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Tolerance and Cadmium (Cd) Immobilization by Native Bacteria Isolated in Cocoa Soils with Increased Metal Content

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Abstract: Twelve cadmium native bacteria previously isolated in soils of cocoa farms located in the western Colombian Andes (Santander), and tolerant to 2500 µM $CdCl_2$ (120 mg Cd/L), were chosen in order to test their tolerance and Cd immobilization using liquid culture medium (Nutritive broth) at different concentrations of heavy metals. Furthermore, in the greenhouse experiments, the strains *Exiguobacterium* sp. (11-4A), *Klebsiella variicola* sp. (18-4B), and *Enterobacter* sp. (29-4B) were applied in combined treatments using CCN51 cacao genotype seeds grown in soil with different concentrations of Cd. All bacterial strains' cell morphologies were deformed in TEM pictures, which also identified six strain interactions with biosorption and four strain capacities for bioaccumulation; FT-IR suggested that the amide, carbonyl, hydroxyl, ethyl, and phosphate groups on the bacteria biomass were the main Cd binding sites. In the pot experiments, the concentration of Cd was distributed throughout the cacao plant, but certain degrees of immobilization of Cd can occur in soil to prevent an increase in this level in roots with the presence of *Klebsiella* sp.

Keywords: bacteria; native; soil; cacao; Cd; TEM; FT-IR

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1. Introduction

One of the heavy metals in the earth's crust is cadmium (Cd). It can be found in salt compounds, which have been shown to be chronically harmful to both humans and the environment. The World Health Organization has set a maximum daily intake of 2.5 mg/Kg for these compounds [1]. Because bacteria in particular have a defense mechanism to counteract Cd's toxicity, this heavy metal could be biostabilized in soil by using microorganisms. There is information regarding microbial communities that have been isolated from the soil in different Asian and European countries, with a focus on their behavior when it comes to bioaccumulation and biosorption of Cd [2–4].

Active plants and microorganisms accumulate metals as a result of typical metabolic processes via exchange in the cell wall, complexation reactions in the cell wall, or intracellular precipitation [5]. Bioaccumulation entails the absorption of metals from media contaminated by living or dead organisms, inactive biomass, and active plants. The first method for metal absorption by inactive biomass is through ionic groups on the cell surface, and metal accumulation in biomass is more efficient than some ion exchange resins at eliminating metals from water [6]. A number of variables, including pH, temperature, biomass concentration and type, the presence of different metal ions in solution, and contact time, have an impact on the biosorption process [3].

Numerous reports discuss the use of local bacteria in paddy and soybean crops as a means of preventing both geogenic and anthropogenic Cd transfer to the plant. *Cupriavidus* sp. WS2 can survive in high Cd conditions and immobilize Cd by biosynthesizing intracellular Cd-containing nanoparticle inclusions, which decreases the accumulation

of Cd in rice seedlings [7]. *Burkholderia* sp. Y4 inoculation may indirectly alter the availability of micronutrients and inhibit Cd accumulation in rice by preferential Cd-biosorption [8]. The effects of the acidophilic strain *Pseudomonas putida* 62BN and alkalophilic strain *Pseudomonas monteilli* 97AN on the remediation of Cd and the subsequent effects on soybean to improve plant growth in the presence of Cd, and reduce the concentration in plant and soil in acidic and alkaline soils, respectively, have been reported in soybean crops [9].

The presence of Cd has a negative impact on cocoa crops, and research is being carried out to determine what causes the high concentration of Cd in cocoa seeds from Latin America [10]. The manufacturing and processing of cocoa derivatives, as well as local and international sales that adhere to standards for Cd concentration in food, are all impacted by this circumstance, which also limits the places suitable for cultivation (soils with lower Cd concentrations) [11]. Meanwhile, different reports on isolated bacteria have been linked to geogenic Cd soil in Colombia's cocoa crops [12,13]. The processes of natural strains against Cd concentration and in vitro studies using cocoa pots to determine the mechanisms of native strains against concentrations of Cd, as well as cocoa pot experiments to know the possibility of counteracting the Cd transfer to the cocoa plants, are not mentioned though.

The goal of this study is to assess the mechanisms underlying the response of cell morphology and analyze the cadmium tolerance of 12 native bacteria strains in in vitro assays when they are inoculated with CCN51 cacao genotype seeds under various concentrations of Cd.

2. Materials and Methods

2.1. Bacteria Strains and Growth Conditions

Native bacteria strains used in this study were previously isolated from non-rhizospheric soil cocoa farms in lands with cocoa crops between 15–20 years old; the farms located in the municipality of San Vicente de Chucurrí, department of Santander, Colombia [13]. Cadmium-resistant bacterium was isolated by adding to the media a cadmium concentration of 2500 µM $CdCl_2$ (120 mg Cd/L), the growing strains were purified to obtain a pure culture, and then stored in cryovials with 10% (*v/v*) of glycerol at −20 °C for further study [13]. The native bacteria strains were selected whose taxonomic identification is summarized in Table 1.

Table 1. Homology of the 16S rDNA sequences of the native cadmium-tolerant soil bacteria.

Phylogenetic Affiliation (Related Reference Sequence)	Gram Stain	GenBank Accesión Number
Bacillus toyonensis	+	MN587894
Burkholderia arboris	–	MN587896
Cupriavidus necátor	–	MN587892
Escherichia fergusonii	–	MN587901
Exiguobacterium acetylicum	+	MN587893
Ralstonia solanacearum	–	MN587895
Serratia marcescens	–	MN587899
Dermacoccus barathri	–	MN587890
Enterobacter tabaco	–	MN587891
Klebsiella variicola	–	MN587897
Lactococcus lactis	+	MN587898
Staphylococcus capitis	+	MN587900

2.2. Effect of Cadmium on Bacterial Growth

An inoculum (50 mL) was grown for all the strains at 32 °C for 24 h until reaching an optical density of 1.0 at 600 nm (2×10^7 CFU/mL). They were inoculated at 1% *v/v* in nutrient broth at 32 °C in a shaker (150 rpm) at different Cd concentrations (0, 10, and 15 mg Cd/L), previously prepared the stock solution (500 mg Cd/L) dissolving $CdCl_2$ in sterile deionized water by diluting to desired concentrations. The bacteria strains growth (each curve by triplicate) was determined every 3 h by the optical density at 600 nm. *Cupriavidus metallidurans* DSM 2839 was used as a positive control strain [3]. We calculated

the μ, g, and K growth variables for each native bacteria in exponential stage; the μ is the specific growth rate constant (h^{-1}) ($\log N - \log N0 = +/2303\ (t - t_0)$); g (generation time) is the time it takes for a population of bacteria to double in number ($g = 0.693/\mu$); the inverse of the generation time is called growth rate (K), and it is expressed as generations /h ($K = 1/g$) [14].

2.3. Cadmium Uptake Ability of Bacteria Strains

Growing cell suspensions (2.5 mL) was introduced to culture broth (250 mL), modifying the concentration of Cd present in the medium, depending on the tolerance level of the strain, and incubated at 32 °C for 24 h without adjusting broth pH. All cells were precipitated by centrifugation at 4 °C for 10 min at 4000× *g* and the pellet was washed 3 times with sterile deionized water; then, the samples were washed with sterile 10 mM EDTA to remove Cd excess that could bind to the cell surface. The supernatant and cell pellets after centrifugation were used to measure total Cd by atomic flame absorption. All experiments were performed in triplicate and the mean values with standard deviation were reported [15]; furthermore, the ability of each strain to Cd bioaccumulate was evaluated in the calculation of bioaccumulation factor (BF), which is defined as the relationship between the concentration of the element in cellular biomass compared to the concentration of the element in the culture broth (BF = (Metal) biomass cellular/(Metal) culture broth) [16].

2.4. Transmission Electron Microscopy and Analyzer Energy Dispersive X-ray (TEM/EDX)

Bacteria cells were obtained by centrifugation at 4 °C for 5 min at 6000× *g*; afterward, the cells were washed gently 3 times with sterile deionized water, and fixed either with 2.5% glutaraldehyde in phosphate buffer (pH 7.2) at 4 °C for 4 h, washed with the same buffer, then immerse into 1% Osmium tetraoxide in phosphate buffer (pH 7.2) for 2 h. Cells were dehydrated with an ethanol gradient and deposited in ERL resin. The section was stained with 2% uranyl acetate for 1 h and then fixed with lead citrate for 15 min. The samples were them sectioned (50 nm) with a Du Point diamond knife in an LKB ultra microtome EM UC7 LEICA (Leica, Wetzlar, Germany) stained with uranyl acetate and lead citrate (2% *w/v*) and examiner under Tecnai G2 F20 Super Twin transmission electron microscope (TEM, Waltham, MA, USA) with GATAN camera US 1000XP-P (Pleasanton, CA, USA) to observe and photographed cell structures also simultaneous EDX analysis with an energy dispersive X-ray detector Oxford (XMAX Instruments, Oxford, UK) [17].

2.5. Fourier-Transform Infrared (FT-IR) Spectrum Analysis

Strains were grown at 32 °C in the presence and absence of cadmium concentrations (10 or 15 mg/L depend on uptake tolerance in numeral 2.3) for 24 h. The bacteria cells were collected by centrifugation at 4 °C for 10 min at 4000× *g*. Cells were washed with sterile deionized water 3 times and resuspended. The samples were freeze-dried, and cells deposited onto KBr plate and were scanned by IR spectrometer Perkin Elmer 100 (Waltham, MA, USA) with the default analysis settings as recommended by the manufacturer. The resolution for each spectrum FT-IR was 4 cm^{-1} and wavelength from 4000 to 450 cm^{-1} [18].

2.6. Greenhouse Experiment

2.6.1. Location and Experimental Design

The greenhouse location (area 160 m^2) was in Yariguíes farm of Compañía Nacional de Chocolates in Barrancabermeja and San Vicente de Chucurí, department of Santander, (6°54′30″ N, 73°44′08″ W), 620 m above sea level, average temperature of 28 °C, annual rainfall of 2984 mm and daily solar radiation of 5500 w/m^2. The study had experimental completely randomized design blocks with 4 × 2 × 2 factorial design. Four treatments corresponded to bacteria (Bacteria 1 (B1-*Klebsiella* sp. (18-4B)), Bacteria 2 (B2-*Exiguobacterium* sp. (11-4A)), Bacteria 3 (B3-*Enterobacter* sp. (29-4B)) and negative control (without bacteria). The treatments were compared between the two different Cd concentrations (0.332 and 12.95 mg Cd/Kg) two times (60 and 120 days) with 3 repetitions.

2.6.2. Pot Assays

Cacao seeds of CCN-51 were harvested in trees between 7 to 8 years old, which were phytosanitary and healthy belonging to the area planted only with the self-pollination variety of Yariguies farm [19]. After the harvesting pods were disinfected with sodium hypochlorite (100 mg/L), the beans with mucilage were covered, extracted, and cleaned to avoid seed rot [20]. Subsequently, the beans were submerged in sterile deionized water and left 2 days to verify the seed viability and achieve a greater germinating probability in the pots. Once the seeds were selected, we evaluated Cd's total concentration (0.14 mg/Kg). The soil was extracted from the same farm area (depth 0–20 cm), dried at the sun, and sieve through mesh N° 20, to remove the stones and root plants, after being sterilized with humidity steam to destroy insects, larvae, and pathogens present in soil. In treatments for high Cd, it was necessary to increase the basal Cd concentration used, $CdCl_2.5/2H_2O$ (Meyer®, Blue Springs, MO, USA), which was prepared as a standard solution with deionized sterile H_2O (1000 mg Cd/L), applied in soil by the manual atomizer, shaking vigorously to homogenize and showed the soil Cd concentration between 10 to 15 mg Cd/Kg. The black plastic pods used (15 cm × 8 cm; 800 to 900 soil grams capacity) were sterilized with sodium hypochlorite (100 mg/L) and dried with paper towel. Bacteria inoculation in the soil was applied in a manual atomizer (spray) at 10^8 cell/gram soil count. Each treatment sample soil was left to rest for a week in a dark, dry, and cool place [7]. The seeds were sown after placing the soil according to treatment and randomized design in the arranged assembly. Each pod was irrigated and controlled with potable water every day to keep soil moisture (60–70%) at field capacity (Cd in water was not detected). After cacao seeds were germinated, fertilization, fumigation, control, and a monitoring plan was implemented according to commercial greenhouse practices, all the reagents used were evaluated for Cd concentration. We collected, according to the treatment, the leachates for each pot and checked the Cd level for each replication at different harvested times (60 and 120 days).

2.6.3. Physical and Chemical Analysis of Soil Properties

The soil properties (exchangeable elements) were measured following the protocols described in the national standard reference norms of the ISO representative in the country (ICONTEC): pH, electrical conductivity (CE), and cationic interchange effective capacity (C.E.C.I) by NTC 5167; texture (bouyoucos method); OM and C (NTC 5403); P (NTC 5530); K, Ca, Mg and Na according to NTC 5349; Cu, Fe, Mn, Al, and Zn by NTC 5526; S and B following to NTC 5404 and total Cd (NTC 3934).

2.6.4. Determination of Cd in Cacao Plant Parts

After harvesting, the plants were cleaned and roots, stems, and aerial parts were separated, and their fresh weight was registered. After the cacao shoots (roots, stems, and leaves), were used for total dry biomass determination in the stove to 80 °C to obtain constant weight. Soil and vegetal material were Cd analysis ICP-OES before digested in HNO_3:$HClO_4$ 1:6 solution and calculated bioconcentration (BCF) and translocation (TF) factors [21].

2.7. Statistical Analysis

Statistical analysis was performed using R Studio software version 3.6.1 (Vienna, Austria), an analysis of variance (ANOVA) and Tukey proof to compare means to biomass, Cd concentration in parts of the cocoa plant by different harvested time and negative control. Statistical significance was determined at $p < 0.05$.

3. Results

3.1. Bacterial Strain and Growth Curves Measurements

To study the behavior of 12 Cd tolerant strains in NB, their growth curves at different Cd concentrations (0, 10, and 15 mg Cd/L) were researched according to Cd levels in cocoa crop soils as previously reported (Figure 1). The results showed that *Staphylococcus* sp.

(2-3) and *Dermacoccus* sp. (4-3) strains could not grow in both Cd concentrations, the other 10 strains were Cd tolerant at 10 and 15 mg Cd/L. In the presence of Cd, the growth curves were lower than without Cd; it was even observed that some strains clearly distinguish the growth phases (exponential/stationary).

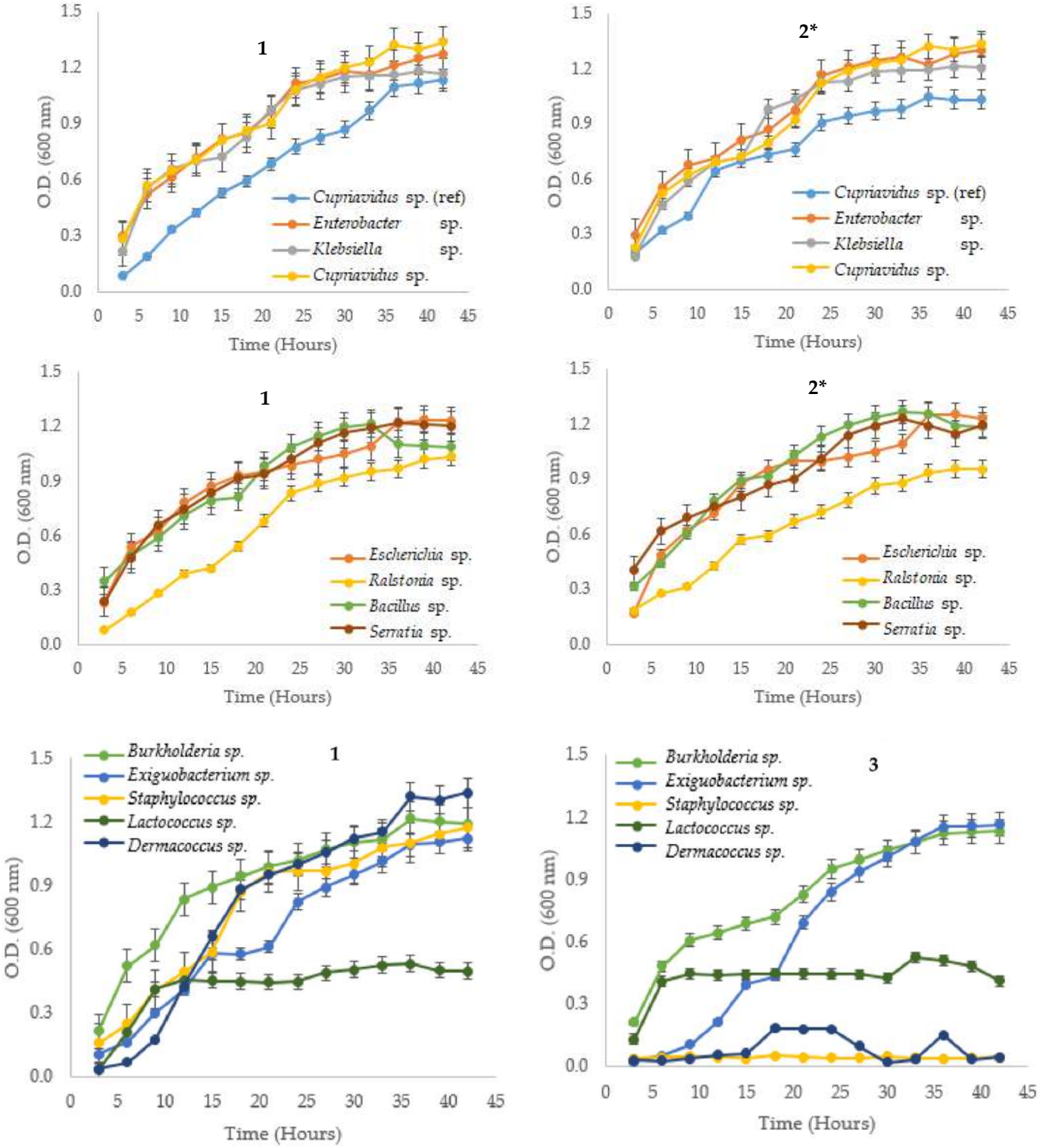

Figure 1. Growth curve native bacteria strains; **1**: 0 mg Cd/L, **2**: 15 mg Cd/L, **3**: 10 mg Cd/L. * Strains grow in both Cd concentrations with the growth curve at the highest concentration.

The lag phase in most strains was shorter, suggesting that these strains have the adequate cellular machinery to hold the Cd level and quickly reach the exponential phase. Table 2 shows the μ, g, and K growth variables.

Table 2. Mean values of growth parameters with and without Cd in nutrient broth.

Native Strain	μ (h^{-1})		g (h)		K (Generation/h)	
	Without Cd	With Cd	Without Cd	With Cd	Without Cd	With Cd
Cupriavidus metallidurans *	0.093	0.058	7.492	11.907	0.133	0.084
Serratia sp. (6-2)	0.076	0.089	9.142	7.388	0.109	0.128
Cupriavidus sp. (15-1)	0.089	0.106	7.760	6.519	0.129	0.153
Klebsiella sp. (18-4B)	0.099	0.126	7.007	5.491	0.143	0.182
Bacillus sp. (10-2)	0.100	0.094	6.944	7.388	0.144	0.135
Ralstonia sp. (16-1)	0.123	0.065	5.639	10.728	0.177	0.093
Escherichia sp. (4-2)	0.066	0.074	10.548	9.365	0.095	0.107
Enterobacter sp. (29-4B)	0.111	0.104	6.249	6.657	0.160	0.150
Exiguobacterium sp. (11-4A)	0.115	0.144	6.005	4.806	0.167	0.208
Burkholderia sp. (17-1)	0.062	0.082	11.124	8.441	0.090	0.118
Lactococcus sp. (22-4)	0.240	0.392	2.885	1.769	0.347	0.565

* Commercial reference strain.

3.2. Cd Bioaccumulation in Native Bacteria Strains

A summary of the behavior of the strains after 24 h of incubation in nutrient broth with Cd can be found in Table 3. The FB (Cd accumulative capacity) for all bacteria strains is high, except for the *Serratia* sp. (6-2).

Table 3. Ability to tolerance and bioaccumulation for the native bacteria strains.

Bacteria	Initial (Cd) (mg/L)	Final pH Nutrient Broth	(Cd) Supernatant (mg/L)	(Cd) Captured (mg/L)	% Cd Capture	Bioaccumulation Factor (BF)
Cupriavidus metallidurans *	3.73 ± 0.31	6.36 ± 0.41	1.70 ± 0.11	1.61 ± 0.07	48.63 ± 0.49	0.43 ± 0.02
Serratia sp. (6-2)		6.25 ± 0.17	3.14 ± 0.09	0.28 ± 0.10	8.02 ± 2.73	0.07 ± 0.03
Cupriavidus sp. (15-1)		6.88 ± 0.30	2.38 ± 0.06	0.78 ± 0.14	24.61 ± 3.35	0.21 ± 0.04
Klebsiella sp. (18-4B)		6.60 ± 0.38	0.69 ± 0.08	2.59 ± 0.40	78.86 ± 2.97	0.69 ± 0.11
Bacillus sp. (10-2)		6.34 ± 0.23	2.20 ± 0.09	0.87 ± 0.19	28.21 ± 5.37	0.23 ± 0.05
Ralstonia sp. (16-1)		6.62 ± 0.25	1.41 ± 0.19	2.17 ± 0.21	60.59 ± 5.59	0.58 ± 0.06
Escherichia sp. (4-2)		6.22 ± 0.42	1.50 ± 0.21	1.78 ± 0.39	53.93 ± 9.09	0.48 ± 0.11
Enterobacter sp. (29-4B)		6.73 ± 0.44	1.56 ± 0.05	1.39 ± 0.04	47.16 ± 1.40	0.37 ± 0.01
Exiguobacterium sp. (11-4A)	3.22 ± 0.27	5.14 ± 0.38	2.92 ± 0.03	0.90 ± 0.09	28.24 ± 2.0	0.24 ± 0.02
Burkholderia sp. (17-1)		6.06 ± 0.20	2.50 ± 0.05	0.68 ± 0.02	21.33 ± 2.21	0.18 ± 0.01
Lactococcus sp. (22-4)		5.16 ± 0.19	1.73 ± 0.04	1.51 ± 0.18	46.38 ± 3.45	0.40 ± 0.05

* Commercial reference strain.

3.3. TEM, EDX, and FT-IR Analysis and Mechanisms of Cd Interaction Using Native Bacteria

3.3.1. TEM Results

TEM was used to determine intra or extracellular Cd accumulation in native bacteria strains. In *Serratia* sp. (6-2), *Exiguobacterium* sp. (11-4A), *Ralstonia* sp. (16-1), *Enterobacter* sp. (29-4B), *Lactococcus* sp. (22-4), and *Bacillus* sp. (10-2), Cd dense granules were observed in external parts and cells bacteria showed morphological deformation (Figure 2A). For *Burkholderia* sp. (17-1), *Cupriavidus* sp. (15-1), *Escherichia* sp. (4-2), *Klebsiella* sp. (18-4B), and reference bacteria *Cupriavidus metallidurans* DSM 2839, Cd dense granules showed the inside of the cytoplasm with a cell morphological deformation (Figure 2B). Native *Staphylococcus* sp. (2-3) produced a sticky substance (exopolysaccharide (EPS)); it interacted with Cd and protected the cell lysis or cellular destruction to Cd concentration, even after 72 h in resuspended nutrient broth at a level of 15 mg Cd/L; the other native strain without Cd growth capacity *Dermacoccus* sp. (4-3) has a yellow–orange coloration intensity, the pellet cellular was intact and suspended even after 48 h in nutrient broth with 15 mg Cd/L concentration (Supplementary Figure S1).

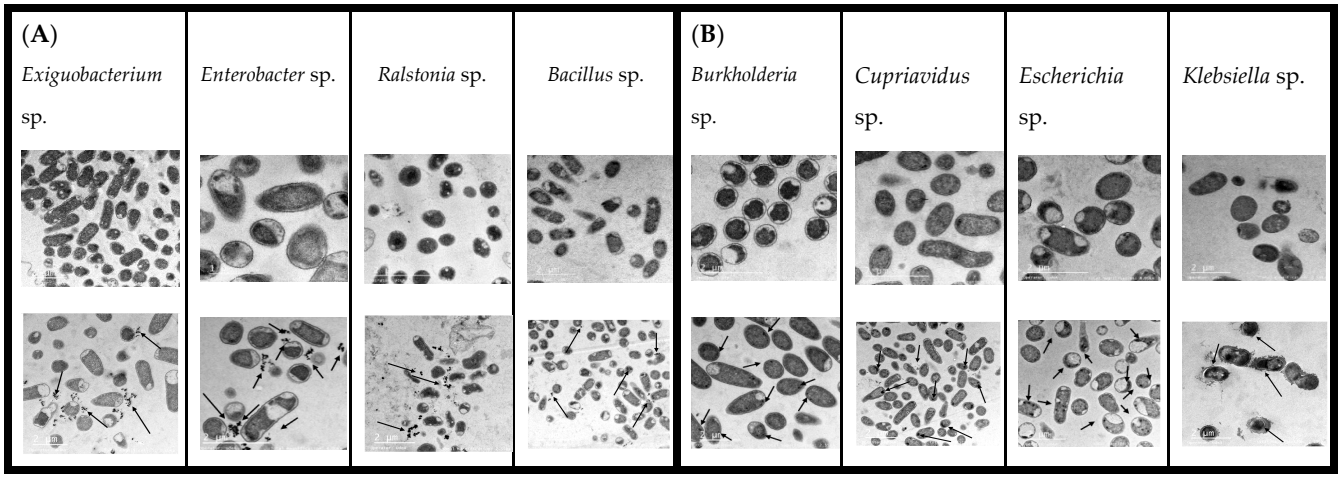

Figure 2. Some TEM pictures for cells without Cd (up), cell tolerance to 15 mg Cd/L (below). (**A**) Biosorption ability; the black arrows indicated external deposits of Cd. (**B**) Intracellular ability; the black arrows indicated intracellular deposits of Cd.

3.3.2. EDX Spectra

EDX scan spectra images showed the Cd identification peaks from 3.2 to 3.4 KeV, the normal range for this heavy metal, and changed the counts in different bioaccumulative abilities. Other present metals in the spectra were C, O, Cl, Pb, Si, Os, and Cu peaks. Cl came from Cd analytic grade reactive ($CdCl_2$); Cl, C, O, and Si were common elements of cell wall constituents and Os and Cu have come from fixation treatment and contrast staining to samples. In addition, in all the strains, an increase in oxygen after Cd biosorption was observed (Supplementary Figure S2).

3.3.3. FT-IR Spectra

FT-IR analysis revealed, before and after Cd binding, that many functional groups were involved in the biosorption process (Supplementary Figure S3). The main functional groups in the cell surfaces of all the spectral were observed changes around 3300 cm^{-1} –OH glucose and N–H proteins were in the range of 1100–1050 cm^{-1} assigned to vibration C–O and C–N stretch, respectively, and also in some samples, identified 1637–1529 cm^{-1} peaks corresponding to the C=O stretch. Table 4 resumed the functional groups and vibration types. The FT-IR results showed that more functional groups were involved in the Cd biosorption process in *Bacillus* sp. (10-2), *Lactococcus* sp. (22-4), *Cupriavidus* sp. (15-1), and *Burkholderia* sp. (17-1).

Table 4. Functional group identification of Cd-tolerant bacteria.

Wavenumber (cm^{-1})	Assignment	Vibration Types
~3275	Amide A	Stretch N-H proteins
2958/2873	Methyl CH_3	Stretch C-H asymmetric lipids/carbohydrates/proteins
2923/2852	Methyl CH_2	Stretch C-H asymmetric lipids/carbohydrates/proteins
~1636	Amide I	Stretch C=O proteins
1529	Amide II	Flexion N-H proteins
1467	Methyl CH_2	Flexion C-H proteins/lipids
1455	Methyl CH_3	Flexion C-H proteins/lipids
1389	Carbonyl	Stretch C=O asymmetric fatty acids and amino acids
~1230/~1060	Phosphate PO^{2-}	Stretch P=O asymmetric phospholipids, nucleic acids
~966	Phosphate PO^{2-}	Stretch P=O symmetric phospholipids, nucleic acids
~914	Ketones	C-O ring vibrations of nucleic acids “sugars”
~860	C-H group	Tri-substituted bending C-H

3.4. Responses of Inoculation to Native Tolerant Cd Bacteria in Young Cacao Plants at Differents Cd Levels in the Soil

3.4.1. Soil Analysis and Cd Concentration Chemicals Used in the Study

Soil properties (pH 4.88, electric conductivity 0.27 dS/m, organic matter 1.53%, total cadmium 0.332 mg/Kg, total nitrogen 0.074%, phosphorous 11.6 mg/g, clay 2%, sand 88%, and silt 10%) were recollected in cacao farm (Yariguies); the textural class of the soil was sandy silt loam according to Bouyoucos method. With respect to Cd content in chemicals employed in the study, only Ridomil Gold N60WP (fungicide) 2.67 mg/Kg and Basacote 12M (fertilizer NPK) 0.534 mg/Kg were detected. For Nilo 350SC (insecticide), Bélico 500SC, Imidogen 350SC (insecticide), Nutremin 200EC (insecticide), and Carrier (encapsulating aid), the Cd content was not detected.

3.4.2. Roots and Aerial Parts Cacao Biomass

Roots and aerial parts of the cacao biomass in each plant part for each harvested time were resumed in Table 5. ANOVA analysis indicated that roots and aerial part the factors to influence biomass were time and Cd concentration in soil. The paired Tukey test refers to the biomass levels with no significant statistical differences between the control (without bacteria) and inoculated samples.

3.4.3. Cd Accumulation in Cacao Plants, Bioconcentration, and Translocation Factors

Effects of *Klebsiella* sp. (18-4B) (Bacteria 1), *Exiguobacterium* sp. (11-4A) (Bacteria 2), and *Enterobacter* sp. (29-4B) (Bacteria 3) on Cd accumulation in roots, sheet, and aerial parts each time (T1-T2). ANOVA analysis in Cd accumulation found no statistical differences in all treatments. Paired Tukey test indicated each time, no significant difference between the control and bacteria inoculated samples. Nevertheless, a decrease in the mean Cd accumulate concentration in roots for T1 (60 days) in *Klebsiella* sp. (18-4B) was 23.38% and 25.12% for T2 (120 days) in the Cd high concentration in soil. In the stems, a decrease in the mean Cd bioaccumulation at T1 for *Klebsiella* sp. (18-4B) was 19.34% and *Enterobacter* sp. (29-4B) was 10.93% at T2. The bacteria effects on Cd bioconcentration in roots showed no statistical differences in Cd soil concentrations and harvested time. However, the bioconcentration factor obtained for all treatments was equal or even less than the control, and especially reported for *Klebsiella* sp. (18-4B) in a different time and Cd level. The translocation factor indicated that the cacao plant was the Cd phytoextractor capability and increased directly proportional to the Cd soil concentration (Table 6). Figure 3 shows the physiological state of cacao plants at 120 days harvest without Cd concentrations.

Table 5. Effects of the bacterial inoculation (control, not inoculated; bacteria, inoculated seedlings) on morphological and physiological parameters of cocoa plants.

Cd Level	Treatment	Fresh Weight (g)						Dry Weight (g)					
		Roots		Stems		Aerial Parts		Roots		Stems		Aerial Parts	
		T1 [a]	T2 [b]	T1 [a]	T2 [b]	T1 [a]	T2 [b]	T1 [a]	T2 [b]	T1 [a]	T2 [b]	T1 [a]	T2 [b]
Low	Control	5.55 ± 1.91	6.58 ± 2.10	7.4 ± 4.2	8.0 ± 1.2	8.3 ± 1.8	10.5 ± 0.2	0.74 ± 0.47	1.17 ± 0.15	1.37 ± 0.55	2.31 ± 0.29	1.89 ± 0.58	3.52 ± 0.49
	Bacteria 1	3.56 ± 0.09	6.13 ± 2.56	4.6 ± 0.1	8.5 ± 2.7	6.9 ± 0.9	12.0 ± 4.9	0.42 ± 0.06	1.14 ± 0.13	1.29 ± 0.63	2.29 ± 0.54	1.57 ± 0.82	3.49 ± 1.52
	Bacteria 2	3.39 ± 0.08	5.70 ± 1.73	5.4 ± 0.9	8.4 ± 0.8	7.7 ± 0.5	12.7 ± 1.9	0.58 ± 0.19	1.13 ± 0.23	1.58 ± 0.47	2.18 ± 0.27	1.85 ± 0.74	3.95 ± 0.96
	Bacteria 3	3.43 ± 0.52	7.34 ± 2.49	5.1 ± 1.5	9.5 ± 2.1	7.7 ± 0.6	14.0 ± 2.3	0.44 ± 0.11	1.35 ± 0.10	1.31 ± 0.43	2.50 ± 0.88	1.78 ± 0.60	4.17 ± 0.84
High	Control	3.83 ± 1.22	4.81 ± 2.03	4.9 ± 1.7	8.6 ± 1.1	6.6 ± 0.5	10.4 ± 0.5	0.44 ± 0.05	1.01 ± 0.36	1.19 ± 0.34	2.47 ± 0.29	1.57 ± 0.65	3.25 ± 0.51
	Bacteria 1	2.53 ± 1.17	3.97 ± 0.70	4.2 ± 0.8	7.0 ± 1.3	5.4 ± 0.7	9.5 ± 1.8	0.40 ± 0.02	0.86 ± 0.18	1.14 ± 0.41	2.04 ± 0.29	1.33 ± 0.78	3.25 ± 0.58
	Bacteria 2	3.18 ± 0.49	4.28 ± 2.36	5.0 ± 0.5	6.2 ± 2.8	5.9 ± 1.0	8.3 ± 3.6	0.48 ± 0.26	0.87 ± 0.14	1.05 ± 0.25	1.81 ± 0.52	1.58 ± 0.95	2.81 ± 1.07
	Bacteria 3	3.13 ± 1.36	3.42 ± 1.28	4.2 ± 1.9	6.1 ± 0.5	5.2 ± 1.4	7.8 ± 1.9	0.50 ± 0.07	0.79 ± 0.41	1.04 ± 0.30	1.92 ± 0.58	1.22 ± 0.76	2.77 ± 0.87
ANOVA (Type II)													
Treatment		8.82	1.91	6.46		5.81	1.53	0.09		0.22		0.16	
Time		12.56	64.09	18.31		18.77	10.91	2.81 ***		10.7 ***		38.81 ***	
Cd level		3.10	32.25*	19.59		21.14	59.92*	0.51 **		0.90		3.75 *	
Treatment: Cd		1.66	5.55	4.68		0.85	29.35	0.02		0.38		1.09	

Values were mean ± standard deviation. Different letters were statistical significance $p < 0.05$ (paired Tukey test). Significance level: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. T1 (60 days); T2 (120 days).

Table 6. Bioconcentration and translocation factors (mean, SD, min.–max.) for all treatments.

Time	Factors Treatment	Bioconcentration (BCF)		Translocatión (TF)	
		Low	High	Low	High
T1	Control	4.92 ± 4.17 (0.39–11.72)	2.54 ± 0.07 (0.97–4.70)	1.13 ± 1.54 (0.25–13.72)	4.30 ± 2.54 (0.97–4.70)
	Klebsiella sp. (18-4B)	3.12 ± 0.70 (0.79–6.27)	1.51 ± 0.07 (0.77–2.16)	0.76 ± 0.68 (0.36–3.29)	8.24 ± 6.61 (0.77–2.16)
	Exiguobacterium sp. (11-4A)	2.75 ± 1.91 (0.93–7.28)	2.39 ± 1.06 (1.40–3.57)	0.24 ± 0.17 (1.65–7.44)	5.18 ± 3.03 (1.40–3.57)
	Enterobacter sp. (29-4B)	3.56 ± 1.64 (0.82–6.75)	2.51 ± 1.36 (0.82–5.16)	0.95 ± 1.20 (0.31–4.62)	3.77 ± 1.31 (0.82–5.16)
T2	Control	4.60 ± 1.49 (0.18–18.57)	3.36 ± 0.90 (0.82–10.39)	5.82 ± 10.83 (0.19–18.57)	7.69 ± 10.47 (0.50–13.75)
	Klebsiella sp. (18-4B)	2.28 ± 0.59 (0.15–7.88)	2.27 ± 0.56 (0.51–5.54)	5.32 ± 7.80 (0.43–7.88)	7.76 ± 9.47 (0.084–15.12)
	Exiguobacterium sp. (11-4A)	4.64 ± 3.19 (0.45–8.01)	2.53 ± 0.28 (0.74–4.94)	5.82 ± 10.83 (0.45–33.84)	6.39 ± 6.70 (0.69–13.49)
	Enterobacter sp. (29-4B)	3.53 ± 3.99 (0.31–17.73)	2.48 ± 0.77 (0.71–3.66)	6.64 ± 11.87 (0.31–17.73)	4.37 ± 1,62 (0.73–7.22)

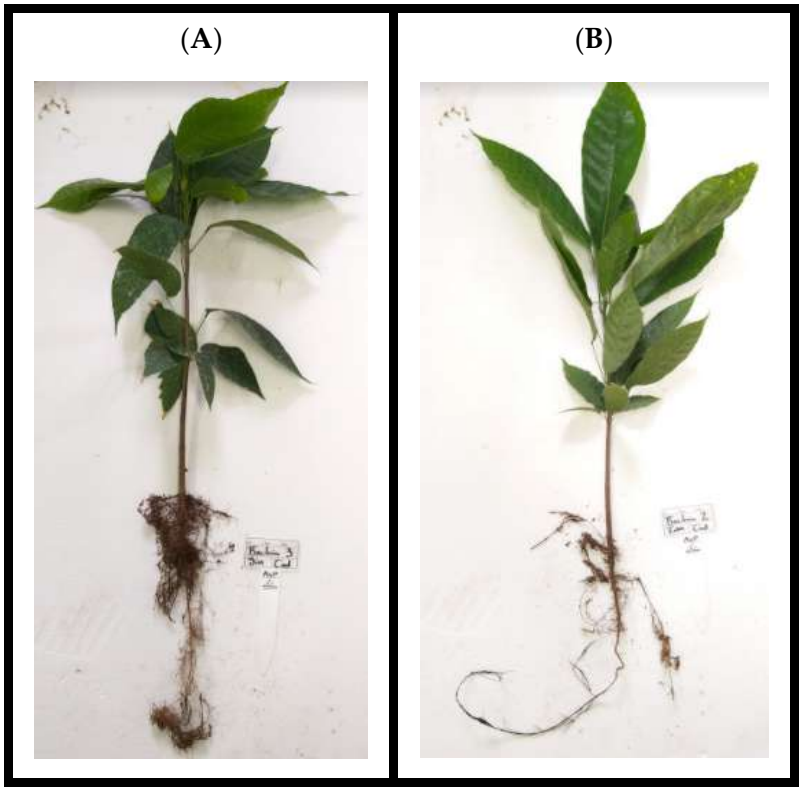

Figure 3. (**A**). Cacao plant without Cd: high biomass aerial, stem large, thick, and various branches; principal root large and straight and abundant secondary roots. (**B**) Cacao plant with Cd concentration: low biomass aerial, stem short, thin; branches cover the top of the stem; principal root was large, thin, curved, and fragile; a few secondary roots.

4. Discussion

The isolation and characterization of native bacteria in complicated ecosystems or high pollution areas has lately become extremely relevant at the scientific level in order to find bacterial genera that display tolerance and advance treatments to address the substance that affects soil or water. There is a great deal of interest in the use of microorganisms in bioremediation since it has advantages, including high efficiency, low cost, and eco-

friendliness. In addition to the fact that microorganisms can tolerate environmental stress through fast mutation and evolution, research of natural bacterial tolerance mechanisms can aid in the development of bioremediation solutions [22].

The present study established how 10 native bacterial strains behaved in nutrient broth at different Cd concentrations (0, 10, and 15 mg Cd/L), which corresponded to Cd levels in soils used to grow cocoa in the Santander Region in Colombia. It also demonstrated how Cd acts on the cell wall side chains and identified interactions between them. The growth curves showed a decrease in optical density conserving times to different phases after Cd was added to the broth; this delayed growth pattern suggested that bacteria strains must reduce growth and likely alter their physiological mechanisms in response to Cd toxicity [3]. Ten strains were grown at Cd concentrations regardless of Cd tolerance capacity, and two strains did not grow to full development, thus showing that Cd concentrations have an impact on growth conditions. The behavior of the positive control, *Cupriavidus metallidurans* DSM 2839, was similar to that which has been previously reported [3,23].

According to μ, the growth factors g and K demonstrated the behavior of each strain against Cd, even though the strains showed lower values with Cd, which gave them a survival advantage in the presence of a growth restrictor such as heavy metals. Regarding the two strains of *Dermacoccus* sp. (4-3) that did not develop in the presence of Cd concentrations, we discovered the yellow pigment generated by this strain in culture media. These microbial pigments, created by in vitro bacteria, have the ability to stop or chelate metal ions and have antioxidant qualities that can be used to prevent their toxic effects. By killing the cell pellet at high Cd levels, this mechanism can be prevented [24].

The other strain, *Staphylococcus* sp. (2-3), produced EPS, which may have anionic properties that promote interaction with cationic heavy metals (including Cd) and result in the formation of EPS-metal complexes [25]. After 24 h in nutrient broth, the native bacteria in this study, decreased the pH between 5.14 and 6.88, and also decreased the Cd absorption capacity from 8% to 79%. The removal rates of the ten strains were specific to the 10 mg/L group. The *Lactococcus* sp. (22-4) biosorption capture rate was larger than *Exiguobacterium* sp. (11-4A), while in the 15 mg/L groups, *Klebsiella* sp. (18-4B) removal was higher than *Ralstonia* sp. (16-1) and *Escherichia* sp. (4-2). These bacterial gene differences have been attributed to mechanisms for Cd interaction with the cell wall, Van der Waals forces, covalent bonds, redox reactions, extracellular precipitation, or combinations of these processes [26].

Another relevant aspect is the characteristics of the bacterial wall cells, which are more complex in Gram-negative bacteria than in Gram-positive ones, and have distinct biosorption capacities [27]. There are various ways that bacteria interact with heavy metals, including mechanism-independent (physicochemical interactions without energy intake) and process-dependent (transporting metal across the cell membrane with energy expenditure), with energy expenditure separating both [16].

Contaminant concentration and speciation have influenced the cumulative capacity, along with pH, which is key for heavy metal biosorption. The solubility, the ionic states of the functional groups on the biosorbent surface, and the competition with other ions are all affected by the pH of the solution [28,29]. According to TEM images, all the strains presented morphological changes, wrinkles, and deformed shapes after Cd exposition, which may be toxic effects conferred by heavy metals. These phenomena were related to the description of colony plates. Some strains exhibit swelling, irregular shapes, and liquid texture, others have a crusty surface, and the halo or colony is enlarged, covering a large plate area. According to Figure 2A, in the six native strains of *Serratia* sp. (6-2), *Exiguobacterium* sp. (11-4A), *Ralstonia* sp. (16-1), *Enterobacter* sp. (29-4B), *Lactococcus* sp. (22-4), and *Bacillus* sp. (10-2), dense Cd granules were observed in external parts.

The ability to accumulate Cd extracellularly has been reported in bacterial genera such as *Serratia* sp. [16], *Bacillus* sp. [30–33], *Ralstonia* sp. [34,35], *Enterobacter* sp. [36,37], *Exiguobacterium* sp. [38], and *Lactococcus* sp. [39]. This mechanism depends on Cd concentration and interaction time. Cd through efflux pumps is used by several bacterial

species to maintain metal ions outside the cell to avoid their toxicity [3]. Another aspect of extracellular biosorption mechanism that also depends on growth conditions to produce Cd detoxification was CdS precipitated by lipoprotein biosurfactant. It conferred heavy metal tolerance and could express metallothioneins attached to membrane cells, also known as Lam B lipoprotein attached to peptidoglucane. Even metallothioneins capture heavy metals and mobilize them at specific sites in the cellular structure [40].

In Figure 2B, the reference bacteria *Cupriavidus metallidurans* DSM 2839 and four native strains, *Burkholderia* sp. (17-1), *Cupriavidus* sp. (15-1), *Escherichia* sp. (4-2), and *Klebsiella* sp. (18-4B) showed dense Cd granules inside the cytoplasm. Members of a number of bacterial species, such as *Cupriavidus* sp. [16], *Klebsiella* sp. [41–43], *Escherichia* sp. [44], and *Burkholderia* sp. [45] have been noted to possess this capacity to intracellularly collect Cd and other metals. This might occur because of the ability of living cells to produce energy. Compared to external bioabsorption, it is a slow absorption mechanism, but intercellular absorption results in higher capacity. Additionally, based on the inherent surface characteristics of cells and the active transport of a tiny quantity of Cd to interior cell structures, the Cd allocations of microbial cells change with contact time [46].

Additional details on elemental quantification in the biomass strains, as well as C, N, and oxygen peaks that are likely present in polysacharides and proteins in the cell envelop the bacterial biomass, are provided by EDX scanning. Ca has a significant impact on cell metabolism, especially when bound to a variety of cell envelope proteins. Due to the fact that Cd is a divalent metal such as Ca, as seen in TEM images [2], it can substitute for Ca and affect the structure of the cell. Thus, an oxide precipitate containing Cd is formed on the surface, as indicated by the increase in oxygen [47].

Through the interaction of metals with native bacterial strains, FT-IR is a method to identify functional groups. Cd was found to be involved in the stretching of functional groups in the cell after 24 h of incubation. There was a slight difference between the control groups and those treated with Cd. Different peaks at 3275, 1634, and 1531 cm^{-1} (amide groups) were identified for the native bacterial strains, which suggests that nitrogen atoms may be the main adsorption sites for Cd binding on the bacteria strains. The peaks at 914, 1057, and 1636 cm^{-1} also showed a shift, suggesting that the band has been broadened and stretched due to the presence of C=O groups. The peaks at 2958, 2923, 2873, 2852, 1467, 1455, and 860 cm^{-1} all show an intriguing alteration. It is possible that the oxygen atoms in the hydroxyl groups in the biomass strain are involved in this absorption process because this region corresponds to both C–H and O–H stretchings [48]. The peaks in the region of 966, 1230, and 1060 cm^{-1} indicate that the phosphate bond is involved in Cd biosorption and that cadmium phosphate can be produced on the biomass surface [2].

The extracellular interaction associated with each group was the amine (N–H) absorption peak at 3276 cm^{-1}, at 2854 cm^{-1} with stretched alkane C–H leaps, and at 1637 cm^{-1} amide stretch signal I. *Serratia* sp. (6-2) had a low percentage of Cd absorption for each one, with a value of BF = 0.07. N–H is mainly stretched at 1529 cm^{-1} in the protein amide II band. The stretched C–H or C–OO groups emit a 1389 cm^{-1} signal, and the P=O bond is connected with a 1227 cm^{-1} band. C–OH is 1054 cm^{-1} and P=O is 1073 cm^{-1}, respectively.

According to Chen and Xu [49], it is believed that the amine group, amide I and II, and the phosphoric acid group are the main groups involved in the Cd adsorption process. *Cupriavidus* sp. (15-1) exhibited intracellular bioaccumulation and had a 24% Cd absorption with a BF = 0.21. However, the FT-IR analysis detected interactions with C–H stretching around the C–OH bond at 2851 cm^{-1}, 1452 cm^{-1}, 1388 cm^{-1}, and 1060 cm^{-1}. It may be associated with metal ion transport mechanisms. The *Klebsiella* sp. (18-4B) strain showed intracellular bioaccumulation and 78% Cd absorption with a value of BF = 0.69.

Since CdS is created outside the cell and enters the cell through the Mg or Mn transporter system, Shamin and Rehman [41] suggested that *Klebsiella pneumoniae* may have an intracellular absorption due to sulfur formation and an increase in organic phosphorus. Furthermore, signals at 3275 cm^{-1}, 1528 cm^{-1}, 1226 cm^{-1}, and 1061 cm^{-1}, which are associated with N–H and P=O groups, and transport systems, also showed alterations in

the FT-IR. *Bacillus* sp. (10-2) exhibited extracellular biosorption, BF = 0.23, and Cd capture at a rate of 28%. In addition, the FT-IR spectra changed to the following wavelengths: 3276 cm^{-1} and 1529 cm^{-1} for the N-H bond, 1455 cm^{-1} for the C-H bond, 1226 cm^{-1} and 1061 cm^{-1} for the stretch P=O bond. These signals were inferred to be phospholipids, carboxyl, amide I, amino, sulfates, and phosphate groups on the surface of strain 10-2, according to Huang and Jia [47].

Moreover, *Ralstonia* sp. (16-1) exhibits a biosorption mechanism with a 60.5% Cd absorption rate and BF = 0.58. According to Park, and Ko [34], Cd sulfite precipitation and an extracellular mechanism are present in *Ralstonia* sp. HM-1. The FT-IR showed 3277 cm^{-1}, 2872 cm^{-1}; 1636 cm^{-1}; 1388 cm^{-1}; 1170 cm^{-1}; 1061 cm^{-1}; 968 cm^{-1} which are mainly linked to cell wall functional groups of C=O, P=O, and N–H in saturated fatty acids, phosphate, and amine groups in proteins, or lipids, thus indicating that the cell wall components generated under Cd stress are involved in enhancing stress adaptation and increasing Cd adsorption capacity. These results were similar to Huang and Liu [50].

Escherichia sp. (4-2) showed intracellular bioaccumulation, BF = 0.48, and 54% Cd capture. Only three signals changed in the FT-IR, showing the C–H stretching and N–H bending vibrations of peptide bonds to membrane proteins or transport systems: 3274 cm^{-1}, 2851 cm^{-1}, and 1635 cm^{-1}. These findings support those of Huang and Jia [47]. Additionally, mainly with amide group II, which represents C–H stretching and N–H bending vibration of peptide bond to membrane protein or transport system. These results agree with Huang and Jia [47]. In addition, 47% of Cd capture, BF = 0.37 with extracellular bioaccumulation potential following the Cd biosorption by *Enterobacter* sp. (29-4B).

Meanwhile, the FT-IR spectra showed changes at 3277 cm^{-1} (stretch N–H proteins), 2957 cm^{-1} (stretched C–H), 1529 cm^{-1} (asymmetric lipids/carbohydrates/proteins), and 1455 cm^{-1} (flexion N–H proteins); 1387 cm^{-1} (flexion C–H proteins/lipids), 1229 cm^{-1} (stretched C=O asymmetric fatty acids/amino acids), and 1169 cm^{-1} (stretched P=O asymmetric phospholipids/nucleic acids). For *Enterobacter* sp. DNB-S2, Sun and Wang [51] identified similar interaction groups with Cd. Meanwhile, *Exiguobacterium* sp. (11-4A) registered a 28% Cd capture rate with a biosorption capacity value of BF = 0.24. Bands 3276 cm^{-1}, which correspond to stretch N-H proteins, 2958 cm^{-1}, 2875 cm^{-1} and 2851 cm^{-1} which are assigned to stretch C-H asymmetric lipids/carbohydrates/and proteins, 1638 cm^{-1} which is assigned to stretch C=O proteins, 1220 cm^{-1}, 1036 cm^{-1}, and 965 cm^{-1} which are assigned to stretch P=O asymmetric phospholipids, and nucleic acids, were all found to change in FT-IR.

The behavior of this native *Exiguobacterium* sp. matched that of Park and Chon strain [38]. Meanwhile, *Burkholderia* sp. (17-1) demonstrated a 21% Cd capture rate, BF = 0.18, and intracellular Cd bioaccumulation, whereas FT-IR espectra showed changes in signals. Stretching N-H proteins at 3274 cm^{-1}, C-H asymmetric lipids/carbohydrates/proteins at 2958 cm^{-1}, 2873 cm^{-1}, 2852 cm^{-1} flexioning C-H proteins/lipids at 1467 and 1455 cm^{-1}, and stretching N-P proteins at 1390 cm^{-1}. Stretch P=O symmetric phospholipids, nucleic acids, 1227 cm^{-1}, 1075 cm^{-1}, 1054 cm^{-1}, and 965 cm^{-1} corresponding to asymmetric fatty acids and amino acids. This behavior resembles capture by biosorption; however, the results showed intracellular accumulation for *Burkholderia* sp. (17-1), maybe both mechanisms of action occur for this strain. According to Zhang and Li [45], *Burkholderia cepacia* GYP1 may have gone through various stages of fast immobilization of Cd(II) on the cell surface coordinated with functional groups, after transport of Cd(II) to cells and accumulation in cytoplasm, efflux of intracellular Cd(II) depended on energy, and entrapment or adsorbed of extracellular Cd(II) by EPS. *Lactococcus* sp. (22-4) had a BF = 0.40 and accumulated biosorption with a 46% Cd capture rate. OH and NH2 groups were, contrary to Sheng and Wang [39], responsible for Cd biosorption in *Lactococcus lactis* subsp. *Lactis*, according to our findings. Other proteins, with similar length to ours, include 1637 cm^{-1} stretch C=O proteins, 1526 cm^{-1} flexion N–H proteins, 1468 cm^{-1} and 1452 cm^{-1} with flexion C-H proteins/lipids, and 1219 cm^{-1}, 1054 cm^{-1}, and 964 cm^{-1}.

Subsequently, for the selection of native strains to be studied in the greenhouse, it was carried out according to the highest growth variables, μ, g, and K. *Exiguobacterium* sp. (11-4A), *Enterobacter* sp. (29-4B), *Lactococcus* sp. (22-4), *Cupriavidus* sp. (15-1), and *Klebsiella* sp. (18-4B) strains grow faster in the presence of Cd. Another aspect of strain selection was the higher Cd concentration, an action mechanism found in this study and reported in the literature, finally selecting *Exiguobacterium* sp. (11-4A), *Enterobacter* sp. (29-4B) and *Klebsiella* sp. (18-4B).

In contrast, the cocoa plants in the greenhouse study were monitored under different treatments and harvest times in order to evaluate the behavior of the three native bacteria strains. The CCN51 cocoa variety was included in the greenhouse design because it was more prevalent in South American crops, had a high level of disease resistance, had an aromatic profile, contained more polyphenols, and had cocoa butter in the beans [52]. Concentration, exposure time, texture, and chemical characteristics of the soil, such as pH and organic matter, among others, all have a direct impact on Cd toxicity in cocoa [53]. Regarding soil texture, it offers benefits including ease of tilling, low compaction, quick water absorption, and high drainage; however, these conditions require permanent irrigation and fertilization. The pH of the soil is acidic due to the leaching of nutrients, which is an important factor in the Cd absorption process.

While the soil has a low percentage of organic matter, Qi and Lamb [54] suggested that Cd can interact with oxides of iron, manganese, and carbonates, which are generally immobile, allowing free mobility, phyto-availability, and Cd exchange. This study has a certain advantage because, according to Shahid and Dumat [55], Cd is directly influenced by humus, the substance responsible for maintaining an elevated pH level and highly reactive fulvic acid content, thereby promoting Cd bioavailability.

In order to contrast the effects of heavy metals on the plants and demand some sort of activity on the inoculating bacteria, we applied cadmium chloride, one of the most stable and water-soluble Cd sources, to enhance the basal Cd in the soil [56]. The fertilizer chosen for use in the greenhouse is crucial because, according to Zug and Huamaní Yupanqui [57], fertilizers regardless of Cd concentration can result in high Cd levels in crops, contaminate them directly, or generate Cd mobility in acid soils, thus allowing plants to grow faster or absorb more Cd through soil micelles. The fungicides and insecticides were added in a rotational manner established on the farm. They had low Cd concentrations, and the doses applied contributed to increasing the Cd total concentration by approximately 0.01 mg/L for each treatment without determining another influence in the experiment. This was carried out to fertilize the soil with NPK, to achieve seed germination, as well as to control possible pests or fungi that could affect the plants. Having innate mechanisms to bind free Cd in the cell wall, complex in the cytoplasm, or excrete chelating organic acids, some bacteria develop potential Cd resistance, dominating rhizospheric conditions, and supporting plant growth in Cd stress situations [58].

The native bacteria strains used in greenhouse experiments, *Klebsiella* sp. (18-4B) (Bacteria 1), and *Enterobacter* sp. (29-4B) (Bacteria 3) belong to the Proteobacteria phyla, they were Gram-negative bacillus and *Exiguobacterium* sp. (11-4A) (Bacteria 2), Gram-positive bacillus which belong to the Firmicutes phyla; these bacteria genera were characterized by fast growth, and high biomass production at 24 h without a supplemental medium. In terms of biomass, the plants were observed to increase in all treatments in T2, with no statistical differences; however, the biomass of roots and aerial parts of plants inoculated with bacteria 3 was slightly higher compared to control. This finding corresponded to Li and Liu [59], who report that *Enterobacter* sp. FM-1 cadmium-resistant bacteria promote the biomass production of plants cultivated in Cd polluted soil.

The root and aerial sections differ significantly in terms of exposure time and Cd concentration, according to variance analysis. Plants were found to have long, thin roots, little secondary root growth, and few aerial parts, which indicated lower dry weight in treatments with high Cd concentrations, symptoms of Cd toxicity from the soil [60]. The results showed a significant translocation rate for Cd, which was absorbed by the root

system and deposited in all cacao plant organs. Aerial part > stem > root was the order of accumulation in plant parts at T1, but stem > aerial part > root was the order at T2. According to Verbruggen and Hermans [61], greater metal accumulation in the roots is a characteristic of intolerant plants, while plants that can translocate metals to the aerial parts are regarded as tolerant factors. The above is referred to as the bioconcentration factor (BCF) and translocation factor (TF), which indicate the relationship between the Cd concentration in the soil and roots (BCF), and the Cd concentration in the stem and roots (TF); BCF < 1 and TF < 1 indicated that the plant excluded the Cd; BCF > 1 and TF > 1 indicate that the plant accumulated the Cd; and BCF > 10 indicates hyperaccumulation [21].

In our study, all T1 and T2 treatments had high Cd concentrations, and the BCF indicated that cocoa plants were capable of accumulating and phytoextracting Cd; this BCF depended on the cacao variety and the soil properties, not just for Cd, but also for trace elements [62,63]. Several publications have cited the existence of proteins that conferred natural resistance to Cd translocation, which may have been mediated by metal ion transporters that increased Cd content in roots and mobilized it into xylem and phloem, according to Arevalo-Hernandez and Arevalo-Gardini [64]. According to final Cd levels in soil and leachates for treatments with low Cd, they were 0.254 and 0.005 mg kg^{-1}; high Cd 12.94 and 0.63 mg kg^{-1} for T1; 0.22 and 0.045 mg kg^{-1}, high Cd 9.78 and 1.17 mg kg^{-1}, respectively, for T2. In contrast, at initial concentrations, Cd translocated in plants came exclusively from the soil.

The findings of this study demonstrated that different bacterial strains recovered from soils on Cd-polluted cocoa farms interact with Cd in distinct ways. The bacterial growth kinetics were studied using the proposed methodology, and the key to understanding the adaptation mechanism was discovered to be an inverse relationship between growth rate and heavy metal concentrations, but in native bacteria tolerant strains, growth is a response to the external restriction [65]. Some strains had morphological changes, which may have been a signal to infer maximum Cd tolerance level independently of genera.

Additional research needs to be carried out to identify metabolic pathways and enhance the ability of this type of bacteria to absorb heavy metals. To reduce the impact of cadmium in agricultural soils, the same methodology should be used to test different criollo cocoa varieties to find which variety has the lowest capacity to translocate Cd. The correct combination of native Cd-tolerant bacterial strains must also be used with different types of amendments, fertilizers, and agrochemicals.

5. Conclusions

Twelve native bacteria strains that had previously been molecularly described and were isolated from soil with Cd concentrations participated in the current study. These bacteria revealed two distinct ways of Cd capture, such as extracellular capacity (biosorption) and extracellular mechanism (Cd cytoplasm precipitation) in different concentrations, that depended on time and incubation conditions. All the genera have been reported in the literature with different Cd capability properties. Furthermore, in the pot assay under controlled conditions, the native strain *Klebsiella* sp. (18-4B) produced a certain degree of Cd immobilization in the soil to prevent the accumulation of Cd in the roots of cacao plants, thus demonstrating that native bacteria is one of the strategies for bioremediation to apply in soil cocoa crops with Cd geogenic presence. We identified the major functional groups on cell surfaces and reported the chemical changes before and after Cd interaction. The most significant issue to be tackled in recent years is environmental contamination, particularly the presence of heavy metals in soil used for crops. The native bacteria strains with action and tolerance to Cd were inoculated with a certain tendency to counteract Cd bioaccumulation in roots, which can reduce the negative effects of Cd on growth and productivity in cocoa crops in soils with cobalt concentrations, which is one of the limitations for the chocolate industry, which is booming due to the quality of Colombian beans.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres13030039/s1, Figure S1: Strains without Cd growth; Figure S2: EDX spectra in some strains; Figure S3: FT-IR spectra.

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