess, Thus Gram-positive bacteria with multiple layers of peptidoglycan containing unique components (teichoic acid, amino acids and meso-diaminopimelic acid) that act as ligands in the uptake of metal ions are more efficient in adsorption of heavy metal ions compared to Gram-negative bacteria (Tayang and songachan., 2021). In scientific studies, specific bacteria like *Pseudomonas, Bacillus* and *Azotobacter* exhibit tolerance towards multiple heavy metals like cadmium, chromium, lead, zinc, nickel etc (Rizvi *et al.,* 2020). Identification of these microorganisms is crucial before assessing their biosorption capacity.

Advanced molecular methods like 16S rRNA sequencing and VITEK-MALDI-TOF aid in microbial identification. In recent years, microbiologists have adopted MALDI-TOF MS for microbial identification. This technique efficiently identifies the microbe using intact cells or their extracts, making it both sensitive and cost-effective. However, its reliance on the spectral database containing peptide mass fingerprints of specific genera, species or strains limits its accuracy for accurate in the identification of new isolates (Singhal *et al.,* 2015). 16S rRNA sequencing is an effective molecular characterization technique to analyze microbial diversity. This technique includes the examination of genetic information encoded in the 16S rRNA gene that enables accurate classification and characterization of microorganisms (Muzammila *et al.*, 2021).

Predominantly, the biosorption capability of these indigenous microbial species is determined using ICP-OES (spectrometry). ICP-OES is a meticulous analytical technique utilized to examine the surface of microbial cells, mapping the distribution of elements on the biomass cell wall and quantifying their concentrations before and after the biosorption process (Long *et al.*, 2021).

Additionally, Exopolysaccharides (EPS) in microbial biofilm are pivotal in bacterial adaptation to diverse stress conditions. One of the significant abilities includes chelating heavy metals from polluted areas and facilitating their growth in heavy metal-affected environments, consequently, these mechanisms offer promising avenues for remediating heavy metal-contaminated areas. Thus, researchers delve into the quantification and characterization of Extracellular polysaccharides (EPS) produced by indigenous bacteria, exploring their potential as potent remediators (Batool *et al.,* 2017).

The core objective of this study is to assess the heavy metal biosorption capacity of indigenous bacterial species sourced from the soil near an industrial contamination area. Collected and cultured industrial soil samples undergo various tests, including morphological characterization, identification by VITEK-MS, 16S rRNA sequencing, and ICP-OES to determine biosorption capacity, cadmium tolerance, and quantification of EPS in the bacterial species. This concludes that bioremediation using indigenous microorganisms acts as a prominent, sustainable and cost-effective bioremediation

technique for addressing cadmium-contaminated environment

MATERIALS AND METHODS

Isolation of cadmium-resistant bacteria

Industrially contaminated soil was sourced from highly heavy metal contaminated sites at GLORY PHARMA GAJULAMANDYAM, Chittoor district of Andhra Pradesh. The samples were serially diluted and plated on minimal salt agar media, followed by incubation at 37°C (Sahith et al. 2024). Enrichment culture methods were employed to extract bacterial strains. Extracted bacterial isolate, designated as GPC1 L was subjected to purification and then underwent morphological characterization.

Identification and molecular characterization of isolate GPC1 L

The isolate GPC1 L was identified using the VITEK-MS MALDI-TOF system and the resulting spectra were interpreted using the knowledge base version 3.0 database and spectral identifier R 2.1.0 software. Any identification and score value provided by VITEK-MS were deemed acceptable for data analysis. Escherichia coli ATCC 8739 served as a calibrant and quality control (Kim *et al.,* 2022). Subsequently, the genomic DNA was extracted from the isolate GPC1 L which was further subjected to the amplification of 16S rRNA gene sequence. This amplified sample was sent to CSIR – National Chemical Laboratory, Pune (India) for 16S rRNA gene sequencing and the sequencing files were analyzed by using BLAST against the closest culture sequence retrieved from the NCBI database (Kumar *et al.,* 2020).

Assessment of Cadmium tolerance of the bacteria:

The tolerance of the bacterium towards the heavy metal cadmium was evaluated by employing metal salts like CdCl2 in particular. Increasing concentrations (20 to 100 mg/l) of this heavy metal was added separately to the modified metal biosorption medium (8.1%NaCl, 0.7%MgCl2, 0.96%MgSO4·7H2O, 0.036%CaCl2, 0.2%KCl, 0.006%NaHCO3, 0.0026% NaBr, 0.5%Yeast extract, 0.3%Glucose) and subsequently inoculated with the culture of "*Bacillus cereus GPC1L*". The culture flasks were incubated at 37ºc for 5 days by maintaining a pH of 7.0. Observations were made at 20,40,60,80 and 100 hours for assessment of growth kinetics and to obtain optical density at 620nm for analysis (Tarangini *et al.,* 2009 and S. Muzammil *et al.,* 2021).

Assessment of the biosorption capacity of cadmium in Bacillus cereus GPC1 L:

The biosorption capacity of *Bacillus cereus* GPC1 L for cadmium was systematically evaluated over a range of concentrations i.e.,2,4,6,8 and 10 mg/l in culture tubes. Each tube was prepared with 50 ml of metal biosorption medium and subsequently inoculated with 20 µl of 24-hour-old bacterial culture. The samples were incubated at an optimum temperature of 37°C for a predetermined period of 20, 40, 60, 80, and 100 hours (Dutta *et al.,* 2016). Upon centrifugation, the supernatants were subjected to Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) to determine the amount of cadmium adsorbed by the biosorbent (*Bacillus cereus*) (Aida *et al.,* 2021) and further the percentage biosorption was determined using the specified formulae (Zhang *et al.,* 2010).

$$\text{Percentage Biosorption (\%)} = \frac{Ci - Cf}{Ci} \times 100$$

Ci = initial concentration of the cadmium in the solution, *Cf* = final concentration of the heavy metal in the solution after biosorption.

Biofilm (EPS) production and quantification

The culture was inoculated into TY broth until it reached stationary phase and then diluted with MGM media to attain an OD of 0.1 at 620nm, 100 µl aliquots were transferred to a 96-well microtiter plate and incubated at 30oc for 48 hrs. Subsequently, the growth was quantified at OD 620nm using a MICROELISA auto reader. Planktonic cells were removed, and the plate was rinsed with distilled water. Aqueous solution of crystal violet (0.1% w/v) was added to the plate and

incubated for 15 min. Rinse the plate and solubilize the biofilm by adding 150ul of 30% (v/v) acetic acid. Samples were transferred to a new 96-well microtiter plate and the OD of crystal violet was measured at 570 nm (Primo *et al.*, 2019).

Statistical Data Analysis:

A regression analysis in Excel was employed to examine the correlation between the dependent variable and X Variable 1. The model, expressed as Y = 2.2812 + 0.2862X, demonstrated statistical significance (P = 0.0192), indicating that the independent variable had a significant impact on the dependent variable (Table 5).

RESULT AND DISCUSSION:

Physiochemical characteristics, according to studies, play an essential influence in the development of metal tolerance in indigenous bacteria of a specific site (Shi *et al.*, 2013). Physiochemical parameters like pH of the industrially contaminated soil sample were assessed at the time of sample preparation. The pH of both the control and the sample was determined to be between 8.2 and 8.3 respectively, which shows the alkalinity of the sample. This elevated pH level in industrial effluents has deleterious effects, including nutritional imbalances in soil and impacts on microflora at the site where effluents were collected (Kaur *et al.*, 2010).

As for heavy metal concentration, the cadmium concentration of the sample was measured as 5.54 mg/l. The high concentrations of cadmium in industrially contaminated soil sample contaminate the waterbodies, posing hazardous effects on aquatic life due to its high toxicity and carcinogenicity. Moreover, cadmium accumulates in the food chain, afflicting all living organisms. Thus, heavy metal cadmium needs to be remediated from all sources of contamination (Pambudiono *et al.*, 2018).

In accordance with morphological characteristics, fourteen bacterial strains were isolated using serial dilution and enrichment culture methods (Behera *et al.*, 2019). Those fourteen isolates were coded as GPC1 A, GPC1 B, GPC1 C, GPC1 D, GPC1 E, GPC1 F, GPC1 G, GPC1 H, GPC1 I, GPC1 J, GPC1 K, GPC1 L, GPC1 M, and GPC1 O respectively. A specific isolate, GPC1 L is chosen for our current investigation as a result of the selection procedure (Figure 1).

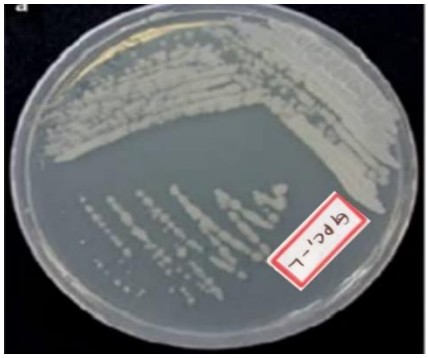


Figure 1: Pure culture of the isolate GPC1 L

The specified bacterial isolate GPC1 L was identified using morphological and molecular characterization like VITEK-MS MALDI-TOF and 16S rRNA gene sequencing. According to the morphological characterization, the Gram nature of the isolate GPC1 L is outlined as Gram-positive rods as in Figure 2, consistent with the standard morphology of Bacillus species (Lu *et al.*, 2018). Molecular characterization like VITEK-MS MALDI-TOF, ultimately affirming its alignment as **"*Bacillus cereus*"** with an identification rate of 99.9% (Table 1, Figure 3)**.** This result is similar to previous findings (Muigg *et al*., 2022; Caldeira *et al.*, 2024).

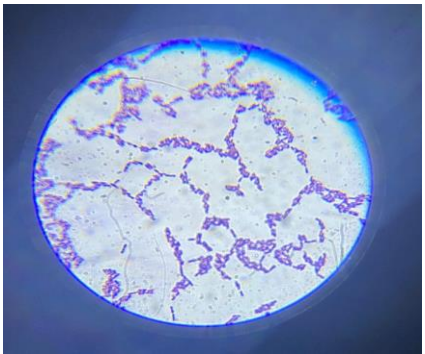


Figure 2: Gram's nature of GPC1 L (Gram positive Bacilli)

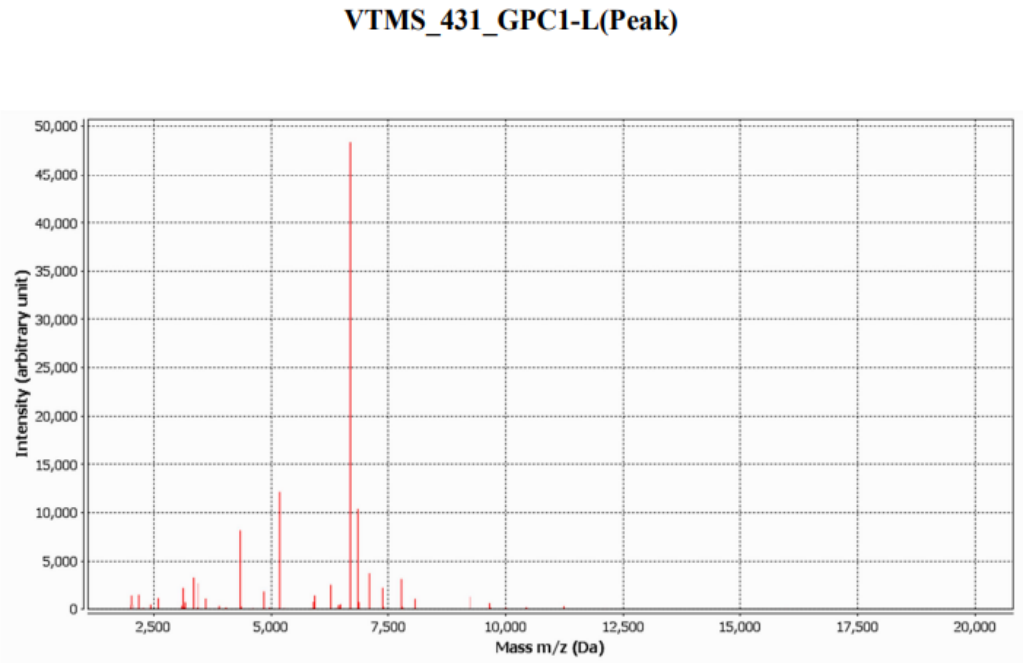


Figure 3: Spectral identification by VITEK-MS:

Table 1: Rapid identification of isolate by VITEK-MS

Sample Id	GPC1 L
Gram nature observed	Gram positive bacilli
Confidence value (%)	99.9
Confidence level	High
VITEK-MS identification	***Bacillus cereus* group**

The isolated genomic DNA underwent PCR to amplify the 16S rRNA gene, specifically targeting the isolate's strain. The resulting amplicons, representing the 16S rRNA gene sequences, were then subjected to sequencing using Sanger's method for the identification of the isolate. The comparison of these generated sequences with known 16S rRNA gene sequences in databases, such as BLAST (Basic Local Alignment Search Tool), facilitated a robust molecular characterization of the isolate. This process included contrasting the sequences with the most similar cultural sequence obtained from the NCBI database (National Centre for Biotechnology Information) (Sahith et al. 2024). This database identifies areas of proximity between sequences (Altschul *et al.,* 1990). This comparative analysis ensures a reliable and accurate assessment of the genetic identity of the *Bacillus cereus* strain, contributing to a comprehensive understanding of its molecular profile. Based on the outcomes derived from 16S rRNA gene sequencing and phylogenegtic analysis, Isolate GPC1 L unveiled a 100% with "***Bacillus cereus***" (Figure 4, 5), delivering a robust genetic outline of the isolate (Hakovirta *et al.,* 2016; Sahith et al. 2024).

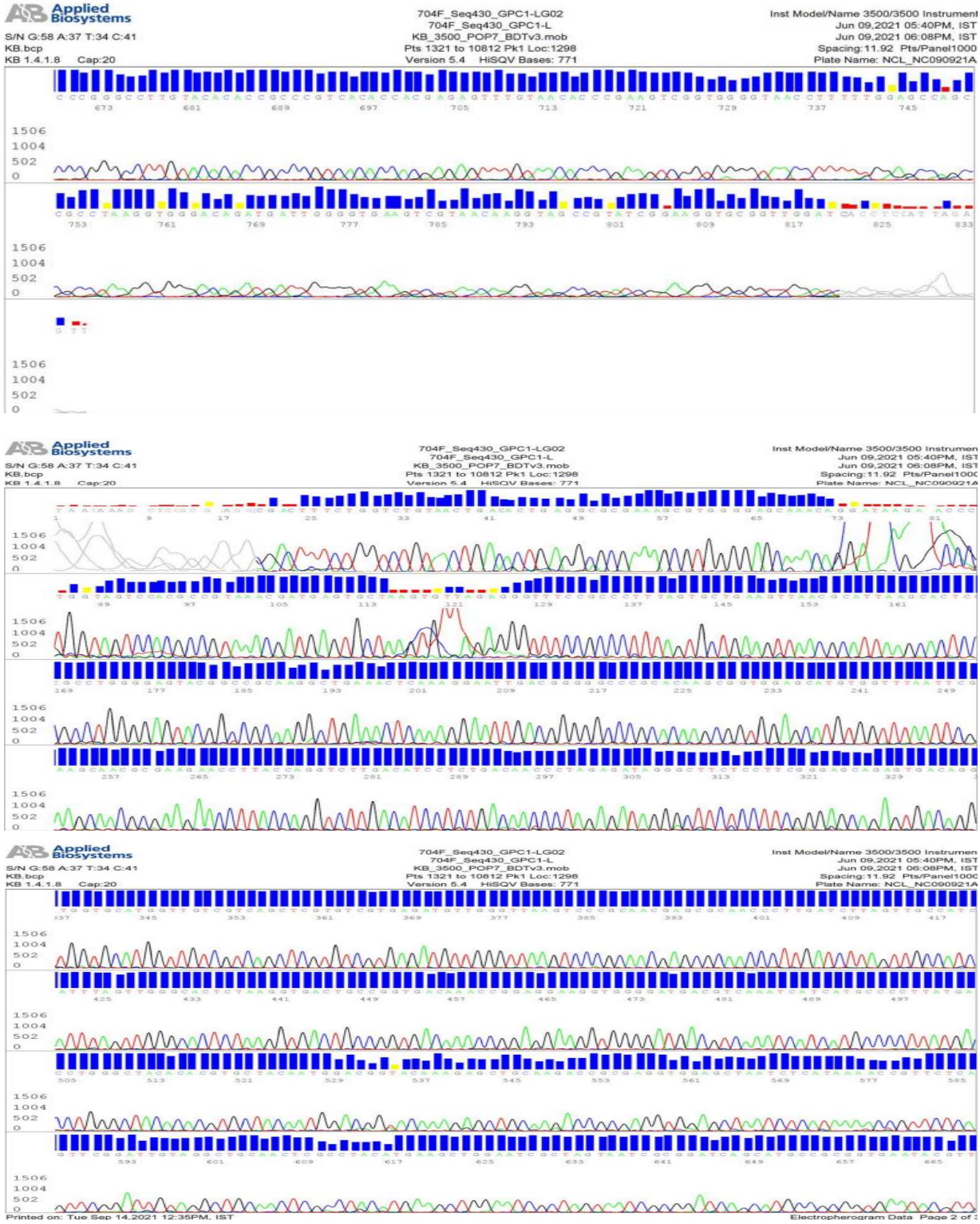


Figure 4: Sequence alignment of Bacillus cereus GPC1 L

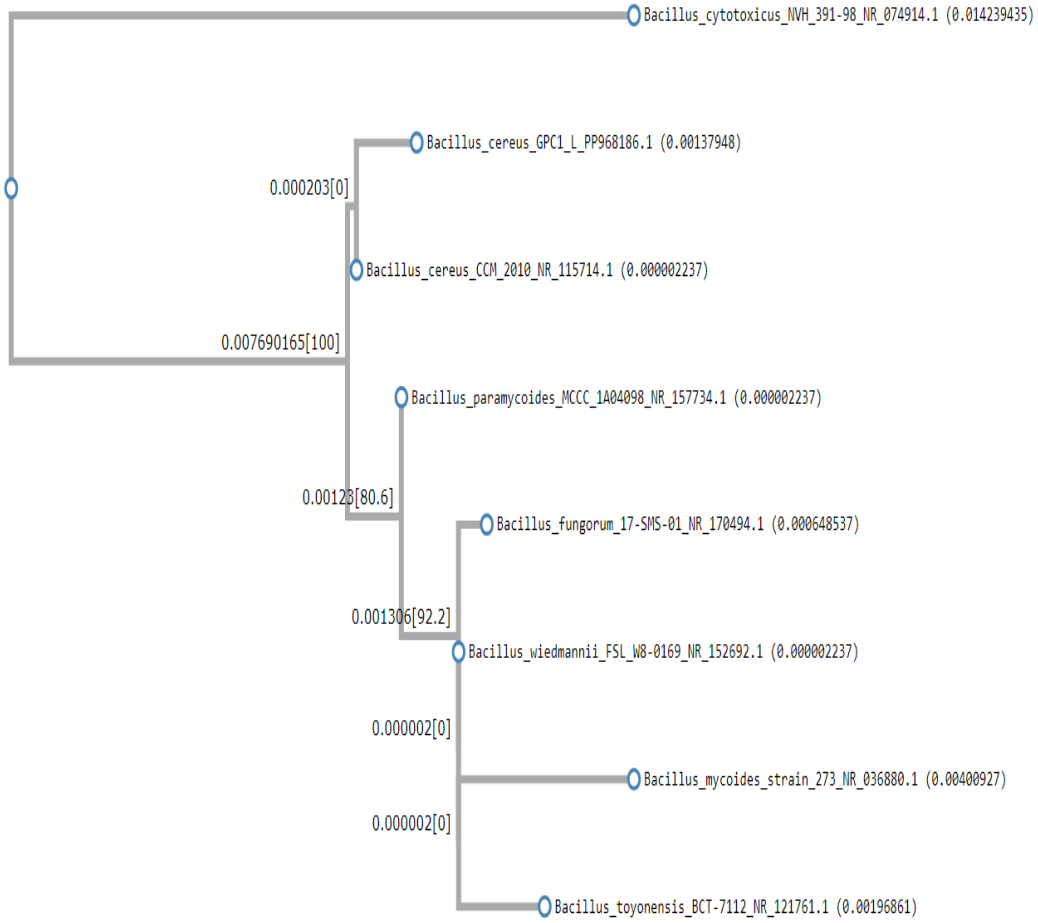


Figure 5: Phylogenetic analysis of GPC1 L

Bacteria may be able to endure certain levels of heavy metal concentrations in their polluted microenvironments; hence, such bacterial species could be potential candidates for heavy metal removal from contaminated habitats (Gonzalez and Ghneim 2021). In the investigation of bioremediation for cadmium heavy metal (Cd2+), the growth patterns of Bacillus cereus were studied at different concentrations of cadmium (2, 4, 6, 8 and 10mg/l). The tolerance test revealed the remarkable ability of the GPC1-L isolate to withstand the presence of cadmium, with the highest tolerance observed at 6mg/l. Significantly, an increase in cadmium concentration corresponded to a reduction in optical density. This decline in optical density serves as a clear indicator of the toxic impact of cadmium on bacterial growth (Table 2) (Figure 6).

Table 2 : Growth of Bacillus cereus in at various concentration of cadmium heavy metal (Cd tolerance)

Cadmium (Cd) concentration (mg/l)	**(Growth) OD values at 660nm**				
	Time of Incubation (hours)				
	20	40	60	80	100
2 mg/L	0.07	0.10	0.15	0.16	0.14
4 mg/L	0.05	0.07	0.11	0.14	0.12
6 mg/L	0.04	0.05	0.09	0.12	0.10
8 mg/L	0.03	0.04	0.06	0.11	0.08

10 mg/L	0.01	0.03	0.05	0.09	0.05

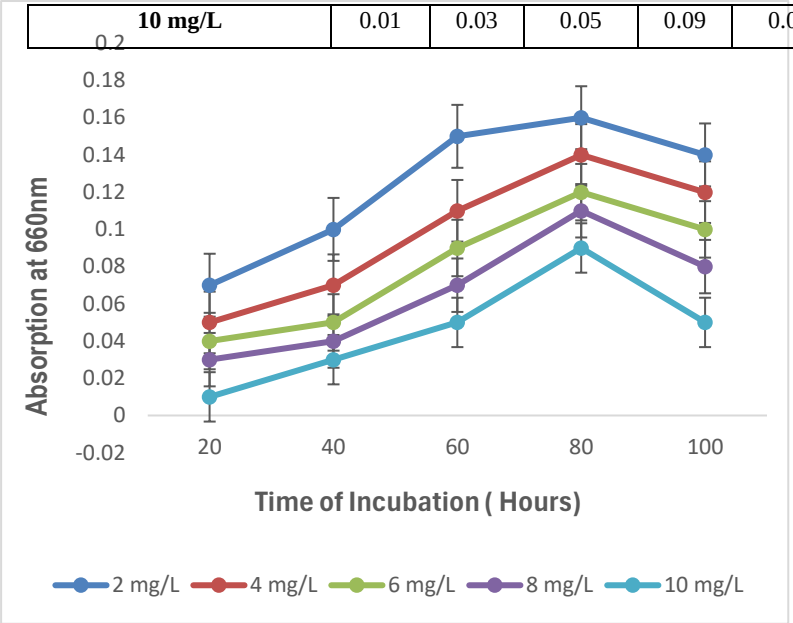


Figure 6: Growth of *Bacillus cereus* GPC1 -L at various concentrations Of $CdCl_2$ with error bars signifies the ± Standard error

Error ((i)Time of incubation vs Percentage Biosorption) and ((ii)Concentration of Cadmium vs Percentage Biosorption) respectively.

Subsequent scrutiny utilizing Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) disclosed distinctive cadmium biosorption phenomena exhibited by ***"Bacillus cereus GPC1 L".*** At an initial cadmium concentration of 2mg/l, the isolate exhibited a gradual increase in cadmium absorption over time. After 20 hours of incubation, it had absorbed 0.844mg/l (42.4% biosorption percentage) of cadmium followed by 1.254mg/l (63.05%), 1.478mg/l (74.34%) at 40, 60 hours respectively and this absorption capacity improved significantly at 80 hours, with the isolate absorbing 1.696mg/l (85.30 %) of cadmium with a subsequent decline value 1.572mg/l (79.03%) at 100 hours incubation period. The percentage biosorption increased to indicate a higher efficiency at 80 hours (85.30%). Likewise, at a higher cadmium concentration of 4mg/l, the isolate demonstrated a rapid cadmium uptake as well. After 20 hours, it had already absorbed 1.604mg/l (40.23%) of cadmium, and this absorption continued to increase during the 40,60,80-hour incubation period, reaching from 2.330mg/l (58.26%), 2.688mg/l (67.17%) to 3.088mg/l (77.23%) with slight decline of 2.887mg/l (72.17%) at 100 hours incubation period. The percentage biosorption of cadmium was notable at both time points, with a slightly higher efficiency observed after 80 hours (77.23%) (Table 3).

When exposed to an even higher cadmium concentration of 6mg/l, the isolate exhibited a slower cadmium uptake rate than before. After 20 hours, it had absorbed 2.348mg/l (39.34%) of cadmium, and this absorption capacity increased to 3.326mg/l (55.62%), 3.721mg/l (62.21%), 4.371mg/l (73.03%) after 40, 60, 80 hours of incubation respectively followed by decline with 3.916mg/l (65.48) at 100 hours of incubation. The biosorption percentage also showed a significant improvement over time, rising from 39.34% at 20 hours to 73.03% at 80 hours (Table 3).

Table 3: Biosorption capacity of *Bacillus cereus* GPC1 -L

Concentration of Cadmium (mg/L)	**Time of Incubation (Hours)**	**Control**	**Test**	**Removal capacity**	**Percentage Biosorption (%)**

2 mg/L	20	1.989	1.145	0.844	42.4
	40	1.989	0.735	1.254	63.04
	60	1.989	0.511	1.478	74.34
	80	1.989	0.293	1.696	85.3
	100	1.989	0.417	1.572	79.03
4 mg/L	20	3.998	2.394	1.604	40.23
	40	3.998	1.668	2.33	58.26
	60	3.998	1.31	2.688	67.17
	80	3.998	0.91	3.088	77.23
	100	3.998	1.111	2.887	72.17
6 mg/L	20	5.986	3.638	2.348	39.34
	40	5.986	2.66	3.326	55.62
	60	5.986	2.265	3.721	62.21
	80	5.986	1.615	4.371	73.03
	100	5.986	2.070	3.916	65.48
8 mg/L	20	7.995	5.436	2.559	32.0
	40	7.995	3.897	4.098	51.25
	60	7.995	3.286	4.709	58.92
	80	7.995	2.712	5.283	66.05
	100	7.995	3.945	4.050	50.74
10 mg/L	20	9.998	7.448	2.550	25.45
	40	9.998	6.098	3.900	39.0
	60	9.998	5.264	4.734	47.4
	80	9.998	4.977	5.021	50.27
	100	9.998	6.348	3.65	36.58

Cadmium biosorption capacity declined further with higher concentrations, at 8mg/l of cadmium concentration the biosorption capacity is as follows 2.559mg/l (32%), 4.098mg/l (51.25%), 4.709mg/l (58.92%), 5.283mg/l (66.05%), 4.050mg/l (50.74%) after 20, 40, 60, 80, 100 hours of incubation period. Where the highest biosorption is observed at an 80-hour incubation period. Furthermore, at 10mg/l cadmium concentration, the biosorption capacity declines compared to lower concentrations with values 2.550mg/l (25.45%), 3.900mg/l (39%), 4.734mg/l (47.4%), 5.021mg/l (50.27%), 3.650mg/l

(36.58%) after 20,40,60,80 and 100 incubation periods respectively.

These findings demonstrate that the isolate's capacity to absorb cadmium is affected by both the initial concentration of cadmium and the duration of incubation.

The experiment yielded conclusive results, affirming that the isolate exhibited its maximum biosorption capacity at lower concentrations, specifically at a concentration of 2mg/l (Figure 7). This discovery highlights the isolate's effectiveness in eliminating cadmium, a heavy metal contaminant, from the surrounding environment. Furthermore, the trial length of 80 hours for incubation was shown to be the most effective period for cadmium elimination. During this extended incubation period, the isolate had sufficient time to interact with and sequester the cadmium ions effectively, resulting in the most efficient removal of this hazardous heavy metal from the environment.

(i) Time of incubation vs Percentage Biosorption **(ii) Concentration of Cadmium vs**

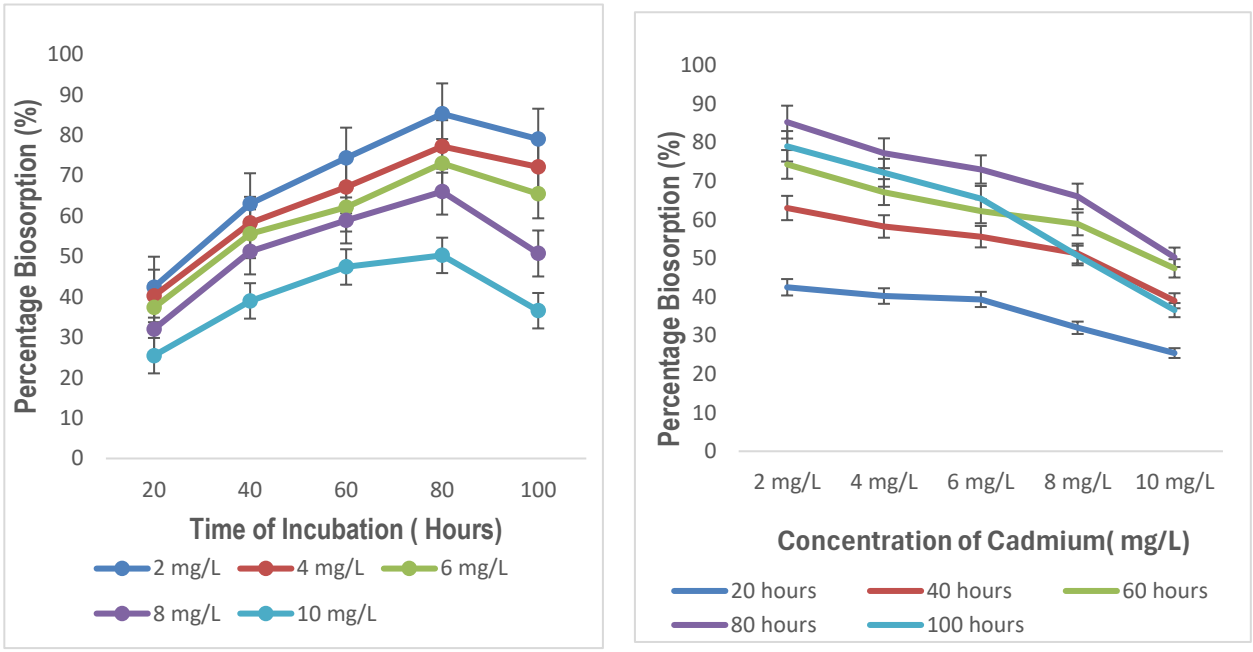


Figure 7: Percentage biosorption of *Bacillus cereus* GPC1 -L with error bars signifies the ± Percentage

Similarly, previous studies made on cadmium adsorption by *Bacillus* species have yielded significant findings. *Bacillus cereus* on eggshell *demonstrated* an adsorption capacity of 137.97 ± 4.031 mg/g (Rasheed *et al.,* 2023). *Bacillus licheniformis exhibited a lower* adsorption capacity of 24.51 mg/g (Baran *et al.*, 2021). Notably, immobilized viable Bacillus cereus RC-1 achieved a biosorption capacity of 158.77 mg/g (Huang *et al.*, 2020).

Furthermore, the inquiry delved into examining the participation of Exopolysaccharides (EPS) in the process of biofilm formation, a fundamental mechanism crucial for bacterial adaptation. Exopolysaccharides (EPS) are polymeric compounds secreted by bacteria when they proliferate in an abundant environment or after establishing in a suppressive environment. They play an important part in the production of bio-films that grow on substrates in situations where other organisms cannot colonize (Sutherland., 2001). In some species, EPS play an important role in instilling antibiotic resistance in the organism by preventing antibiotic permeability (Singh *et al.*, 2021). *"Bacillus cereus GPC1-L"* showcased the proficiency to generate biofilms after a 5-day incubation period, as substantiated by an optical density (OD) measurement of 0.29 at 660nm and a biofilm formation capacity of 0.17 at 570nm. The establishment of biofilms assumes a pivotal role, augmenting the enduring presence and flexibility of bacterial strains within their surroundings. Comprehensive details regarding these discoveries are accessible in Table 4.

Table 4: Evaluation of Biofilm

NAME OF THE ISOLATE	Bacillus cereus GPC1 L
GROWTH OD AT 660nm	0.29
QUANTIFICATION OD AT 570nm	0.17

In conclusion, the study proficiently isolated and comprehensively characterized *Bacillus cereus* GPC1 L, an indigenous bacterial strain from cadmium-contaminated industrial effluents, illustrating its adaptation to elevated heavy metal levels and substantial potential for bioremediation. By employing morphological and molecular characterization, including VITEK-MS MALDI-TOF and 16S rRNA gene sequencing, *Bacillus cereus* was identified with high precision. The strain displayed remarkable cadmium biosorption capabilities, with maximum adsorption efficiency of 85.30% at 2 mg/l cadmium concentration for 80 hours of incubation period. Furthermore, its biofilm formation was observed and quantified, accentuating its adaptability and persistence in cadmium-contaminated environments. These observations emphasize *Bacillus cereus* as a potential indigenous strain that serves as a substantial and sustainable agent to bioremediate cadmium from contaminated sites as well as in combating heavy metal pollution and restoring ecological balance.

Table 5: Statistical data analysis using regression

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.464743
R Square	0.215986
Adjusted R Square	0.181898
Standard Error	1.151721
Observations	25

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	8.404718	8.404718	6.336195	0.019249682
Residual	23	30.50861	1.326461		
Total	24	38.91333			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	2.281215	0.400877	5.690559	8.558E-06	1.451937221	3.110492	1.451937	3.110492
X Variable 1	0.286151	0.113679	2.51718	0.019249	0.050987622	0.521314	0.050988	0.521314

ACKNOWLEDGEMENTS

The author sincerely acknowledges her deep sense of gratitude to project in-charge and supervisor Prof. Ch. Paramageetham, HOD, Department of Microbiology, Sri Venkateswara University, Tirupati for suggesting this project work and valuable guidance. Additionally, the author's gratitude is extended to Zuari Agro Chemicals Ltd for conducting the ICP-OES analysis.

DECLARATION OF POTENTIAL CONFLICTS OF INTEREST

The authors affirm that there is "No conflict of interest."

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