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Cadmium-Tolerant Plant Growth-Promoting Bacteria *Curtobacterium oceanosedimentum* Improves Growth Attributes and Strengthens Antioxidant System in Chili (*Capsicum frutescens*)

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Abstract: The remediation of potentially toxic element-polluted soils can be accomplished through the use of microbial and plant-assisted bioremediation. A total of 32 bacteria were isolated from soil samples contaminated with potentially toxic elements. The isolated bacterial strain DG-20 showed high tolerance to cadmium (up to 18 mM) and also showed bioaccumulative Cd removal properties, as demonstrated by atomic absorption spectroscopy studies. By sequencing the 16S rRNA gene, this strain was identified as *Curtobacterium oceanosedimentum*. Under stress and normal conditions, isolate DG-20 also produced a wide range of plant growth promoting traits, including ammonia production (51–73 µg/mL) and IAA production (116–183 µg/mL), alongside siderophore production and phosphate solubilization. Additionally, pot experiments were conducted to determine whether the strain could promote Chili growth when Cd salts are present. Over the control, bacterial colonization increased root and shoot lengths significantly up to 58% and 60%, respectively. Following inoculation with the Cd-tolerant strain, the plants also increased in both fresh and dry weight. In both the control and inoculated plants, Cd was accumulated more in roots than in shoots, indicating that Chili was phytostabilizing Cd levels. Besides improving the plant attributes, Cd-tolerant bacteria were also found to increase the amount of total chlorophyll, proline, total phenol, and ascorbic acid in the soil when added to the soil. These results suggest that the inoculant provides protection to plants from negative effects. The results of the present study predict that the combined properties of the tested strain in terms of Cd tolerance and plant growth promotion can be exploited for the purpose of the bioremediation of Cd, and for the improvement of Chili cultivation in soils contaminated with Cd.

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1. Introduction

Soil serves as an important foundation for agricultural resources, the environment, and a secure and healthy food supply, and continues to be vital for sustainability in the world [1]. Over the past few decades, the development of industry and urbanization have been a significant factor resulting in the reduction of cultivable land and a rise in soil pollution. In agricultural fields, usage of agrochemicals are gradually increasing, with a higher concentration of potentially toxic elements due to various reasons, such as mineral mining, urban sewage disposal [2], discharge of toxic bi-products from nickel-cadmium battery manufacturing, leather tanning, and metal alloying, etc. Potentially toxic elements, especially Cadmium (Cd) and Lead (Pb), are able to translocate from soil to plants and eventually into higher trophic levels [3,4].

Cd is a potentially toxic element found in nature. It is dangerous to all living organisms, including plants and animals [5,6]. When plants experiences toxic Cd, food chains are also probably affected by it, which may further lead to several human health problems such as renal failure, osteoporosis, liver cirrhosis, and Itai-Itai diseases [7–11]. It inhibits growth; activates or inhibits enzymes; affects water balance and ion transport; interferes with chlorophyll biosynthesis; inhibits various enzymes involved in the Calvin cycle; disrupts the evolution of O_2 over photosystem II (PSII); affects the transfer of electrons between PSI and PSII; and inhibits the activity of a variety of enzymes including carbonic anhydrase, $NADP^+$ glyceraldehyde-3-phosphate dehydrogenase, phosphoenolpyruvate carboxylase, ribulose-1,5-bisphosphate carboxylase oxygenase, fructose bis-phosphatase, and fructose-6-phosphate kinase [12,13]. Therefore, different types of changes can be observed in plant bodies due to Cd toxicity, including morphological [14], physiological, and biochemical [15] and photochemical changes [16–19].

There are a number of reclamation techniques to handle the life-threatening problems resulting from potentially toxic elements [20–22]. The techniques may include vitrification and stabilization in situ and ex situ [23–26]. One disadvantage of these techniques is the cost, as they can only be used on some areas of land and can be extremely expensive. Researchers have begun using hyper-accumulator plants for phytoremediation as an eco-friendly and widely recognized remedy [27,28]. However, plants grow slowly in most of these situations. Problems also exist in growing plants that are not hyper-accumulators of Cd and are not capable of surviving at all in even low levels of potentially toxic elements.

Therefore, some plant growth-promoting rhizobacteria (PGPR) that are Cd-tolerant can be a promising substitute and the future preference for the resilient development of agriculture systems under potentially toxic elements polluted conditions [5,29]. Among the different methods that are used to deal with metal stress, bacteria resistant to Cd use biosorption and bioaccumulation within cells [30,31], as well as metal volatilization, oxidative/reduction/enzymatic reduction, and biological precipitation are also possible [32]. There are different types of PGPR that stimulates phytoextraction [33–35], while others release metal chelating agents into the soil to prevent metal mobility to plant parts [36–38].

The PGPR can increase plant growth through either direct or indirect means [39,40]. These PGPR have the ability to promote growth through a variety of mechanisms, including the fixation of nitrogen [40], solubilization of insoluble phosphate [39], and chelation of iron by iron chelating compounds, known as siderophores. The process of enhancing plant growth can be indirectly accomplished by the induction of antibiosis and systemic resistance. PGPR also produces enzymes to degrade cell wall materials, they can produce antifungal compounds, and can reduce iron rhizospherically. In soil contaminated with Cd or Pb, a variety of strains of bacteria that promote plant growth have been found [33,41,42]. Some of these includes *Bacillus*, *Micrococcus*, *Klebsiella*, *Bradyrhizobium*, *Enterobacter*, and

Pseudomonas. Presently, PGPRs resistant to potentially toxic elements have been isolated and identified in nature from diverse sources [5,43–47]. Nonetheless, it remains to be seen how these resources can be used effectively on a large scale. Consequently, further research is needed to explore previously described potentially toxic element tolerances, such as Cd. PGPR strains that are able to survive in adverse ecological conditions contribute to sustainable agriculture. The Daman Ganga riverside in Vapi has been highly contaminated with many industries and urban effluents, which have potentially toxic elements in them [5], and according to the study of the Central Pollution Control Board in India, the effluent that emerged from Vapi's common effluent treatment plant (CETP) does not meet the stipulated standards. Previous research has shown that bacterial strains isolated from the polluted rhizospheric soils are able to tolerate different toxic metals, and also promote plant growth under heavy metal stress conditions [5,43]. Consequently, PGPRs were isolated from the rhizosphere of *Salix purpurea* L., collected from the metal contaminated site in the study. Therefore, the present study was designed to characterize, identify, and explore the possibilities of PGPR species that have potent biosorption abilities and are Cd-tolerant, among the species cultured.

2. Materials and Methods

2.1. Sample Collection and Isolation

The four composite soil samples were collected from Daman Ganga riverside (20°20′29.72″ N; 72°54′15.74″ E), Vapi, Gujarat. The collected rhizospheric soil was dark black in color and clay in texture. A sterile bag was used to collect the samples and ice boxes were used to transport them to the laboratory. As per the Indian standard methods of test for soils, soil physicochemical properties were determined. Using sterile distilled water, 1 g of soil sample was serially diluted up to 10^{-8}, and the last three dilutions (100 µL) were spread on nutrient agar for bacterial isolation. Each plate was incubated for 48 h at 37 °C. After collecting, purifying, and maintaining the morphologically distinct bacteria (size, shape, margin, and elevation) on the same nutrient agar medium at 4 °C, subsequent experiments were conducted [48].

2.2. Screening for Cd-Tolerant Ability

During the subsequent screening process, 32 morphologically distinct bacterial isolates isolated from rhizospheric soil samples were tested for Cd tolerance. In sterile distilled water, 1 M stock solution of $CdCl_2.H_2O$ (Mwt-201.32) was used to prepare initial metal concentrations of 1 mM. The isolates were transferred from 1 mM to the next concentration (1 mM interval), once they had been grown at a given Cd concentration. For further testing, the isolate DG-20 with the highest Cd tolerance (18 mM) was selected based on screening.

2.3. Bioaccumulation of Cd in DG-20

The bioaccumulations of Cd in bacterial cells were determined based on the method outlined in Chiboub et al. (2016) [49], with some modifications. Briefly, the pure active culture of DG-20 (OD/absorbance at 600 nm = 0.8) was inoculated into 100 mL of nutrient broth, having 1 mM Cd^{2+} and incubated at 37 °C under shaking conditions at 120 rpm. Media with growing cells were collected at regular 12 h time intervals and centrifuged for 10 min at 10,000 rpm. The cell pellets were washed with sterilized distilled water and further agitated (120 rpm) with 10 mM sterilized EDTA at 30 °C for 20 min to remove Cd ions from the surface of the cell. After incubation, cell suspensions were centrifuged for 20 min at 10,000 rpm and cells were re-suspended in 5 mL of 0.1 M HNO_3, and then again centrifuged at 10,000 rpm for 30 min. For cell wall-bound (cell wall) Cd^{2+}, the supernatant was used, whereas dry cell pellets (intracellular, digested with 3:1 H_2SO_4-$HClO_4$ at 110 °C for 3 h and diluted with distilled water) were used for the determination of intracellular accumulated Cd^{2+}.

2.4. Plant Growth-Promoting Properties of DG-20

Under normal and stressed conditions, plant growth promoting properties such as indole-3-acetic acid (IAA), ammonia, siderophore production, phosphate and potassium solubilization, and enzyme production were evaluated. For stress conditions, 9 mM Cd was added to each PGP test.

2.4.1. IAA Production

A Luria broth (LB) containing 5 µg/mL of L-tryptophan was used to produce IAA by DG-20 isolate. In order to analyze IAA production, the inoculated broth was incubated for 72 h. Centrifugation was carried out to collect the supernatant following incubation. A supernatant (2 mL) was mixed with Salkowski reagent (4 mL) (50 mL—35% perchloric acid, 1 mL—0.5 M $FeCl_3$) and orthophosphoric acid (2 drops) and then incubated for 30 min at 37 °C. The absorbance at 530 nm was measured using a spectrophotometer. Using an IAA standard, the concentration of IAA production was determined in µg/mL [50].

2.4.2. Ammonia Production

Ammonia was produced by inoculating the isolate for five days on peptone broth at 37 °C. After incubation, the culture supernatant was centrifuged, and 0.5 mL was mixed with 1 mL of Nessler's reagent. With the addition of ammonia-free distilled water, the mixture volume was adjusted up to 10 mL. A spectrophotometer was used to measure absorbance at 450 nm [51]. A standard of ammonium chloride (0–50 µg) was used to calculate the amount of ammonia produced, which was expressed in µg/mL.

2.4.3. Phosphate Solubilization

The ability of the isolate to solubilize phosphate was studied using Pikovskaya medium supplemented with 0.5 g/100 mL of tricalcium phosphate. After streaking, plates were incubating at 37 °C for 72–96 h. Clear zones around the colonies indicated that the inorganic phosphate had been solubilized [52].

2.4.4. Potassium Solubilization

The solubilization of potassium in Aleksandrov medium was determined by adding mica powder (100 mg/100 mL) to the mixture [53]. Plates were incubated for 72–96 h at 37 °C. Clear zones surrounding the colony indicated potassium solubilization.

2.4.5. Siderophore Production

Chrome azurol S (CAS) agar plates were used to test the bacteria's ability to produce siderophore. To prepare the agar plate, it was amended with CAS dye (60.5 mg), iron III solution (1 mM $FeCl_3.H_2O$), and hexadecyltrimethylammonium bromide (72.9 mg). The active culture of bacteria was streaked and incubated at 37 °C for 72–96 h. The development of yellow orange zones around the colonies indicated that siderophores were being produced [54].

2.4.6. Screening of Extracellular Enzyme Production

The extracellular hydrolytic enzymes (chitinase, pectinase, amylase, and cellulase) have all been measured on agar plates using modified CMC, pectin, starch, and colloidal chitin [55]. Isolate DG-20 was streaked on the respective (normal and 9 mM Cd incorporated) plates and incubated at 37 °C for 2–3 days. The presence of a clear zone around the bacterial colony indicated a positive result.

2.5. Identification of Potent Bacterial Strain DG-20

In order to isolate DNA from the bacterial strain DG-20, nutrient broth medium was used as a growth medium. In line with the method described by Wilson (2001) [56], the DNA was extracted by the NaCl-CTAB method. In 20 µL of TE buffer, extracted DNA was dissolved and used as a template for 16S rRNA gene amplification. Amplification

of the target DNA was performed in a volume of 50 µL by mixing 20 ng of the template DNA with 2.5 mM concentration of dNTPs, 1 mM concentration of each universal primer (reverse-1492r and forward-27f), and 3U of Taq DNA polymerase in 10X Taq buffer (Thermo Fisher Scientific, Bengaluru, India). The amplification procedure was performed using a GeneAmp PCR system 9700 (Applied Biosystems, Waltham, MA, USA) with the conditions described by Desai and Patel (2019) [57]. As part of the Sanger sequencing process, a purified PCR amplicon was sequenced at Eurofins Genomic India Pvt Ltd., Bangalore, India. The obtained sequence was subjected to sequence match analysis using Nucleotide Basic Local Alignment Search Tool (BLASTn) on NCBI.

2.6. Effect of Cd-Tolerant Bacterial Strain DG-20 on Chili Growth

The soil samples for the pot experiment were collected from the Veer Narmad South Gujarat University's greenhouse. Drying, sieving, and sterilization of the collected soils was carried out at 121 °C for 15 min at 15 lbs. In each pot there was 1 kg of sterile soil, which was artificially contaminated with 2 g $CdCl_2.H_2O$ (Himedia®, Mumbai, India) and then thoroughly mixed. Initially, the surface of the Chili seeds was sterilized with 70% ethanol for two minutes, followed by 2% sodium hypochlorite for two minutes; it was then washed twice with sterile distilled water for three minutes and dried on sterile filter paper. The pure culture of isolate DG-20 was grown in nutrient agar broth at 37 °C for 24 h and then diluted in sterile saline water to a final concentration of 10^8 CFU/mL. The surface sterilized seeds were immersed for two hours in a Cd-tolerant DG-20 isolate and then dipped into distilled water (control); it was then air dried before being sown (15 seeds per pot). The following treatments were performed in three replicates: (1) C1, control-1 (without bacterial and Cd treatment); (2) C2, control-2 (Cd alone); (3) T1, Cd + DG 20; and (4) T2, DG 20 alone. The pots were arranged in a completely random factorial design. Watering was carried out regularly to maintain moisture levels and temperature was maintained between 30 and 37 °C, along with a relative humidity of 58%, in a greenhouse with a natural light cycle of 13–14 h in summer. From the pots, the seedlings were removed after 30 days and washed for measuring root and shoot length and dry and wet weight, as well as for assessing Cd accumulation in the plant and its physiological properties [58].

2.7. Estimation of Cd in Plant Sample

In the oven, all plant samples were dried at 70 °C after being washed with distilled water. Acid digestion was performed on dried samples using a mixture of 2 M HCl and 1 M HNO_3 for 6 h [43]. Then, a 0.45 µm syringe filter was used to filter the digested samples. The Cd content in the plant parts were estimated by atomic absorption spectrophotometer. Atomic absorption measurements were performed with an air-acetylene flame. The following conditions were used: slit widths of 6 nm, an absorption line of 228.8 nm, and lamp currents of 4 mA. Using conventional aspiration, 500 µL of the final sample solution was introduced into the nebulizer. A different concentration of Cd standard solution was used for calibration. The blank solution was also subjected to a similar procedure and measured in parallel to the sample solutions.

2.8. Influence of Cd-Tolerant Bacterial Strain DG-20 on Physiological Properties of Chili

2.8.1. Chlorophyll Content Estimation

The chlorophyll a and b content was determined via the method described by Panda et al. (2008) [59]. Leaf samples of 0.5 g were homogenized in 5 mL of 80% acetone (v/v). A centrifuge at 5000 rpm for five minutes was used to separate the mixture. The step was repeated until a colorless residue was obtained. The absorbance was measured at 645 nm and 663 nm. Using the following formula, chlorophyll content was determined:

$$Chl^a = (12.7 \times A_{663} - 2.69 \times A_{645}) \times V/(1000 \times w) \quad (1)$$

$$Chl^b = (22.9 \times A_{645} - 4.68 \times A_{663}) \times V/(1000 \times w) \quad (2)$$

where the volume of the total extract is V and the fresh weight of the leaf is w.

2.8.2. Proline Content Estimation

Proline content was determined from leaves following the protocol by Upadhyay et al. (2012) [60]. To begin the assay, 0.5 g of leaves were ground in 5 mL of 3% sulphonyl salicylic acid ($C_7H_6O_6S$) at 4 °C. The mixture was then centrifuged for 10 min at 10,000 rpm. Afterwards, 2 mL of the supernatant was mixed with equal volumes of glacial acetic acid (60% v/v) and ninhydrin solution (1% w/v). In a boiling water bath, the reaction mixture was kept for a period of 1 h. A cooling bath was used after incubation to stop the reaction. Each reaction tube was then mixed thoroughly with 4 mL of toluene. After collecting the aqueous phase, the absorbance at 520 nm was measured. Proline content was expressed as µg/g of tissue.

2.8.3. Total Phenol Content Estimation

For determining the concentration of phenol, the Folin–Ciocalteu method was used [61]. A fresh leaf sample was oven dried at 75 °C and ground using a mortar and pestle. To prepare the alcoholic extract, the homogenate sample was boiled in 10 mL of 80% ethyl alcohol in a water bath for 10 min. The extract was cooled and centrifuged for 10 min at 6000 rpm. In 2 mL of supernatant, Folin–Ciocalteu reagent at 0.5 mol/L and 20% Na_2CO_3 were added. The absorbance was measured at 760 nm. A gallic acid equivalent (mg gallic acid/g fresh weight) was used to express the phenol content.

2.8.4. Ascorbic Acid Estimation

1 g of leaf sample was homogenized in 5% ice-cold trichloroacetic acid to determine ascorbic acid content. At 4 °C, the homogenized mixture was centrifuged at 10,000 rpm for 10 min. After collecting the supernatant, 0.5 mL of it was mixed with 0.2 mL of 0.66% sodium molybdate, 0.2 mL of 0.05 N H_2SO_4, and 0.1 mL of 0.025 mM sodium phosphate before being incubated for 40 min at 60 °C in a water bath. The mixture was cooled and centrifuged for 5 min at 5000 rpm at the end of incubation. After that, the absorbance was measured at 660 nm. The concentration of ascorbic acid is expressed in µg/g of wet weight [62].

2.9. Statistical Analysis

All experiments were carried out in triplicate. Results are presented as mean ± SD of the number of experiments performed. The significance of the results was determined among the treatments using one way ANOVA followed by Tukey's post hoc test and Student t-tests at $p < 0.05$. The analyses were carried out using the software Graph Pad Prism 5.0.

3. Results

3.1. Physicochemical Properties of Soil Sample

The physicochemical properties of the soil sample and the levels of potentially toxic elements were determined (Table 1). It was determined that the pH of soil was nearly neutral (7.7 ± 0.05). The high content of organic carbon (2.25 ± 0.04%) and phosphorous (423.45 ± 12.05 mg/kg) were the most prominent elements in the sample, followed by potassium (267 ± 15.13 mg/kg), magnesium (45.63 ± 2.92 mg/kg), sulfur (40.16 ± 1.4 mg/kg), calcium (21.56 ± 3 mg/kg), and zinc (5.04 ± 0.32 mg/kg). Several potentially toxic elements were found in the sample, including arsenic, chromium, cadmium, nickel, and cobalt, with concentrations of 0.016 ± 0.001, 0.012 ± 0.001, 0.023 ± 0.002, 0.032 ± 0.002, and 0.015 ± 0.001 ppm, respectively.

Table 1. Physicochemical properties and potentially toxic elements analysis of rhizospheric soil sample collected from Daman Ganga (river in Western India) riverside.

Properties	Values
pH	7.7 ± 0.05
Organic carbon (%)	2.25 ± 0.04
Phosphorous (mg/kg)	423.66 ± 12.05
Potassium (mg/kg)	267 ± 15.13
Magnesium (mg/kg)	45.63 ± 2.92
Sulphur (mg/kg)	40.16 ± 1.4
Calcium (mg/kg)	21.56 ± 3.0
Zinc (mg/kg)	5.04 ± 0.32
Arsenic (ppm)	0.016 ± 0.001
Chromium (ppm)	0.012 ± 0.001
Cadmium (ppm)	0.023 ± 0.002
Nickel (ppm)	0.032 ± 0.002
Cobalt (ppm)	0.015 ± 0.001

3.2. Isolation, Screening, and Identification of Potent Cd-Tolerant Bacterium

In the present study, a total of 32 morphologically distinct bacterial strains were isolated from potentially toxic element-polluted rhizosphere soils collected from Daman Ganga riverside (Vapi, Gujarat, India). Primary screening of all 32 isolates in the presence of 8mM of Cd revealed that 50% were tolerable to Cd. Increased Cd concentration led to choosing an isolate, DG-20, with the capability to tolerate maximum Cd concentrations up to 18 mM. It was chosen for further study of its PGP activities and its capacity to mineralize Cd. After sequencing of the 16S rRNA gene and comparison with sequences available in NCBI, strain DG-20 was identified as *Curtobacterium oceanosedimentum* (*C. oceanosedimentum*). Under accession number OM471784, a partial sequence of 1400 bp of 16S rRNA gene has been submitted to the NCBI, GenBank database.

3.3. Estimation of Cd Bioaccumulation in C. oceanosedimentum

It was determined that Cd accumulation varied with incubation time. Cells accumulated the most Cd at 36 h, reaching 57.30 ± 18.24 mg/g DW (dry weight) (Figure 1). Cd concentrations in cell walls were lowest at the initial stage (12 h), whereas it reached its maximum at 36 h (74.62 ± 1.36 mg/g DW).

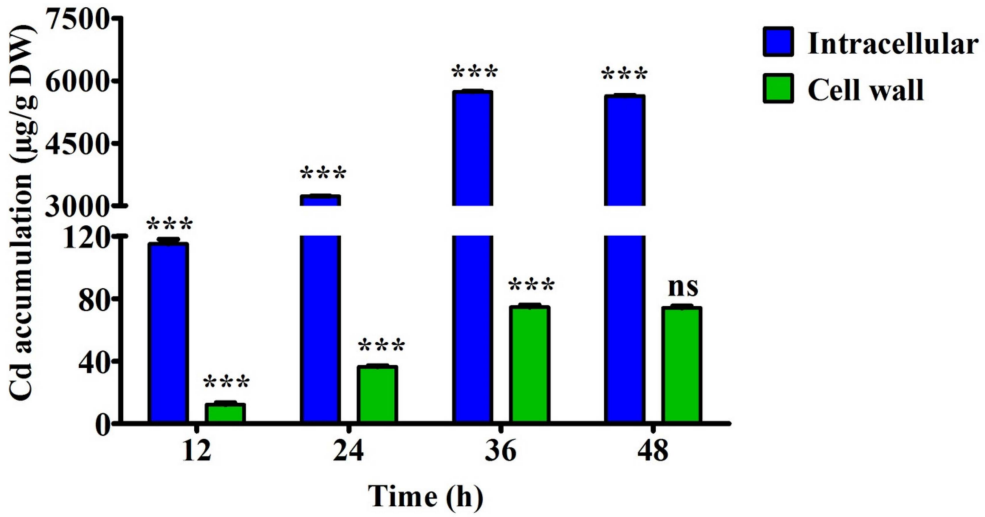

Figure 1. Cd accumulations in two compartments of *C. oceanosedimentum*. Error bars indicate SD (standard deviation) of three independent experiments. Significance; ns > 0.05, * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$.

3.4. Plant Growth Promotion Properties of C. oceanosedimentum

The testing of different PGP properties was conducted under normal and stress conditions, including production of IAA, ammonia, siderophore, phosphate, and potassium solubilization (Table 2). Qualitative results indicate that, under normal conditions, *C. oceanosedimentum* was capable of producing phosphate, potassium, and siderophore. Under stress conditions, it also showed this ability, except for potassium solubilization. Under normal conditions, production of IAA and ammonia by *C. oceanosedimentum* was found to be 183.66 ± 1.52 µg/mL and 73 ± 2 µg/mL, respectively, whereas 116.33 ± 2.08 µg/mL and 51.66 ± 1.52 µg/mL were found under stressed conditions, respectively.

Table 2. Different plant growth-promoting properties of isolate DG-20 under normal and stress (Cd treated) conditions.

Conditions	IAA (µg/mL)	Ammonia (µg/mL)	Siderophore	Phosphate Solubilization	Potassium Solubilization	Amylase	Cellulase	Pectinase	Chitinase
Normal	183.66 ± 1.52	73 ± 2	+	+	+	+	+	-	+
Stress (Cd treated)	116.33 ± 2.08	51.66 ± 1.52	+	+	-	+	+	-	+

Key legend: (+ = positive, - = negative).

A potent PGP bacterium is also known for its ability to produce extracellular enzymes. In normal and stress conditions, *C. oceanosedimentum* can produce amylase, cellulose, and chitinase, but not pectinase (Table 2).

3.5. Influence of Cd-Tolerant C. oceanosedimentum on the Growth of Chili

In normal as well as under Cd stress conditions, seeds treated with *C. oceanosedimentum* showed considerably different results in shoot length, root length, and wet and dry biomass of the plants ($p < 0.05$) (Figure 2A–C and Figure 3). In artificially contaminated soil, the effects of *C. oceanosedimentum* on Chili growth was evaluated by pot assay. In the absence of stress, the bacteria-treated seedlings showed higher root (58%) and shoot (60%) lengths compared to the control. Under stress conditions, the bacteria-treated seedlings had longer roots (86%) and shoots (52%) compared to the control (Figure 2C). The wet and dry biomass of the plant (roots and shoots) increased with the treatment of *C. oceanosedimentum*+ Cd, compared to the control-1 (C1) (without bacterial and Cd treatment) and the control-2 (C2) (Cd alone) (Figure 3).

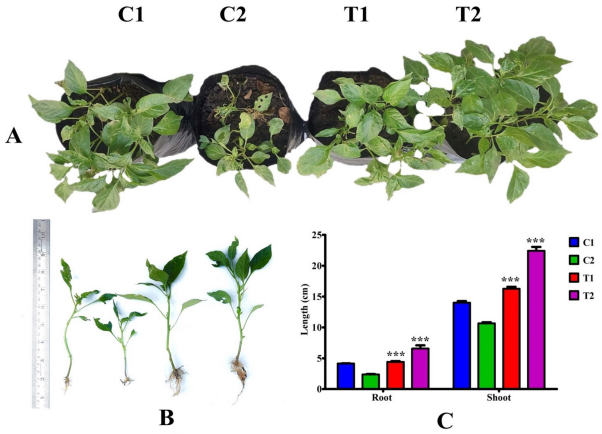

Figure 2. Effect of inoculation with plant growth-promoting *C. oceanosedimentum* on the growth of Chili. The phenotypic appearance of plants in different conditions (**A**), morphology of plants grown 30 days after germination (**B**), root and shoot length of plants (**C**). C1—without DG-20 and Cd treatment, C2—without DG-20 and with Cd treatment, T1—with DG-20 and Cd treatment, T2 only DG-20 treatment. Error bars indicate SD (standard deviation) of three independent experiments. Significance; ns > 0.05, * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$.

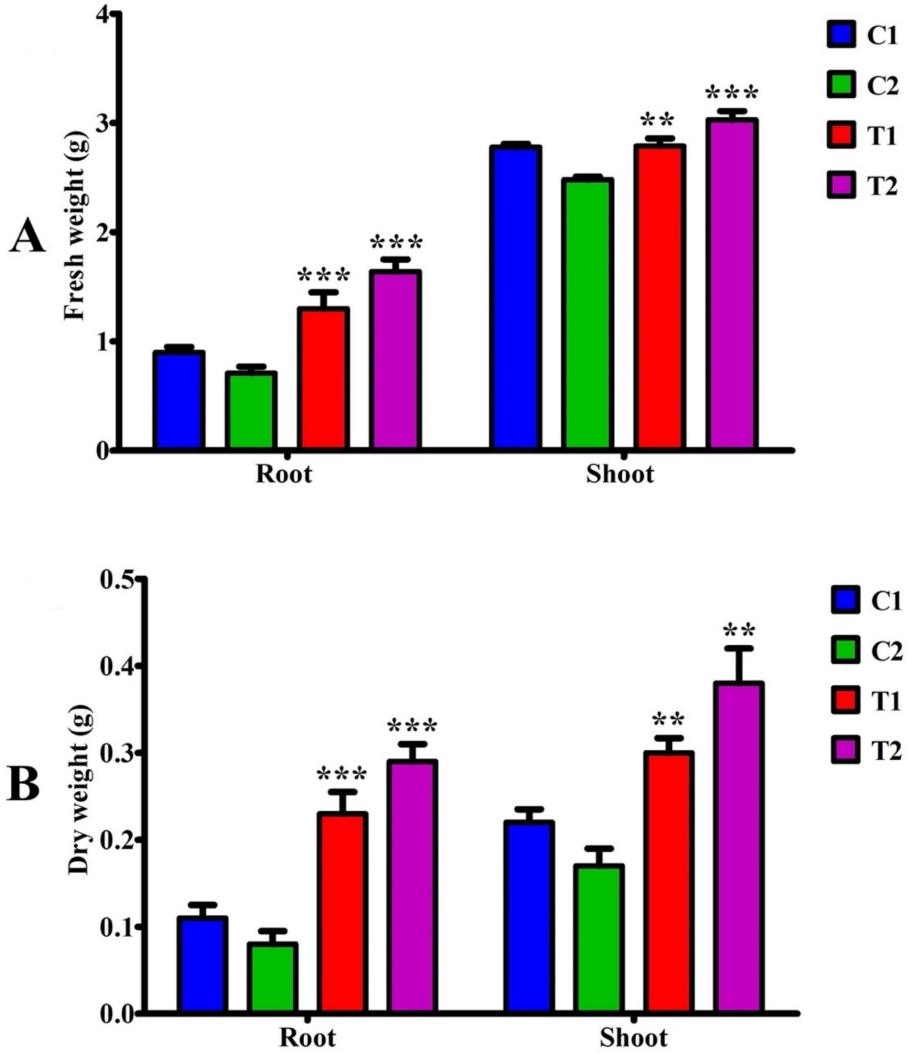

Figure 3. Effect of Cd stress and *C. oceanosedimentum* inoculation on Chili root and shoot (**A**) Fresh weight and (**B**) Dry weight. C1—without DG-20 and Cd treatment, C2—without DG-20 and with Cd treatment, T1—with DG-20 and Cd treatment, T2 only DG-20 treatment. Error bars indicate SD (standard deviation) of three independent experiments. Significance; ns > 0.05, * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$.

3.6. Cd Accumulation in Chili Roots and Shoots Tissues

The concentration of Cd in the root and shoots (including leaves) of Chili grown on artificially contaminated soils was determined (Figure 4). The roots (0.93 ± 0.12 µg/mL) and shoots (0.57 ± 0.07 µg/mL) of Chili plants treated with stress and *C. oceanosedimentum* mobilized more Cd than the control-2 (without bacterial and with Cd treatment). As a result of the bacterial strain causing soil metal mobilization to be augmented, the concentrations of Cd was elevated in Chili plants.

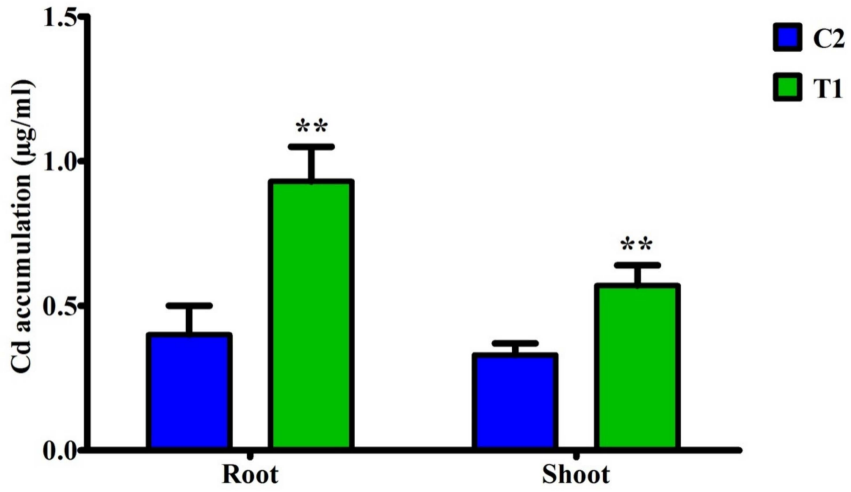

Figure 4. Effect of *C. oceanosedimentum* on Cd contaminated soil on the uptake of Cd by Chili roots and shoots grown for 30 days. C2—without DG-20 and with Cd treatment and T1—with DG-20 and Cd treatment. Error bars indicate SD (standard deviation) of three independent experiments. Significance; ns > 0.05, * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$.

3.7. Effects of C. oceanosedimentum on Physiological Properties of Chili

In comparison to control plants (C1 and C2), plants treated with *C. oceanosedimentum* showed improved chlorophyll a and b synthesis. In the inoculated plants (T1 and T2), chlorophyll a and b showed an increase in photosynthetic pigments to 55.54% and 26.15% and 35.09% and 22.99%, respectively (Figure 5A).

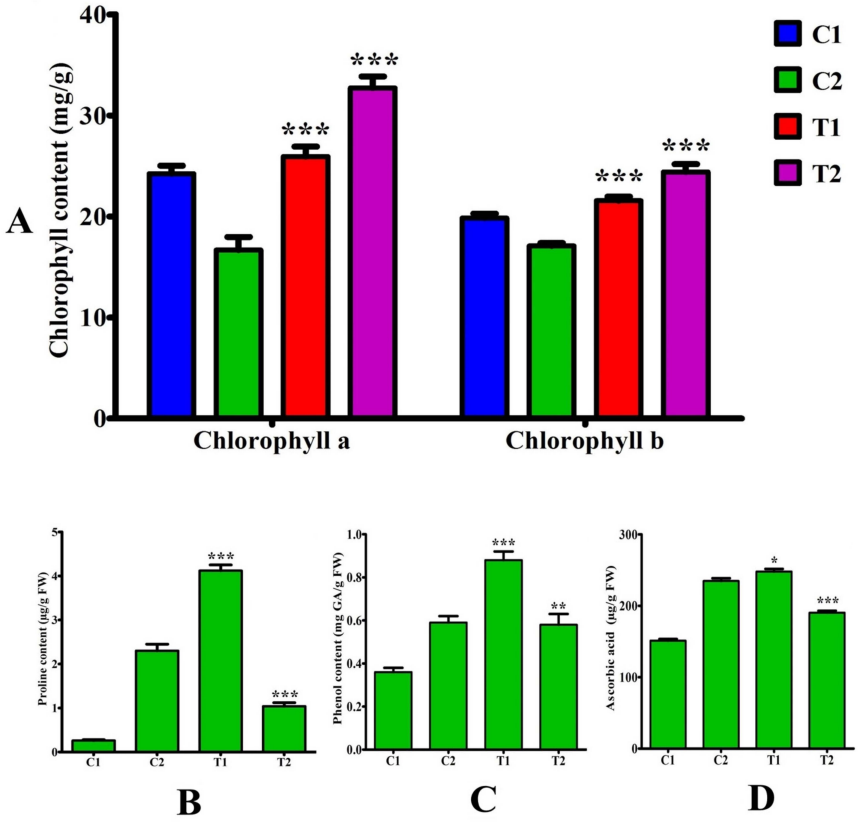

Figure 5. Effect of *C. oceanosedimentum* on (**A**) chlorophyll content (**B**) proline content (**C**) phenol content (**D**) ascorbic acid content under Cd stress. Error bars indicate SD (standard deviation) of three independent experiments. Significance; ns > 0.05, * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$.

Cd-stressed plants had higher proline content compared to unstressed plants, and plants treated with DG-20 had higher proline content than uninoculated plants (Figure 5B). As compared to control plants, proline content increased in T1 (4.22 ± 0.13 µg/g FW (fresh weight), followed by T2 (1.04 ± 0.08 µg/g FW).

As a result of determining the total phenol content of plants under normal and Cd stress conditions, it was found that phenol content was significantly higher in plants that were exposed to Cd rather than control plants ($p < 0.05$). Compared to control plants, T1 (0.88 ± 0.04 mg GA/g FW) showed the highest level of phenol, followed by T2 (0.58 ± 0.05 mg GA/g FW) (Figure 5C).

According to the results of the determination of ascorbic acid, the bacterial cultures that were treated with Cd-stressed Chili seeds had a significantly increased content of ascorbic acid. In this experiment, higher levels of ascorbic acid were found in T1 (248.06 ± 3.62 µg/g FW) and T2 (190.5 ± 2.59 µg/g FW), compared to control plants (Figure 5D).

4. Discussion

It is generally accepted that the presence of potentially toxic elements imposes significant burdens on local biodiversity and microbes [63]. Metal-tolerant bacteria thrive in these environments, and they can be used for bioremediation [64]. Compared to unpolluted environments, isolated bacteria from polluted soils are well-known to be resistant to higher concentrations of potentially toxic elements [65,66]. Consequently, plant growth-promoting bacteria are currently being investigated as potential candidates for remediating chemically affected soils and meeting global agricultural needs [67]. In the last few decades, various bacterial strains with plant growth-promoting properties have been isolated and characterized [68–70].

The present study describes Cd-tolerant bacteria isolated from the Daman Ganga riverside, Vapi, Gujarat, along with their molecular identification and analysis of their bioaccumulation potential. The isolated Cd-tolerant strain DG-20 was found to be *C. oceanosedimentum*. Through inductively coupled plasma-optical emission spectrometry (ICP-OES), the percentage accumulation of Cd concentration in whole cells was measured, which suggested potentially toxic element tolerance and possible mechanisms [71]. Liaquat et al. (2020) [72] also observed Cd accumulation by *Stenotrophomonas maltophilia* (*S. maltophilia)* from the mine. According to some studies, the *S. maltophilia* strain can tolerate potentially toxic elements such as Cd, cobalt, Pb, zinc, mercury, copper, etc. [73]. In a study by Gao et al. (2013) [74], it was found that *S. maltophilia* can decompose organic pollutants such as phenanthrene as its sole carbon source. Cd can be effectively sorbed by growing *Bacillus cereus* M116 [75]. *Pseudomonas aeruginosa* showed good biosorption potential against nickel, chromium, Cd, and Pb according to Raja et al. (2006) [76]. According to Haq et al. (1999) [77], *Enterobacter cloacae* and *Klebsiella* species are resistant to chromium, Cd, and Pb. After isolating *Klebsiella variicola* from industrial effluents, Afzal et al. (2017) [78] also observed the metal tolerance potential of bacterial isolates against nickel and cobalt. Selvi et al. (2012) [79] isolated a number of metal tolerant bacteria, which were reported to tolerate Pb, zinc, copper, mercury, and copper.

During the present study, the growth-promoting properties of strain DG-20 were tested under normal and stressed conditions. Until now, very few studies have been conducted on the screening of PGP activities of metal-tolerant isolates under stress and non-stress conditions. PGP properties of strain DG-20 includes the ability to produce IAA, ammonia, siderophore, phosphate, and potassium solubilization, as well as its ability to produce various extracellular enzymes, including amylase, cellulose, and chitinase. Several studies have shown that the low levels of IAA produced by the PGPR enhances plant growth by stimulating the growth of cells in the roots by promoting cell division [80], whereas high levels of IAA increase the formation of lateral and adventitious roots and inhibit the growth of primary roots [81]. By promoting the development of lateral and adventitious roots, IAA enhances plant growth and tolerance to potentially toxic elements. A Cd-resistant *Bacillus*

megaterum (SaN1) has also been screened by Pan et al. (2017) [82]. Similar results have been reported for copper resistance [83]. Several strains of *Azotobacter*, *Pseudomonas*, and *Bacillus* have been reported as copper resistant by Ahmad et al. (2008) [84]. IAA production has been demonstrated by *Kocuriarosea* [85], *Kocuria* [86], and *Kocuriaturfanensis* sp. [87].

The strain DG-20 is capable of solubilizing organic phosphates. The P-solubilizing activity of a microbe is actually a biochemical process that allows for the release of organic acids in the surroundings [88]. Plants' phosphate assimilation can be increased by PGPR's solubilization of phosphate. Additionally, iron can be sequestered from rhizosphere soil by PGPR siderophores, inhibiting phytopathogen growth and expediting plant iron uptake [89]. In the DG-20 strain, siderophore production was found to be positive. Plant growth can be promoted, and potentially toxic element stress can be reduced by using PGPR, because it produces siderophore, which relieves the stress to the plants and provides iron to them [90]. Potassium is one of the most important aspects of essential nutrients for crop productivity. Several enzymes are involved, including those involved in photosynthesis, respiration, starch synthesis, and protein synthesis, and they are activated by potassium ions. Additionally, potassium concentration influences the opening and closing of stomatal guard cells or daily changes in leaf orientation. By producing organic acids and other chemicals, potassium solubilizing bacteria improve soil potassium availability, stimulating plant growth and mineral uptake in plants. It was also found that strain DG-20 was capable of solubilizing K. Moreover, DG-20 was also able to produce ammonia, which is useful for plants, directly or indirectly, in their growth. *C. oceanosedimentum* also has a hydrolytic enzyme producing ability. Several enzymes, such as amylase, cellulase, and chitinase, can be used to promote the degradation of organic matter in soil, promotes plant growth, and controls the proliferation of pathogenic fungi through hydrolysis of their cell walls [91,92]. Under a Cd-stress environment, the present study demonstrated the real potency of bacterial strain DG-20. The Cd-tolerant PGPR improved metal bioavailability around the root zone by inducing acidification and thus facilitated plant Cd uptake [5].

When bacterial isolates have more than one growth-promoting trait, this can facilitate the increase of growth of the plant [93]. Pre-treatment of the seeds of Chili with the DG-20 strain led to better plant growth and seed germination. It is possible that strain DG-20 can produce auxin, which promotes root growth in young plants. After strain DG-20 inoculation, the shoot and root dry weights of Chili plants increased significantly. A similar result was observed by Jiang et al. (2008) [94] in tomato plants (*Solanum lycopersicum* L.) and corn plants (*Zea mays* subsp. *mays* L.). The increased IAA production and higher photosynthetic pigment levels are likely responsible for the increased biomass of plants inoculated with DG-20 (Figure 3) [81,95,96].

The contents of chlorophyll (Chl a and Chl b) were significantly increased in the leaves of Chili plants inoculated with strain DG-20. Consequently, higher chlorophyll levels in leaves should benefit growth and photosynthesis under Cd stress. Furthermore, the Chl a/b ratio of Chili plants increased significantly. The Chl a/b ratio correlated positively with the early light-induced proteins (ELIPs) and the light-harvesting chlorophyll-protein complex (LHCII) [97]. Free radicals are prevented from forming by the production of ELIPs in the plant photosynthetic system. By increasing the content of ELIP in leaves, plants are able to adapt to the environmental stress conditions [97]. The ratio of Chl a/b is normally lowered because of a lesser relative sensitivity of LHCII due to Cd toxicity or decrease in the amount of PSII [98]. Likewise, Wan et al. (2012) [96] stated that *Serratia nematodiphila* strain LRE07 significantly increased the content of photosynthetic pigments in *S. nigrum* L. leaves grown in Cd-polluted conditions.

A combination of plants and microbes used for phytoremediation to enhance extraction (phytoextraction) or stabilization (phytostabilization) of metals has been recognized to be a sustainable and dependable method for the remediation of metals from soil to improve the quality of soil [99,100]. The efficiency of phytoremediation can be affected substantially by interactions between heavy metals, PGPR, and plants. Plant exudates and microbial activities can influence soil heavy metal bioavailability in plants [101,102]. Plants have

developed mechanisms for immobilizing, mobilizing, or transforming heavy metals for survival and adaptation in metal-stressed environments, rendering them inactive and non-toxic for their own development, resulting in either enhanced or repressed metal transfer from soil to plants [103]. To establish successful phytoremediation systems, it is necessary to carefully select and test specific plant-PGPR associations, mechanisms of their interactions, and the therapeutic effects of these interactions [104]. Currently, little research has been conducted on the interactions of plants, PGPR strains, and metals.

As well as enhancing plant growth and phytoextraction of Cd, inoculation of *C. oceanosedimentum* also increases ascorbic acid, proline, and total phenol levels in Chilies. The amount of proline accumulated in the Chili leaves differed significantly between control and treated plants. In this study, proline production was higher in soil contaminated with Cd. Kartik et al. (2021) [105] concluded that proline production reflects a plant's tolerance to stress due to contaminated soil. As an antioxidant and a metal chelator, proline is imperative during stress [106,107]. Plants have been shown to utilize phenolic compounds for a variety of purposes. Phenolic compounds have antioxidant properties that prevent lipid peroxidation and chelate metals [108,109]. The exposure of potentially toxic elements such as nickel, Cd, and copper have been reported to induce phenolic biosynthesis in plants [110–112].

Inoculated plants showed a similar increase in ascorbic acid biosynthesis compared with the control plants. Ascorbic acid plays a crucial role in enhancing photosynthetic pigments, oxidative defense, and transpiration [113]. Ascorbic acid is also known to trigger an array of functions in plants subjected to stress [113]. The enhanced antioxidant molecules in the inoculated plants may have acted as a trigger to reduce Cd stress, thereby increasing the growth and uptake of Cd (Figure 5). In addition, metal-polluted soils have been reported to induce polyphenol synthesis in plants. Additionally, inoculation with appropriate PGP bacteria induces phenylalanine ammonia lyase expression, an enzyme crucial to polyphenol synthesis [114].

5. Conclusions

Cd is one of the environment's most toxic contaminants, responsible for crop field contamination, leading to decreased productivity and related toxicity challenges in plants. Based on the results of the present study, it can be concluded that *C. oceanosedimentum*, isolated from rhizospheric soil samples contaminated with potentially toxic elements from Daman Ganga riverside, India, has a high Cd tolerance ability and biosorption capacity with plant growth-promoting traits. In the future, the isolated DG-20 strain of *C. oceanosedimentum* has the potential to be used to bioremediate soil that has been contaminated with Cd and may result in the better growth of Chili plants.

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