Effect of Zn/Cd Tolerant Endophytic Bacterial Inoculation on Phenolic Profiles of *Gynura pseudochina* (L.) DC. Grown in Zn and Cd Contaminated Soil

Ruttanakorn Munjit[1], Aphidech Sangdee[1], Dawn Alnold[2], Nitra Nuengchamnong[3], and Woranan Nakbanpote[1,*]

[1]Department of Biology, Faculty of Science, Mahasarakham University, Khamriang, Kantarawichai, Maha Sarakham 44150, Thailand
[2]Harper Adams University, Newport, Shropshire TF10 8NB, United Kingdom
[3]Science Laboratory Centre, Faculty of Science, Naresuan University, Phitsanulok 65000, Thailand
Email: ruttanakorn.munjit@gmail.com (R.M.); aphidech.s@msu.ac.th (A.S.); darnold@harper-adams.ac.uk (D.A.); nitran@nu.ac.th. (N.N.); woranan.n@msu.ac.th (W.N.)
[*]Corresponding author

Manuscript received July 3, 2024; revised August 2, 2024; accepted August 16, 2024; published December 17, 2024

***Abstract*—*Gynura pseudochina* (L.) DC. is a native Zn and Cd hyperaccumulative plant. It can be applied to rehabilitate an old zinc mine by cooperating with a select Zn/Cd tolerant plant endophyte that contains plant growth-promoting properties. Therefore, this research aims to study the inoculation effects of selected Zn/Cd tolerant endophytic bacteria on the growth and phenolic profiles of *G. pseudochina* growing in Zn plus Cd contaminated soil. *Curtobacterium* sp. GLD03, *Stenotrophomonas sp.* GSD10 and *Chryseobacterium* sp. GTID13 were separately inoculated on tubers before growing in nutrient-rich commercial soil supplemented with Zn (1000 mg L^{-1}) and Zn (50 mg L^{-1}) for two months. The excess Zn and Cd resulted in phytotoxicity (yellow and dead leaves) and a decrease in dry weight. GSD10 had a direct impact on Zn and Cd accumulation in the underground (tubers and roots) part, while GLD03 and GTID13 tended to promote Zn and Cd accumulation in shoots. Interestingly, GLD03 and GTID13 seemed to degrade phenolic compounds in leaves. The total phenolic content (TPC) and total flavonoid content (TFC) decreased by 68-76% and 70-80%, respectively, when compared to the non-inoculated plants. The TPC and TFC of GTID13 decreased by 20% and 12%, respectively. HPLC and LC-MS/MS analysis confirmed the results. Moreover, the phenolic profile of GTID13-inoculated plants was not different from that of uninoculated plants. Caffeoylquinic acid and caffeic acid likely played a key role in reducing Zn/Cd stress. The other involved compounds were salicylic acid, ethyl chlorogenate, dicaffeoylquinic acid, ferulic acid, and methyl 3,4-dicaffeoylquinate. The results obtained can be used as information for selecting endophytes to be applied in the field.**

***Keywords*—bioaugmentation, cadmium, endophyte, phenolic compounds, Zinc**

I. Introduction

Phytomanagement can be used to restore an old zinc mine that has been contaminated with zinc tailing. Planting native plants that are tolerant of both zinc (Zn) and cadmium (Cd) is appropriate because Cd is a guest element in zinc ores [1]. *Gynura pseudochina* (L.) DC., a perennial herb in the Asteraceae family, is a Zn/Cd hyperaccumulative plant. In addition, *G. pseudochina* is a native plant found in the zinc resource area in Tak Province, Thailand [1, 2]. Zn is an essential micronutrient for normal plant growth at a low concentration, whereas Cd is not an essential element [3]. However, a supra-optimal Zn concentration cannot only be toxic to plants but also aggravate the toxic effects of Cd by inducing oxidative stress [4, 5]. Phenolic compounds are antioxidants that can scavenge reactive oxygen species (ROS), catalyze oxygenation reactions by forming metallic complexes, and inhibit oxidizing enzyme activities [6, 7]. An *in vivo* system clearly indicated that *G. pseudochina* responded to an excess of Zn and/or Cd concentrations by increasing TPC and TFC, especially caffeic acid and rutin [5]. In addition, the phenolic compounds that played a role in the esterification of the cell wall, especially chlorogenic acid, *p*-coumaric, and caffeic acid, contributed to the accumulation of Zn and Cd in the cell wall [4]. Under the stress of Cd and Zn in *Kandelia obovata*, phenolic acids were mainly synthesized with the properties of scavenging free radicals and involved in the process of Zn and Cd uptake [8]. Besides, the Cd toxicity caused *Gynura procumbent* [9] and *Malva parviflora* [10] to have more TPC, TFC, and antioxidant activity. *Vaccinium corymbosum* showed an increase in chlorogenic acid when exposed to Cd stress [11]. Flavonoids, flavonols, hydrolyzable tannins, and condensed tannins in *Phaseolus vulgaris* were also induced by Cd [12].

Endophytic bacteria colonize plant tissues without causing disease or negative effects on the host. Plant growth-promoting endophytic bacteria (PGPEB) can directly improve plant growth by producing phytohormones and siderophores and getting increased limiting nutrients (phosphate, nitrogen, and potassium) [13]. Metal-resistant PGPEB can associate with their host hyperaccumulating plants to enhance phytoremediation [14]. Furthermore, Endophytic bacteria have an important role in secondary metabolites and can either decrease or increase metal accumulation in herbal plants [15].

Our research isolated Zn/Cd tolerant endophytic bacteria from *G. pseudochina* growing in the zinc mine tailing. The endophytic bacterial strains of *Curtobacterium* sp. GLD03, *Stenotrophomonas* sp. GSD10, and *Chryseobacterium* sp. GTID13 contained some plant growth-promoting properties. Reinoculation of these endophytic strains into *G. pseudochina* could be beneficial for plant growth during zinc mine rehabilitation. However, it is necessary to understand the relationship between endophytes and host plants, as well as the potential applications for achieving their cooperation in reducing metal stress [14].

Therefore, this research aims to study the effects of inoculation of *Curtobacterium* sp. GLD03, *Stenotrophomonas* sp. GSD10, and *Chryseobacterium* sp. GTID13 in *G. pseudochina* plants growing in soil contaminated with Zn plus Cd. The investigation focused on

plant growth, phytotoxicity, Zn and Cd accumulation, TPC, TFC, antioxidant activity, and phenolic compounds.

II. Materials and Methods

A. Preparation of Zn plus Cd Contaminated Soil

A commercial compost soil (Levington R-M2, ICL, UK) (pH 5.3-6.0, N: 192, P: 98 K: 319, and conductivity 228-414 µs) was used as a medium in the pot experiment. The soil was air-dried and sieved to obtain 0-4 mm particle size. The Zn plus Cd contaminated soil was prepared by thoroughly mixing the soil with a number of metal solutions to obtain the concentration of Zn 1,000 mg kg^{-1} soil and Cd 50 mg kg^{-1} soil. The solutions were prepared from $ZnSO_4.7H_2O$ and $CdSO_4$. The contaminated soil was left in the greenhouse for 7 days to stabilize the metals before being sterilized at 121 °C for 15 min over three consecutive days.

B. Zn plus Cd Tolerant and PGPEB Properties

Curtobacterium sp. GLD03, *Stenotrophomonas* sp. GSD10, *Chryseobacterium* sp. GTID13 were isolated from the stems, leaves, and tubers of healthy *G. pseudochina* growing in the former zine mine of Padaeng Industry Public Company Limited (PDI), Phatat Phadaeng sub-district, Mae Sot, Tak Province, Thailand (N 16°39′6.6″, E 98°39′41.2″). They were identified based on 16S rRNA gene sequencing analysis. Details of the modified DNA extraction and identification were presented in Ratthanapolsan *et al.* [16]. The partial 16S rRNA gene nucleotide sequences were deposited in the National Center for Biotechnology Information (NCBI) database under accession numbers MZ314061, MZ314069, and MZ314075. Bacterial colonies were analyzed for their characteristics on trypticase soya agar (TSA) and blood agar [17]. Zn- and/or Cd-tolerant properties were investigated on TSA supplemented with Zn (25-1000 mg L^{-1}) and/or Cd (5-100 mg L^{-1}). Plant growth-promoting properties were studied under control (metal-free) and Zn plus Cd contamination (200+50 mg L^{-1}). Indole-3-acetic acid (IAA) production was investigated by following a modified method of Gordan and Weber [18]. Qualitative analysis of nitrogen fixation, phosphate solubilization, and siderophore production was carried out by inoculating bacteria on a nitrogen-free malate medium [19], National Botanical Research Institute's phosphate growth medium (NBRIP) [20], and chrome azurol S (CAS) agar [21], respectively.

Each bacterial strain was cultivated in Luria-Bertani broth (LB broth) (Fisher, UK) at 30 °C, 18–24 h. The cell pellet was collected by centrifugation at 13,000 rpm for 1 min and washed twice with the ¼ Ringer solution (Sigma, Germany). Then, the pellet was re-suspended in the ¼ Ringer solution and adjusted OD_{600} to 0.1 (10^8 CFU mL^{-1}).

C. Plant Materials and Pot Experiment

A specimen of *G. pseudochina* (L.) DC. (KKU No. 28875) was deposited in the Herbarium of Khon Kaen University (KKU), Thailand. The plant tubers (5–6 g) were surface sterilized by sequentially immersing in 70% (v/v) ethanol for 1 min and 3% (w/v) NaOCl for 3 min and washed three times with sterilized deionized water. Deionized water was sterilized by autoclaving at 121 ºC for 15 min. Then, the tubers were separately soaked for 2 h in each bacterial suspension and ¼ Ringer solution (control). Each treated tuber was transplanted into a plastic pot containing 250 g of the Zn plus Cd contaminated soil and non-contaminated soil (control). The plants were grown in the Envirothon greenhouse at UWE Bristol, UK. The conditions were 25 ºC, 16:8 h day/night regime. Plants were watered with 20 mL of sterilized deionized water for three days per week. Each treatment was performed in six replicates. After growing the plants for 2 months, the plants were carefully harvested. Total chlorophyll concentration per fresh weight (FW) was determined by extraction with 95.5% (v/v) acetone [22]. Dry weight (DW) per plant was carried out to evaluate plant growth. The roots were immersed in 10 mM ethylenediaminetetraacetic acid (EDTA) for 30 min and rinsed with an excess of deionized water to remove the surface adsorbed metals [23]. A modified nitric-perchloric acid digestion method [24] was used to determine the total amounts of Zn and Cd accumulated in biomass on a dry weight basis.

D. Plant Extraction

The leaves were collected and washed with deionized water before blotting and freeze-drying. Then, the dried leaves were ground into a fine powder. A 3-mL disposable syringe (NIPPO, Japan) was used to perform percolation and solvent partition. A series of solvents was in the order of increasing polarity, 95% (v/v) Hexane, 99.5% (v/v) ethanol, 99.9% (v/v) methanol, and 50% (v/v) methanol. A 0.30 g of leaf powder was packed into a syringe to obtain 0.5 mL bed volume. An effluent of 10 mL per loading was collected as a fraction for each solvent. A vacuum manifold was used to control the flow rate to 0.1–0.2 mL per minute. Each extract was stored at -20 °C until analysis in an amber glass bottle with a tight stopper.

E. TPC, TFC, and FRSA Determination

TPC was measured using the modified Folin Ciocalteu method [25]. A test used 100 µL of samples or a standard Gallic acid (GA) solution. A 500 µL of 10% (v/v) Folin–Ciocalteu phenol reagent, 100 µL of 7.5% (w/v) Na_2CO_3, and 300 µL of distilled water were added to the sample. After incubating in darkness for 90 min, the absorbance was measured at 731 nm. The TPC value was expressed in terms of a GA equivalent (µmol GAE g^{-1} dry weight).

TFC was evaluated by a modified colorimetric method [26]. The analysis used 100 µL of sample or standard epicatechin (EC) solution, 500 µL of deionized water, and 30 µL of 5% (w/v) $NaNO_2$. The mixture was incubated in the dark for 5 min. Then, 60 µL of 10% (w/v) $AlCl_3$ was added before incubation for 6 min. Finally, 200 µL of 1 M NaOH and 110 µL of deionized water were added and incubated in the dark for 5 min. The absorbance was measured at 510 nm. The TFC values were presented in terms of an EC equivalent (µmol ECE g^{-1} dry weight).

FRSA was investigated using the 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) free radical method [27]. A 20 µL sample was mixed with 180 µL of 0.08 mM DPPH solution and allowed to incubate in the dark for 30 min. The absorbance was investigated at 515 nm. FRSA was assayed by DPPH in terms of IC_{50} (half-maximal inhibitory concentration) (mg crude extract L^{-1} reaction).

F. HPLC and LC-MS/MS Analysis

Phenolics and flavonoids in the extracts were primarily investigated by high-performance liquid chromatography (HPLC). Briefly, an HPLC (Shimadzu SIL-10AD, Japan) with a C-18 guard column (4.6 mm×250 mm×5 μm) (VetiSepTM UPS C-18, Thailand) and a C-18 reversed-phase column (4.6 mm×25 mm×5 μm) (GL Science Lab InsertSustain C-18, Japan) was performed. The mixture of 3% (v/v) acetic acid in water (solvent A) and 99.9% (v/v) methanol (solvent B) was used to form a gradient mobile phase [28]. The temperature of the column was 40 °C, and the flow rate was 1 mL per minute. A 0.22-μm nylon filter (Whatman, GE Healthcare, UK) was used to filter the extract. A 20 μL of the sample was injected. The HPLC chromatogram was detected by a UV-diode array detector (SPD-M20A, Shimadzu, Japan) at 280 nm.

LC-MS/MS, with a quadrupole time of flight (QTOF) mass analyzer, was used to investigate unknown compounds [4]. Briefly, an Agilent HPLC 1260 series coupled with a QTOF 6540 UHD accurate mass (Agilent Technologies, Waldbronn, Germany) was used for analysis. A Luna C18 (2) 150×4.6 mm, 5 μm (Phenomenex, USA) was used to separate the 5 μL of the filtrated extract. The mobile phase consisted of gradient elution between water (solvent A) and acetonitrile (solvent B), which both contained 0.1% v/v formic acid. The flow rate and temperature of the column were controlled at 500 μL per minute and 35 °C, respectively. A QTOF 6540 UHD with accurate mass was used for the mass analysis. The data was analyzed using Agilent Mass Hunter Qualitative Analysis Software B06.0 (Agilent Technologies, CA, USA).

G. Data Analysis

The data was analyzed using a one-way analysis of variance (ANOVA). Duncan's new multiple range test (DMRT) was used to analyze variance and the separation of means. The statistical analysis was performed using IBM SPSS Statistics 29.0 for Windows (IBM, USA).

III. Results

A. Endophytic Identification and PGPEB Properties

The phylogenetic analysis of *Curtobacterium* sp. GLD03, *Stenotrophomonas* sp. GSD10, and *Chryseobacterium* sp. GTID13 was shown in Fig. 1. The endophytes tolerated high Zn and Cd concentrations, and they were able to grow on TSA supplemented with 1000 mg L^{-1} of Zn or 100 mg L^{-1} of Cd. Table 1 showed that the three endophytes grew well on TSA supplemented with 200 mg L^{-1} of Zn plus 50 mg L^{-1} of Cd. However, the Zn plus Cd stress led to a decrease in IAA production and N_2 fixation properties of GLD03. Meanwhile, the metal stress stimulated GSD10 to produce more IAA and stimulate siderophore gene expression. In addition, the N_2 fixation property of GSD10 was still effective under the metal stress. The IAA and siderophore production of GTID13 under metal-free and the Zn plus Cd stress were unchanged. The pathogenic test on the *G. pseudochina* leaves showed no symptoms by the three strains. Due to the abilities of GLD03, GSD10, and GTID13, especially in terms of Zn/Cd tolerance and IAA production, reinoculation of the endophytes into *G. pseudochina* should be studied as a guideline for promoting plant growth in Zn/Cd contaminated soil.

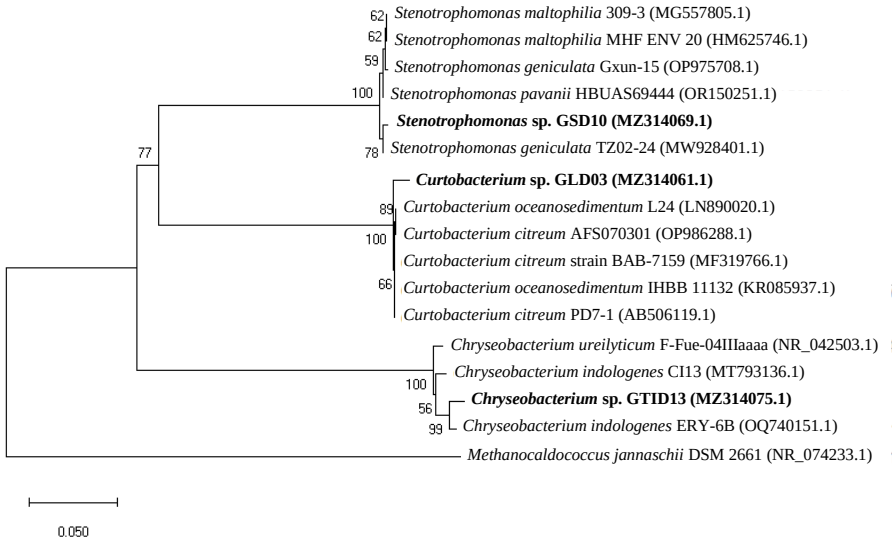


Fig. 1. Phylogenetic analysis of 16S rRNA gene sequencing of three endophytic bacteria isolated from *G. pseudochina* and sequences from NCBI databases using neighbor joining statistical method with 1000 bootstrap raplicates. There were in 1545 positions in final dataset. Evolutionary analyses were conducted with MEGA XI. Bootstrap values are indicated at node. Bar indicates 0.05 substitutions per nucleotide position.

Table 1. Characteristics of plant growth promoting properties of three Zn/Cd endophytic bacteria under control (metal-free) and Zn (200 mg L^{-1}) plus Cd (50 mg L^{-1}) stress, and pathogenicity test on *G. pseudochina* plant

Endophytic bacteria	Plant pathogenicity	Growth on TSA containing Zn + Cd [a]	Plant growth-promoting properties under control, and Zn plus Cd							
			IAA production[b] (mg L^{-1})		N_2 fixation[c]		P solubilization[d]		Siderophore production[e]	
			Control	Zn+Cd	Control	Zn+Cd	Control	Zn+Cd	Control	Zn+Cd
Curtobacterium sp. GLD03	No symptoms	+++	17.52±0.69	7.58±1.19	+, slowly weak*	-	-	-	-	-
Stenotrophomonas sp. GSD10	No symptoms	+++	13.03±0.04	21.16±2.38	++, slowly weak*	+, slowly weak*	-	-	-	++
Chryseobacterium sp. GTID13	No symptoms	+++	12.34±0.15	14.74±0.87	-	-	-	-	++	++

[a] The symbol of growth: − no growth, ± growth/weak, + low growth, ++ moderate growth, +++ high growth. Growth on TSA containing Zn 200 mg L^{-1} + Cd 50 mg L^{-1}

[b] Indole acetic acid (IAA) production is expressed as mean±SD (n=3).

[c] Nitrogen fixation: − no color change from green to blue in nitrogen-free malate medium, + little, ++ moderate, +++ high production

[d] Phosphate solubilization: − not able to solubilize phosphate (tricalcium phosphate), + little, ++ moderate, +++ high production

[e] Siderophore production: − not able to change color in chrome azurol S (CAS) agar, + little, ++ moderate, +++ high production

[*] Slowly weak is expressed as able to produce at 48-72 h.

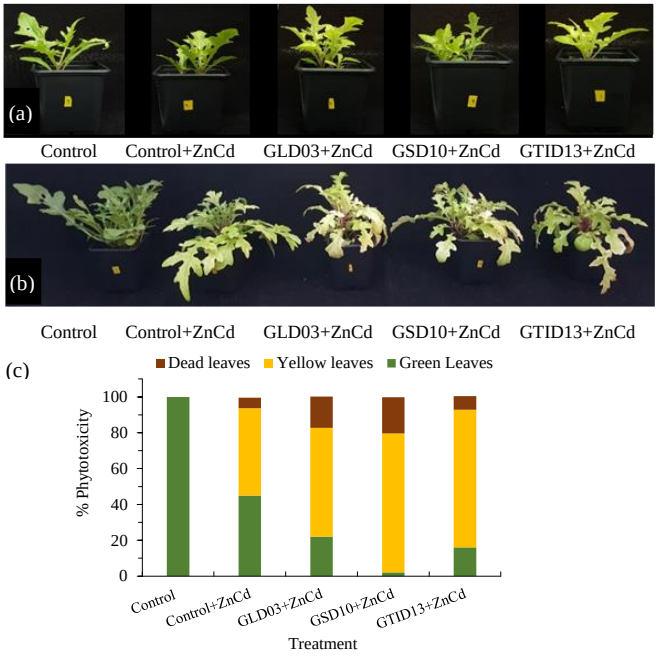


Fig. 2. Morphology of *G. pseudochina* plants growing for (a) one month and (b) two months under non-contaminated soil, Zn plus Cd contaminated soil, and the plants inoculated with GLD03, GSD10, and GTID13 growing in the contaminated soil, and (c) percentage of phytotoxicity.

Fig. 3 shows the dry weight, total chlorophyll content, and the accumulation of Zn and Cd in the plant biomass. The dry weight of plants grown in soil contaminated with Zn plus Cd was lower than those of control plants (Fig. 3(a)). Moreover, a greater decrease in dry weight was significantly caused by inoculation with the endophytic strains. The plants grown in the Zn plus Cd contaminated soil showed a significant decrease in total chlorophyll a and b compared to control plants (Fig. 3(b)). However, the total chlorophyll content of the inoculated plants was not significantly different from the plants of Control+ZnCd. The accumulation of Zn and Cd in the plant biomass was shown in Fig. 3(c) and Fig. 3(d), respectively. For the plants of Control+ZnCd, Zn was accumulated in both the above-ground (shoot) part and underground (tuber+root) part, and Cd accumulation was mainly in the shoot part. GSD10 inoculation had a direct impact on Zn and Cd accumulation in tubers and roots, while GLD03 and GTID13 tended to promote Zn and Cd accumulation in shoots.

The production of phenolic compounds was a main Zn and Cd tolerant mechanism of *G. pseudochina* [5]. Therefore, the impact of endophytic strains on plant growth, phytotoxicity, and Zn/Cd accumulation could be linked to modifications in phenolic profiles.

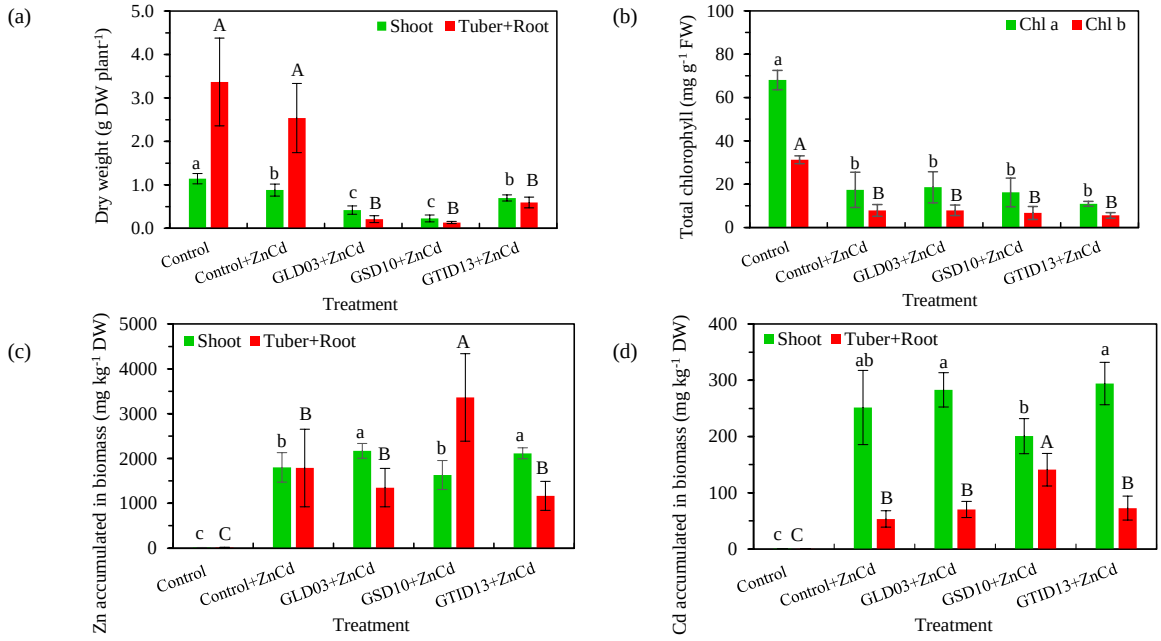


Fig. 3. The growth of *G. pseudochina* on fertile soil (control), fertile soil contaminated with Zn plus Cd (Control+ZnCd), and the contaminated soil inoculated with GLD03, GSD10, and GTID13. Dry weight per plant (a), total chlorophyll content (b), Zn, and Cd accumulated in biomass (c, d) after two months of growing in a pot system. The data are given as the means ± SD (n = 5). The different letters (s) are significant differences.

B. Analysis of phenolic compounds

Table 2 shows TPC, TFC, IC_{50}, crude extract amount, and some phenolic compounds identified by HPLC. The crude extracts obtained from the leaves of the plants grown in the Zn plus Cd contaminated soil were higher than the crude extracts obtained from control plants. In addition, inoculation of the endophytic strains also resulted in a tendency for higher amounts of crude extracts from leaves, especially in the GSD10+ZnCd. In addition, the crude extracts from Control and Control+ZnCd did not have significant differences in TPC, TFC, and IC_{50}. A high IC_{50} indicates a low level of antioxidant activity. The crude extracts from plants inoculated with GTID13 had a slight decrease in TPC, TFC, and IC_{50} values when compared with Control+ZnCd. On the other hand, the TPC, TFC, and IC_{50} of the crude extracts from the plants inoculated with GLD03 and GSD10 were dramatically decreased.

In comparison with Control, an increase in chlorogenic acid (CGA) and a decrease in caffeic acid (CA) in the crude extracts of Control+ZnCd might involve the Zn/Cd tolerant mechanism of *G. pseudochina* (Table 2). The amounts of CGA, CA, and rutin (RUT) seemed to be consistent with the results of TPC, TFC, and IC_{50}. The low levels of TPC, TFC, and antioxidant activity of the crude extracts from

GLD03+ZnCd and GSD10+ZnCd might be caused by the low content of CGA, CA, and RUT in the extracts. The TPC and TFC of GLD03+ZnCd and GSD10+ZnCd significantly decreased by 68-76% and 70-80%, respectively, when compared to those values of Control+ZnCd. The CGA, CA, and RUT decreased by 82-83%, 37-59%, and 54-56%, respectively. Moreover, the areas of unknown compounds in the crude extracts of GLD03+ZnCd and GSD10+ZnCd were also decreased. In the case of GTLD13+CdZn, the TPC and TFC values decreased by 20% and 12%, respectively, when compared to those values of Control+ZnCd. In addition, the amounts of CGA, CA, and RUT in the crude extracts of GTID13+ZnCd were close to those found in the crude extracts of Control+ZnCd. The peak area of the unknown compound was not significantly different from that of the control. The increase in crude extracts and decrease in phenolic compounds could be a sign of changes in phenolic profiles.

Table 2. Crude content, TPC, TFC, IC50, CGA, CA, and RUT in the leaf extracts of *G. pseudochina* growing in non-contaminated soil and Zn plus Cd contaminated soil, without and with Zn/Cd tolerant endophytic inoculation

Treatment	Crude content (mg g^{-1} DW)	TPC (μmol GAE g^{-1} DW)	TFC (μmol ECE g^{-1} DW)	IC_{50} (mg crude extract L^{-1} reaction)	CGA (mg g^{-1} DW)	CA (mg g^{-1} DW)	RUT (mg g^{-1} DW)	Unknown (Area)
Control	205.46 ± 38.36^{c}	96.86 ± 16.66^{a}	53.01 ± 6.03^{a}	83.8 ± 30.2^{a}	1.34 ± 0.01^{c}	0.49 ± 0.01^{a}	1.04 ± 0.04^{a}	3,656,563 ± 122,344^{b}
Control+ZnCd	230.91 ± 47.05^{bc}	93.88 ± 2.37^{a}	50.07 ± 1.40^{a}	108.5 ± 47.5^{a}	1.77 ± 0.01^{a}	0.27 ± 0.02^{b}	0.99 ± 0.01^{a}	4,308,547 ± 7,246^{a}
GLD03+ZnCd	265.50 ± 17.36^{ab}	29.50 ± 3.00^{c}	15.43 ± 2.78^{c}	342.0 ± 83.0^{b}	0.32 ± 0.05^{d}	0.11 ± 0.01^{d}	0.45 ± 0.00^{b}	762,286 ± 201,649^{d}
GSD10+ZnCd	298.80 ± 25.66^{a}	21.90 ± 0.36^{c}	10.24 ± 0.25^{c}	533.5 ± 52.5^{c}	0.29 ± 0.00^{d}	0.17 ± 0.00^{c}	0.43 ± 0.01^{b}	1,130,462 ± 8,2136^{c}
GTID13+ZnCd	272.15 ± 18.22^{ab}	75.39 ± 3.80^{b}	43.63 ± 1.29^{b}	116.5 ± 9.5^{a}	1.53 ± 0.10^{b}	0.27 ± 0.02^{b}	1.01 ± 0.07^{a}	3,844,917 ± 99,834^{b}

Note: Differences in the letters (a-c) in the same column are significant differences according to Duncan's test (p<0.05). Data are given as means ± SD (n=3).

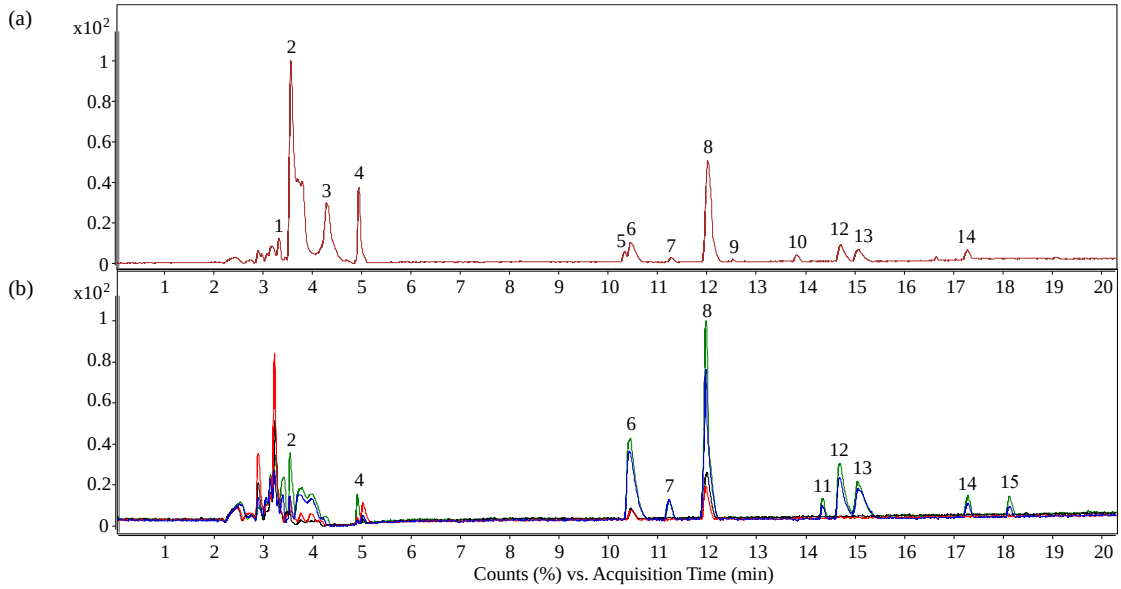


Fig. 4. LC-ESI base peak chromatogram (BPC) of *G. pseudochina* leaf extracts obtained from the combination of ethanol, methanol, and 50% (v/v) methanol fractions, (a) the plant growing in non-contaminated soil (control), and (b) the plant growing in Zn plus Cd contaminated soil (Control+ZnCd) and inoculated with Zn/Cd tolerant endophytes (GLD03, GSD10 and GTID13). For determination of the main peaks, see Table 2.

Table 3. LC-ESI-QTOF-MS/MS analysis of phenolic compounds from *G. pseudochina* leaf extracts obtained from non-contaminated soil (control) and the plant growing in Zn plus Cd contaminated soil (Control+ZnCd) and inoculated with Zn/Cd tolerant endophytes (GLD03, GSD10, and GTID13)

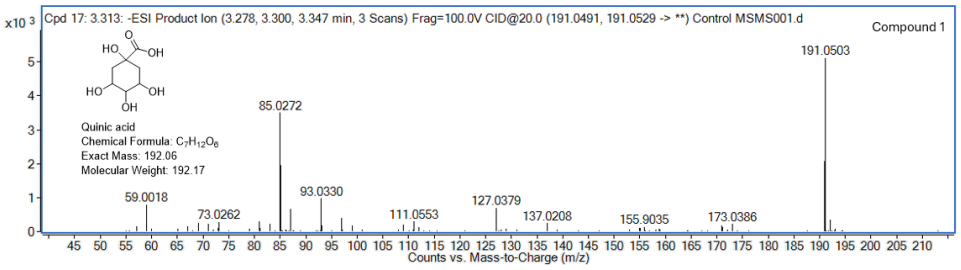
Peak no.	RT (min)	ESI-MS m/z [M-H]-	MS/MS fragment	Tentative identification	Formula	Error (ppm)
1	3.32	191.05	127.03, 93.03, 85.02, 59.01	Quinic acid	$C_7H_{12}O_6$	-7.75
2	3.58	133.01	115.00, 71.01	Malic acid	$C_4H_6O_5$	-7.86
3	4.29	133.01	115.00, 72.99, 71.01, 59.01	Malic acid	$C_4H_6O_5$	-7.11
4	4.94	191.01	111.00, 87.00, 57.03	Citric acid	$C_6H_8O_7$	0.66
5	10.33	359.07	271.09, 243.06, 179.03, 135.04, 71.01	Rosmarinic acid	$C_{18}H_{16}O_8$	-4.89
6	10.46	353.09	191.05, 135.05, 85.02	Caffeoylquinic acid (CGA)	$C_{16}H_{18}O_9$	-12.41
7	11.28	137.02	119.01, 108.02, 81.03, 65.04	Salicylic acid	$C_7H_6O_3$	11.08
8	12.06	179.03	135.04, 89.03	Caffeic acid (CA)	$C_9H_8O_4$	-10.65
9	12.53	475.18	429.17, 325.12, 205.07, 163.05, 101.02	Phenethyl rutinoside	$C_{20}H_{30}O_{10}$	2.95
10	13.82	313.07	269.07, 159.04, 147.04, 109.02	Salvianolic acid F	$C_{17}H_{14}O_6$	3.39
11	14.34	381.11	179.03, 135.04, 85.02	Ethyl chlorogenate	$C_{18}H_{22}O_9$	3.16
12	14.71	515.12	353.08, 191.05, 179.03, 135.04, 85.02	Dicaffeoylquinic acid isomer 1	$C_{25}H_{24}O_{12}$	-6.98
13	15.09	515.12	353.08, 191.00, 179.03, 135.04	Dicaffeoylquinic acid isomer 2	$C_{25}H_{24}O_{12}$	6.78
14	17.29	193.05	161.02,149.05,133.02,106.04,77.03	Ferulic acid	$C_{10}H_{10}O_4$	-10.19
15	18.13	543.15	381.11, 161.02, 135.04	Methyl 3,4-dicaffeoylquinate	$C_{27}H_{28}O_{12}$	-3.13

D. Phenolic Identification by LC-MS/MS

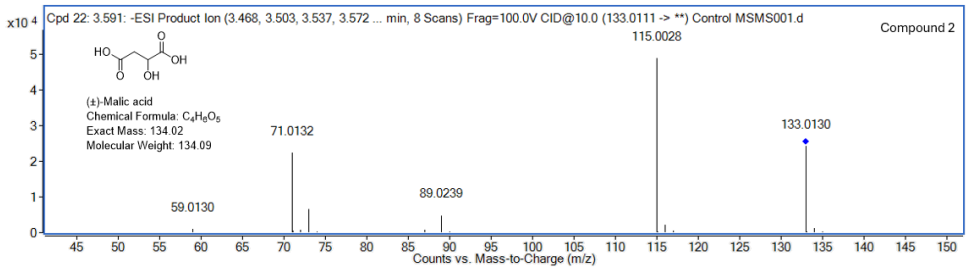
Samples were prepared to contain the same crude extract mass/solvent volume ratio for quantitative comparisons. LC-MS/MS chromatograms are shown in Fig. 4, and the main peaks of chemical profiles are identified as shown in Table 3. The MS/MS fragmentation patterns and structures of the fifteen compounds are shown in Fig. 5 (a)-(o). An increase in peak height was observed for phenolic compounds in the crude extracts of Control+ZnCd (Fig. 4(b), but the peak height of the quinic, malic, and rosmarinic acids was lower

than the peaks found in the Control (Fig. 4(a)). In addition, the peaks of ethyl chlorogenate and methyl 3,4-dicaffeoylquinate were found in the plants of Control+ZnCd. Interestingly, the peaks of extracts from GTID13+ZnCd were not different from those found in Control+ZnCd (Fig. 4(b)). The peaks of CGA, CA, salicylic acid, and dicaffeoylquinic acid were increased.

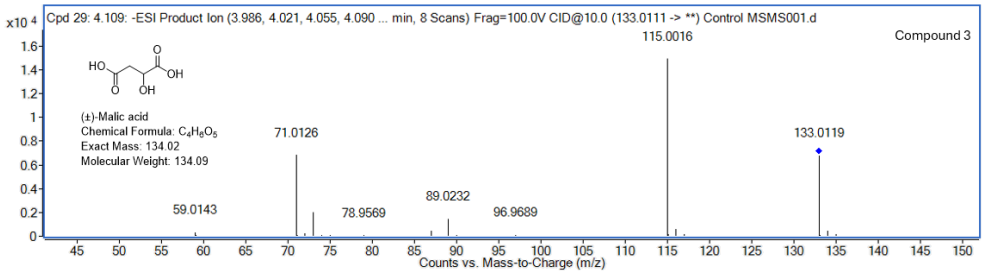
In contrast, only peaks of CGA and CA were clearly found in the extracts of GLD03+ZnCd and GSD10+ZnCd (Fig. 4(b)). Although there are a little content of rutin in the extracts from Control, Control+ZnCd, and ZnCd+GTID13 (Table 2), the rutin peak was not found in the samples prepared for LC-MS/MS. The phenethyl rutinoside, a side chain of rutin, was presented only in the control plant.



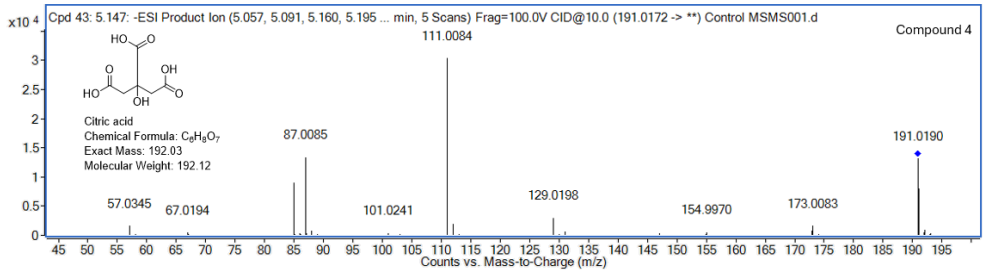
(a)



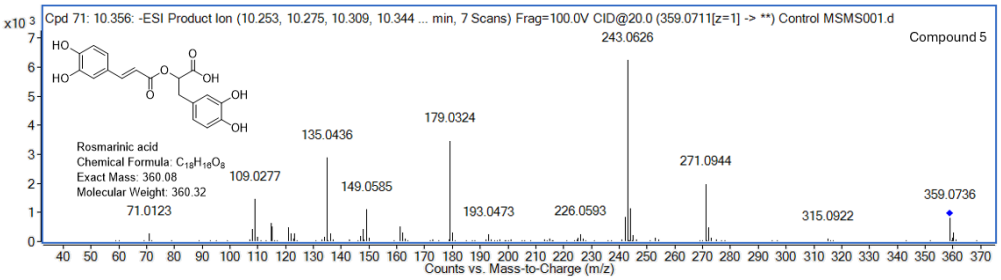
(b)



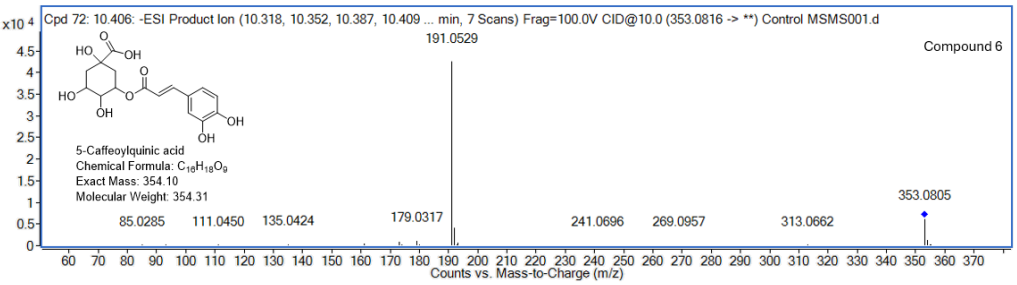
(c)



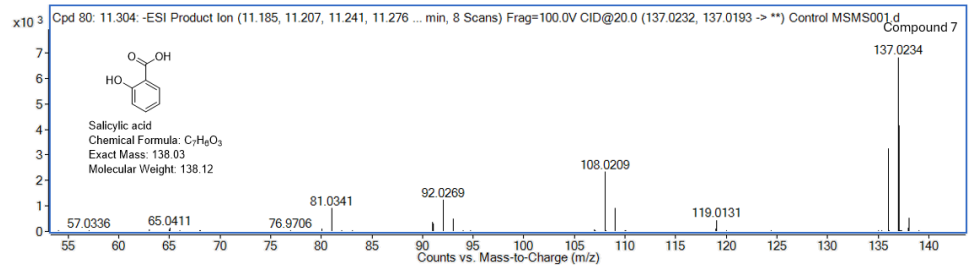
(d)



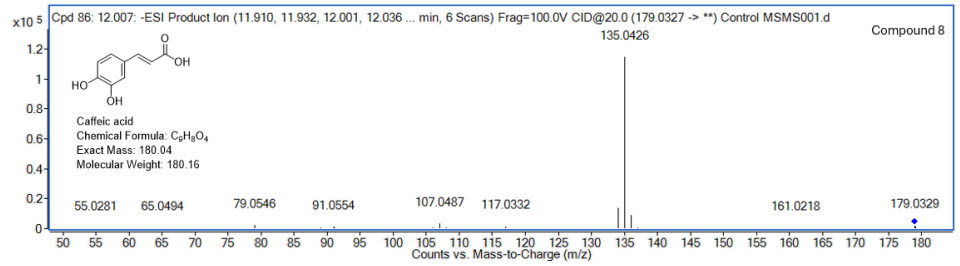
(e)



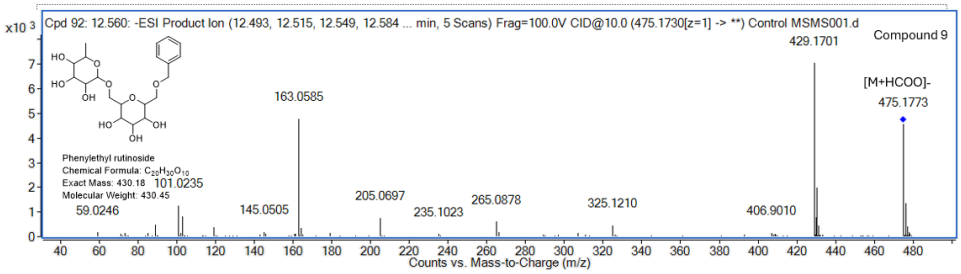
(f)


Cpd 80: 11.304: -ESI Product Ion (11.185, 11.207, 11.241, 11.276 ... min, 8 Scans) Frag=100.0V CID@20.0 (137.0232, 137.0193 -> **) Control MSMS001.d
Compound 7
137.0234
Salicylic acid
Chemical Formula: C7H6O3
Exact Mass: 138.03
Molecular Weight: 138.12
108.0209
92.0269
81.0341
119.0131
57.0336
65.0411
76.9706
Counts vs. Mass-to-Charge (m/z)

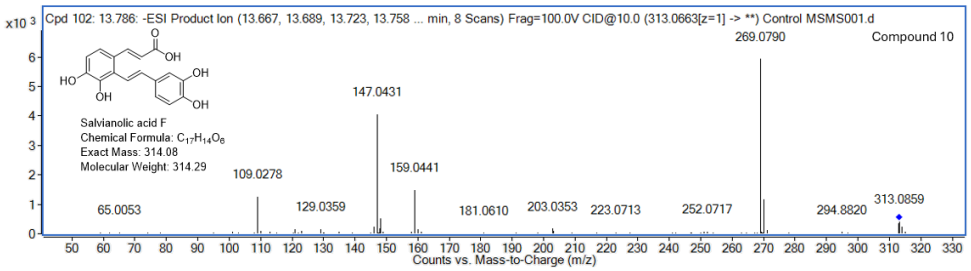
(g)


Cpd 86: 12.007: -ESI Product Ion (11.910, 11.932, 12.001, 12.036 ... min, 6 Scans) Frag=100.0V CID@20.0 (179.0327 -> **) Control MSMS001.d
Compound 8
135.0426
Caffeic acid
Chemical Formula: C9H8O4
Exact Mass: 180.04
Molecular Weight: 180.16
55.0281
65.0494
79.0546
91.0554
107.0487
117.0332
161.0218
179.0329
Counts vs. Mass-to-Charge (m/z)

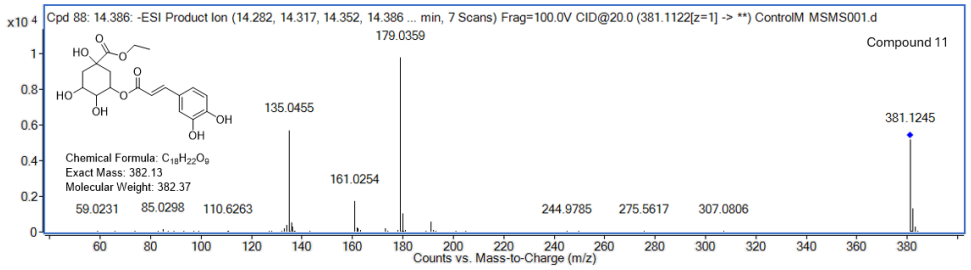
(h)


Cpd 92: 12.560: -ESI Product Ion (12.493, 12.515, 12.549, 12.584 ... min, 5 Scans) Frag=100.0V CID@10.0 (475.1730[z=1] -> **) Control MSMS001.d
Compound 9
429.1701
[M+HCOO]-
475.1773
163.0585
Phenylethyl rutinoside
Chemical Formula: C20H30O10
Exact Mass: 430.18
Molecular Weight: 430.45
101.0235
59.0246
145.0505
205.0697
235.1023
265.0878
325.1210
406.9010
Counts vs. Mass-to-Charge (m/z)

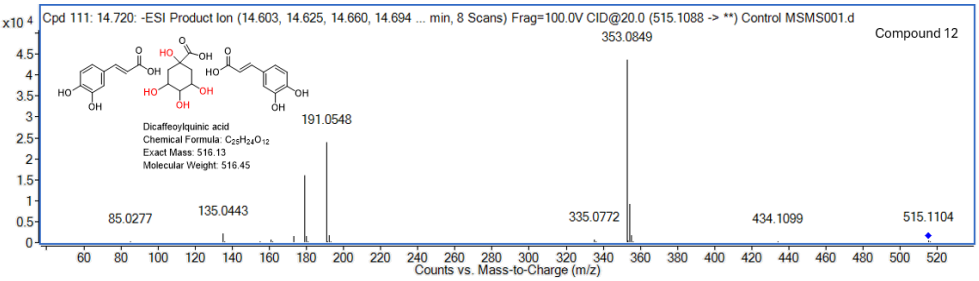
(i)


Cpd 102: 13.786: -ESI Product Ion (13.667, 13.689, 13.723, 13.758 ... min, 8 Scans) Frag=100.0V CID@10.0 (313.0663[z=1] -> **) Control MSMS001.d
Compound 10
269.0790
147.0431
Salvianolic acid F
Chemical Formula: C17H14O6
Exact Mass: 314.08
Molecular Weight: 314.29
109.0278
159.0441
65.0053
129.0359
181.0610
203.0353
223.0713
252.0717
294.8820
313.0859
Counts vs. Mass-to-Charge (m/z)

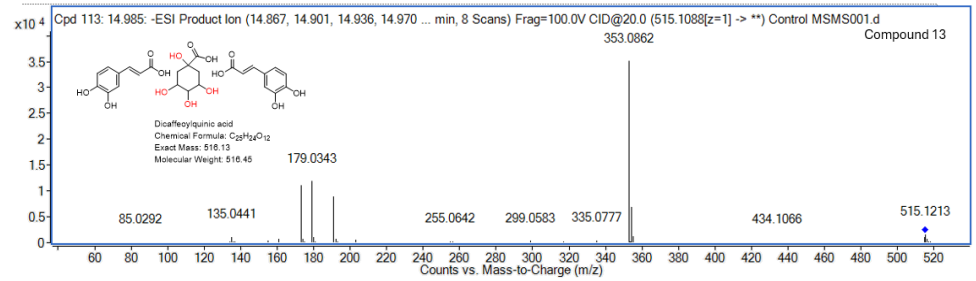
(j)


Cpd 88: 14.386: -ESI Product Ion (14.282, 14.317, 14.352, 14.386 ... min, 7 Scans) Frag=100.0V CID@20.0 (381.1122[z=1] -> **) ControlM MSMS001.d
Compound 11
179.0359
135.0455
381.1245
Chemical Formula: C18H22O9
Exact Mass: 382.13
Molecular Weight: 382.37
161.0254
59.0231
85.0298
110.6263
244.9785
275.5617
307.0806
Counts vs. Mass-to-Charge (m/z)

(k)


Cpd 111: 14.720: -ESI Product Ion (14.603, 14.625, 14.660, 14.694 ... min, 8 Scans) Frag=100.0V CID@20.0 (515.1088 -> **) Control MSMS001.d
Compound 12
353.0849
Dicaffeoylquinic acid
Chemical Formula: C25H24O12
Exact Mass: 516.13
Molecular Weight: 516.45
191.0548
85.0277
135.0443
335.0772
434.1099
515.1104
Counts vs. Mass-to-Charge (m/z)

(l)


Cpd 113: 14.985: -ESI Product Ion (14.867, 14.901, 14.936, 14.970 ... min, 8 Scans) Frag=100.0V CID@20.0 (515.1088[z=1] -> **) Control MSMS001.d
Compound 13
353.0862
Dicaffeoylquinic acid
Chemical Formula: C25H24O12
Exact Mass: 516.13
Molecular Weight: 516.45
179.0343
85.0292
135.0441
255.0642
299.0583
335.0777
434.1066
515.1213
Counts vs. Mass-to-Charge (m/z)

(m)

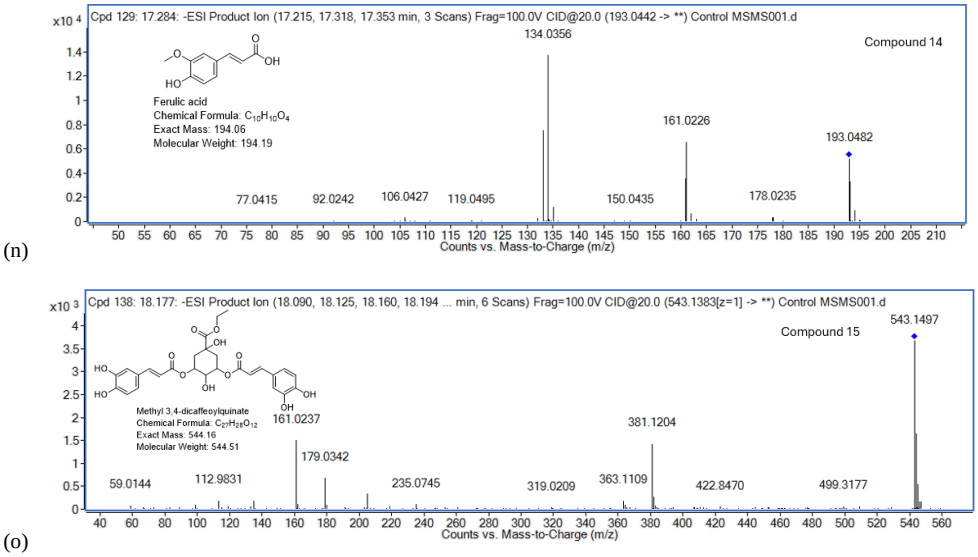


Fig. 5 MS/MS fragmentation patterns and structures of (a) compound 1: quicid acid, (b) compound 2: malic acid, (c) compound 3: malic acid, (d) compound 4: citric acid, (e) compound 5: rosmarinic acid, (f) compound 6: caffeoyl quinic acid, (g) compound 7: salicylic acid, (h) compound 8: caffeic acid, (i) compound 9: Phenethyl rutinoside, (j) compound 10: Salvianolic acid F, (k) compound 11: ethyl chlorogenate, (l) compound 12: dicaffeoylquinic acid isomer 1, (m) compound 13: dicaffeoylquinic acid isomer 2, (n) compound 14: ferulic acid, and (o) compound 15: methyl 3,4-dicaffeoylquinate

IV. Discussion

Understanding the impact of metal-tolerant endophytic bacteria on host plants in a pot experiment is important before applying them to the field. *Curtobacterium* sp. GLD03, *Stenotrophomonas* sp. GSD10 and *Chryseobacterium* sp. GTID13, which produced IAA under Zn/Cd toxicity (Table 1), were investigated in this experiment. Endophytes releasing IAA can influence the plant-endophyte association and plant development in metal-contaminated soil [14]. The toxic effects of Zn plus Cd or endophytic inoculation on *G. pseudochina* plants were investigated by growing them in nutrient-rich commercial soil for two months to fully demonstrate their effects. According to the Zn plus Cd dose-response curve, concentrations greater than 100 mg L^{-1} of Zn plus 15 mg L^{-1} of Cd began to cause a decrease in the dry weight of *G. pseudochina* [4]. Therefore, the decrease in dry weight of plants in Control+ZnCd was due to the toxicity of Zn and Cd (Fig. 2(a)). Besides, endophytic inoculation did not appear to promote plant growth under the high Zn plus Cd-contaminated soil. The plant morphology and phytotoxicity (number of yellow and dead leaves) (Fig. 2) corresponded to the total chlorophyll content (Fig. 3 (b)). The toxicity of heavy metals can cause deficiencies and nutrient imbalance by interfering with the uptake and distribution of essential nutrients in plants [29]. Cd tolerance and toxicity are closely related to the homeostasis of essential metals, such as Ca, Fe, and Zn [29]. Metal stress significantly impacted total chlorophyll content, particularly Cd toxicity [30]. Chlorosis of leaves and wilting are visible symptoms of Cd toxicity, which usually arises when the total concentration of Cd in plant tissues exceeds 30 mg kg^{-1} [7].

Under the condition of Control+ZnCd, *G. pseudochina* was able to accumulate 1801±328 mg L^{-1} of Zn and 251±66 mg L^{-1} of Cd in the shoot part. The amounts of Zn and Cd that were accumulated were comparable to those previously studied in a tissue culture system [4]. *Stenotrophomonas* sp. GSD10 significantly increased the amount of Zn and Cd accumulated in the underground (tuber+root) part, while *Curtobacterium* sp. GLD03 and *Chryseobacterium* sp. GTID13 tended to promote the accumulation of metal in shoots (Fig. 3 (c)-(d)). The increased metal accumulation may be due to the unique properties of the three endophyte species. *Stenotrophomonas maltophili* was able to tolerate high concentrations of Cd [31–32], and *S. maltophili* Sm777 accumulated up to 4% of its biomass by detoxifying Cd to CdS [31]. *Curtobacterium* sp. GX_31 biomasses could adsorb Cd^{2+} by both live and dead onto the cell surface [33]. *Curtobacterium oceanosedimentum* DG-20 had high Cd tolerance and bioaccumulation properties [34]. *Chryseobacterium* DEMBc1 acted as stabilizers for Cd [35], and *Chryseobacterium* cells and extracellular polymeric substances (EPS) functioned as biosorbents [36]. In this experiment, *Chryseobacterium* sp. GTID13 was able to produce a mucoid that could act as a metal biosorber and assist in cell protection.

The activation of the phenylpropanoid biosynthetic pathway occurs under abiotic stress conditions [6]. Therefore, the impact of environmental stresses on plants can be analyzed by measuring the level of phenolic compounds [37]. Phenolic compounds play an important role as antioxidants and metal detoxification [6, 7]. The amount of Zn in the Zn plus Cd treatment could inhibit Cd-induced oxidative stress in *G. pseudochina* [4]. Thus, it might be the reason that the TPC, TFC, and IC_{50} of Control+ZnCd were not different from those obtained from the Control (Table 2). The CGA, CA, and RUT amounts tended to be consistent with TPC and TFC. Interestingly, a significant decrease of TPC, TFC, CGA, CA, RUT, and the unknown peak area was observed in the leaf extracts of GLD03+ZnCd and GSD10+ZnCd (Table 2), which might indicate the breakdown or degradation of the compounds.

The missing peaks in the LC-ESI base peak chromatogram (Fig. 4(b)) of GLD03+ZnCd and GSD10+ZnCd clearly confirmed that phenolic compounds in the plant cell might be degraded by the endophytic strains of *Curtobacterium* sp. GLD03 and *Stenotrophomonas* sp. GSD10. Several symbiotic relationships between plants and microorganisms are based on the reciprocity of benefits for both partners. Possible abilities to degrade phenolic compounds of *Curtobacterium* and *Stenophomonas* have been reported.

Curtobacterium has the potential to decompose and recycle organic material, and it can hydrolyze various polysaccharides [38]. Khleifat *et al.* [39] reported the biodegradation of phenol by *Curtobacterium flaccumfaciens*. *Stenotrophomonas* sp. has potential application in bioremediation [40]. Gao *et al.* [41] showed that *S. maltophilia* was able to decompose organic pollutants such as phenanthrene as its sole carbon source.

Chryseobacterium species are classified as aerobic chemoorganotrophs that can grow on commercial organic media and produce polysaccharide-degrading enzymes [42–44]. *Chryseobacterium* are also considered to play important roles in the degradation of polycyclic aromatic hydrocarbons [45] and 2,4-dinitrotoluene in soil [46]. However, the results in Table 2 and the chemical profiles (Fig. 3) obtained from GTID13+ZnCd and control+ZnCd were slightly different. Additionally, the genome analysis of *Chryseobacterium* revealed superoxide dismutase, hydrogen peroxidase, and catalase [47–49], which could assist in reducing metal-induced oxidative stress in plants. Furthermore, *Chryseobacterium* sp. MDR7 [16], *Chryseobacterium* sp. DEMBc1 [35] and *Chryseobacterium* sp. LKS04 [50] were characterized as Zn and/or Cd-resistant plant growth promotion of endophytes and well recolonized to their host plants.

The LC-ESI base peak chromatogram (Fig. 4) clearly confirmed that *G. pseudochina* produced phenolic compounds to reduce toxicity caused by Cd and excess Zn. This study also revealed that only *Chryseobacterium* sp. GTID13 was able to maintain phenolic content in leaf extracts. In addition, GTID13+ZnCd did not generate any new peaks that differed from Control+ZnCd. The phenolic profile in the extracts of plants grown in a tissue culture system [4] was slightly different from the results of the phenolic profiles found in this study. Citric acid and malic acid are in the metabolic pathway of the tricarboxylic acid cycle. The shikimic acid pathway is the metabolism of carbohydrates and aromatic amino acids. Then, phenylalanine from the Shikimic acid pathway is the starting point of the phenylpropanoid pathway. The phenylpropanol pathway is the precursor for the biosynthesis pathway of caffeic acid, ferulic acid, and salicylic acid [51]. Rosmarinic acid is an ester of caffeic acid and 3, 4-dihydroxyphenyllactic acid [52]. Salvianolic acid, which is formed by rosmarinic acid, is a caffeic acid dimer [53]. Phenethyl rutinoside is an o-gylcosyl compound. Caffeoylquinic acid is an ester of caffeic acid and (-)-quinic acid. Dicaffeoylquinic acid contained two caffeic acid molecules, which had several isomers in our extract. Methyl 3,4-dicaffeoyl quinate is the formal condensation of hydroxy groups at positions 3 and 4 of methyl quinate with two molecules of trans-caffeic acid. Ethyl chlorogenate is a structure of caffeoyl quinic acid [54].

Rosmarinic acid, salvianolic acid, and phenethyl rutinoside were found only in the Control (Fig. 4 (a)). According to the peaks in Fig. 3 (b), caffeoyl quinic acid, salicylic acid, caffeic acid, ethyl chlorogenate, dicaffeoylquinic acid, ferulic acid, and methyl 3,4-dicaffeoylquinate involved with Zn/Cd tolerant mechanism of *G. pseudochina*. In particular, caffeoylquinic acid and caffeic acid remained in GLD03+ZnCd and GSD10+ZnCd.

V. Conclusion

Curtobacterium sp. GLD03, *Stenotrophomonas* sp. GSD10, and *Chryseobacterium* sp. GTID13 were selected for this study because of their plant growth-promoting abilities, especially IAA production, under the stress of 200 mg L^{-1} of Zn plus 50 mg L^{-1} of Cd. Each endophyte was separately inoculated in *G. pseudochina*, and the impact of both inoculation and Zn plus Cd toxicity was. The results obtained from this study indicated that inoculation of PGPEB to host plants under metal stress may not always be beneficial for host plants. GLD03 and GSD10 caused plants to experience increased phytotoxicity, a decrease in dry weight, and degradation of phenolic compounds. The more significant accumulation of Zn and Cd in plants might be due to the unique properties of the endophytes as metal biosorbents. The loss of phenolic compounds occurred in *Curtobacterium* sp. GLD03 and *Stenotrophomonas* GSD10 have been reported to have properties related to the degradation of phenolic compounds. *Chryseobacterium* sp. GTID13 was the only strain in this study that caused a small reduction in TPC and TFC, as confirmed by HPLC and LC-MS/MS. The phenolic profiles of *G. pseudochina* regarding Zn/Cd tolerance both without and with GTID13 inoculation were mainly composed of caffeoylquinic acid, caffeic acid, and their derivatives. Finally, a metabolomics analysis study can clearly understand the complex interaction between endophytes and plant metabolites [55], which should be studied further.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

R.M. and W.N. proposed and coordinated the general approach of the article. R.M and N.N. analyzed the data. R.M and W.N. wrote the paper. A.S. and D.W. proofread and revised the manuscript. All authors had approved the final version.

Funding

Royal Golden Jubilee PhD Programme (RGJ); no. PHD/0212/2556 under The Thailand Research Fund (TRF); and Newton Fund under British Council; no. GA/PhD/Scholar/Year3/010.

Acknowledgment

The authors would like to thank colleagues at the Sustainable Development Laboratory (SDL) at Mahasarakham University and the UWE laboratory at the University of the West of England.

References

[1] M. N. V. Prasad, W. Nakbanpote, A. Sebastian, A. Panitlertumpai, and C. Phadermrod, "*Phytomanagement of Padang zinc mine waste, Mae sot district, Tak Province, Thailand*," in *Soil Remediation and Plants Prospects and Challenges*, K. Hakeem, M. Sabir, M. Ozturk, A. R. Mermut, Eds. London: Academic Press, 2015, ch. 23., pp. 661–687.

[2] N. Panitlertumpai, W. Nakbanpote, A. Sangdee, K. Thumanu, I. Nakai, and A. Hokura, "Zinc and/or cadmium accumulation in *Gynura pseudochina* (L.) DC. studied in vitro and the effect on crude protein," *J. Mol. Struct*., vol. 1036, pp. 279-291, Dec. 2012.

[3] A. Kabata-Pendias, and H. Pendias, *Trace Elements in Soils and Plants*, 4th ed., Boca Raton, New York: CRC Press, 2011, ch. 5, pp. 93-121.

[4] B. Mongkhonsin, W. Nakbanpote, A. Hokur, N. Nuengchamnong, and S. Maneechai, "Phenolic compounds responding to zinc and/or cadmium treatments in *Gynura pseudochina* (L.) DC. extracts and biomass," *Plant Physiol. Biochem*., vol. 109, pp. 549-560, Dec. 2016.

[5] M. Tkalec, P. P. Štefanić, P. Cvjetko, S. Šikic, M. Pavlica, and B. Balen, "The effects of cadmium-zinc interactions on biochemical responses in tobacco seedlings and adult plants," *PloS One*, vol. 9, no. 1, e87582, Jan. 2014.

[6] A. Sharma, B. Shahzad, A. Rehman, R. Bhardwaj, M. Landi, and B. Zheng, "Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress," *Molecules*, vol. 24, no. 13, 2452, Jul. 2019.

[7] E. A. Goncharuk, and N. V. Zagoskina, "Heavy Metals, Their Phytotoxicity, and the Role of phenolic antioxidants in plant stress responses with focus on cadmium: Review," *Molecules*, vol. 28, no. 9, 3921, May. 2023.

[8] S. Chen, R. Lin, H. Lu, Q. Wang, J. Yang, J. Liu, and C. Yan, "Effects of phenolic acids on free radical scavenging and heavy metal bioavailability in *Kandelia obovate* under cadmium and zinc stress," *Chemosphere*, vol. 249, 126341, Mar. 2020.

[9] M. H. Ibrahim, Y. Chee Kong, and N. A. Mohd Zain, "Effect of cadmium and copper exposure on growth, secondary metabolites and antioxidant activity in the medicinal plant Sambung Nyawa (*Gynura procumbens* (Lour.) Merr)," *Molecules*, vol. 22, no. 10, 1623, Oct. 2017.

[10] P. Zoufan, Z. Azad, A. R. Ghahfarokhie, and M. Kolahi, "Modification of oxidative stress through changes in some indicators related to phenolic metabolism in *Malva parviflora* exposed to cadmium," *Ecotoxicol Environ. Saf*., vol. 187, 109811, Jan. 2020.

[11] K. Manquián-Cerdaa, E. Crucesb, M. Escudeya, G. Zúñiga, and R. Calderón, "Interactive effects of aluminum and cadmium on phenolic compounds, antioxidant enzyme activity and oxidative stress in blueberry (*Vaccinium corymbosum* L.) plantlets cultivated in vitro," *Ecotoxicol Environ. Saf*., vol. 150, pp. 320-326, Apr. 2018.

[12] K. Benhabiles Ait El Hocine, Y. Bellout, F. Amghar, "Effect of cadmium stress on the polyphenol content, morphological, physiological, and anatomical parameters of common bean (Phaseolus vulgaris L.)," *Applied Ecology and Environmental Research*, vol. 18, no. 2, pp. 3757-3774, Feb. 2020.

[13] I. Afzal, Z. K. Shinwaria, S. Sikandar, and S. Shahzad, "Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants," *Microbiol. Res.*, vol. 221, pp. 36-49, Apr. 2019.

[14] Y. Ma, M. Rajkumar, C. Zhang, and H. Freitas, "Beneficial role of bacterial endophytes in heavy metal phytoremediation," *J. Environ. Manage*., vol. 174, pp. 14-25, Jun. 2016.

[15] P. P. Tshikhudo, K. Ntushelo, and F. N. Mudau, "Sustainable applications of endophytic bacteria and their physiological/biochemical roles on medicinal and herbal plants," *Microorganisms*, vol. 11, 453, Feb. 2023.

[16] L. Rattanapolsan, W. Nakbanpote, and A. Sangdee, "Zinc- and cadmium-tolerant endophytic bacteria from *Murdannia spectabilis* (Kurz) Faden. studied for plant growth-promoting properties, in vitro inoculation, and antagonism," *Arch. Microbiol*., vol. 203, pp. 1131-1148, Nov. 2020.

[17] H. Prescott, Laboratory exercises in microbiology, 5th ed. New York: McGrraw Hill companies., 2002, ch 15, pp. 92-98.

[18] S. A. Gordon, and R. P. Weber, "Colorimetric estimation of indoleacetic acid," *Plant Physiol*, vol. 26, no. 1, pp. 192–195, Jan. 1951.

[19] I. Watanabe, W. L. Barraquio, M. R. De Guzman, and D. A. Cabrera, "Nitrogen-fixing (acetylene reduction) activity and population of aerobic heterotrophic nitrogen-fixing bacteria associated with wetland rice," *Appl. Environ. Microbiol*., vol. 37, no. 5, pp. 813–819, May 1979.

[20] C. S. Nautiyal, "An efficient microbiological growth medium for screening phosphate solubilizing microorganisms," *FEMS Microbiol. Lett*., vol. 170, no. 1, pp. 265–270, Jan. 1999.

[21] S. Pérez-Miranda, N. Cabirol, R. George-Téllez, L. S. Zamudio-Rivera, F. J. Fernández, "O-CAS, a fast and universal method for siderophore detection," *J. Microbiol. Methods*., vol. 70, no. 1, pp. 127–131, Jul. 2007.

[22] N. Shabala, S. I. Shabala, A.I. martynenko, O. Babourina, I. A. Newman, "Salinity effect on bioelectric activity, growth, na+ accumulation and chlorophyll fluorescence of maize leaves: a comparative survey and prospects for screening," *Aust. J. Plant Physiol*., vol. 25, no. 5, pp. 609-616. May 1998.

[23] L. Chen, S. Luo, X. Xiao, H. Guo, J. Chen, L. B. Wan, T. Xu, Q. Xi, C. Rao, C. Liu, and G. Zeng, "Application of plant growth-promoting endophytes (PGPE) isolated from *Solanum nigrum* L. for phytoextraction of Cd-polluted soils," *Appl. Soil Ecol*., vol. 46, no. 3, pp. 383-389, Nov. 2010.

[24] R. O. Miller, "*Nitric-perchloric acid wet digestion in an open vessel*," in *Handbook of Reference Methods for Plant Analysis*, Y.P. Kalra, Ed. Florida: CRC Press, 1998, pp.57-60.

[25] N. Cicco, M. T. Lanorte, M. Paraggio, M. Viggiano, and V. Lattanzio, "A reproducible, rapid and inexpensive Folin-Ciocalteu micro-method in determining phenolics of plant methanol extracts," *Microchem. J*., vol. 91, no. 1, pp. 107-110, Jan. 2009.

[26] K. M. Yoo, C. H. Lee, H. Lee, B. Moon, and C. Y. Lee, "Relative antioxidant and cytoprotective activities of common herbs," *Food Chem.*, vol. 106, no. 3, pp. 929-936, Feb. 2008.

[27] W. Brand-Williams, M. E. Cuvelier, and C. Berset, "Use of a free radical method to evaluate antioxidant activity," *LWT-Food Sci. Technol*., vol. 28, no. 1, pp. 25-30, Jun. 1994.

[28] Y. Zuo, H. Chen, and Y. Deng, "Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and pu-erh teas using HPLC with a photodiode array detector," *Talanta*, vol. 57, no. 2, pp. 307-316, May 2002.

[29] R. K. Sharma, and G. Archana, "Cadmium minimization in food crops by cadmium resistant plant growth promoting rhizobacteria," *Appl. Soil Ecol*., vol. 107, pp. 66-78, Nov. 2016.

[30] H. Grajek, D. Rydzyński, A. Piotrowicz-Cieślak, A. Herman, M. Maciejezyk, and Z. Wieczorek, "Cadmium ion-chlorophyll interaction – examination of spectral properties and structure of the cadmium-chlorophyll complex and their relevance to photosynthesis inhibition," *Chemosphere*, vol. 261, 127434, Dec. 2020.

[31] D. Pages, J. Rose, S. Conrod, S. Cuine, P. Carrier, T. Heulin, and W. Achouak, "Heavy metal tolerance in *Stenotrophomonas maltophilia*," *PloS one*, vol. 3, no. 2, e1539, Feb. 2008.

[32] S. Wierzba, "Biosorption of lead (II), zinc (II) and nickel (II) from industrial wastewater by *Stenotrophomonas maltophilia* and *Bacillus subtilis*," *Pol. J. Chem. Technol*., vol. 17, no. 1, pp. 79-87, Mar. 2015.

[33] X. Li, Z. Zhao, Q. Xiao, N. He, J. Kong, D. Zhang, R. Li, and Q. Shao, "Potential application of *Curtobacteium* sp. GX_31 for efficient biosorption of cadmium: Isotherm and kinetic evaluation," *Environ. Technol. Inno*., vol. 30, 103122, May. 2023.

[34] M. Patel, K. Patel, L. A. Al-Keridis, N. Alshammari, R. Badraoui, A. M. Elasbali, W. A. Al-Soud, M. I. Hassan, D. K. Yadav, and M. Adnan, "Cadmium-tolerant plant growth-promoting bacteria *Curtobacterium oceanosedimentum* improves growth attributes and strengthens antioxidant system in chili (*Capsicum frutescens*)," *Sustainability*, vol. 14, no. 7, 4335, Apr. 2022.

[35] M. Majewska, S. Wdowiak-Wróbel, M. Marek-Kozaczuk, A. Nowak, and R. Tyśkiewicz, "Cadmium-resistant *Chryseobacterium* sp. DEMBc1 strain: characteristics and potential to assist phytoremediation and promote plant growth," *Environ. Sci. Pollut. Res*., vol. 29, no. 55, pp. 83567-83579, Jun. 2022.

[36] R. K. Sharma, and G. Archana, "Cadmium minimization in food crops by cadmium resistant plant growth promoting rhizobacteria," *Appl. Soil Ecol*., vol. 107, pp. 66-78, Nov. 2016.

[37] S. Chen, Q. Wang, H. Lu, J. Li, D. Yang, J. Liu, and C. Yan, "Phenolic metabolism and related heavy metal tolerance mechanism in *Kandelia Obovata* under Cd and Zn stress," *Ecotoxicol. Environ. Saf*., vol. 169, pp. 134-143, Mar. 2019.

[38] I. Dimkić, V. Bhardwaj, V. Carpentieri-Pipolo, N. Kuzmanović, and G. Degrassi, "The chitinolytic activity of the *Curtobacterium* sp. Isolated from field-grown soybean and analysis of its genome sequence," *PloS one*, vol. 16, no. 11, e0259465, Nov. 2021.

[39] K. Khleifat, M. Magharbeh, M. Alqaraleh, M. Al-Sarayrah, I. Alfarrayeh, Y. Al Qaisi, A. Alsarayreh, and M. A. Al-kafaween, "Biodegradation modeling of phenol using *Curtobacterium flaccumfaciens* as plant-growth-promoting bacteria," *Heliyon*, vol 8, no.9, e10490, Aug. 2022.

[40] S. D. Gregorio, S. Lampis, and G. Vallini, "Selenite precipitation by a rhizospheric strain of *Stenotrophomonas* sp. isolated from the root system of *Astragalus bisulcatus*: a biotechnological perspective," *Environ. Int*., vol. 31, no. 2, pp. 233-241, Nov. 2004.

[41] S. Gao, J. S. Seo, J. Wang, Y. S, Keum, J. Li, and Q. X. Li, "Multiple degradation pathways of phenanthrene by *Stenotrophomonas maltophilia* C6," *Int. Biodeterior. Biodegrad*., vol. 79, pp. 98-104, Apr. 2013.

[42] T. Nishioka, M. M. Elsharkawy, H. Suga, K. Kageyama, M. Hyakumachi, and M. Shimizu, "Development of culture medium for the isolation of *Flavobacterium* and *Chryseobacterium* from rhizosphere soil," *Microbes Environ*., vol. 31, no. 2, pp. 104-110, Apr. 2016.

[43] S.L. Wang, Y. C. Liang, and T. W. Liang, "Purification and characterization of a novel alkali-stable α-amylase from

Chryseobacterium taeanense TKU001, and application in antioxidant and prebiotic," *Process Biochem.*, vol. 46, no. 3, pp. 745-750, Mar. 2011.
[44] S. Rattanasuk, and M. Ketudat-Cairns, "*Chryseobactrium indologenes*, novel mannanase-producing bacteria," *Songklanakarin J. Sci. Technol*., vol. 31, no. 4, pp. 395-399, Jul.-Aug. 2009.
[45] S. Lladó, E. Gràcia, A. M. Solanas, and M. Vinas, "Fungal and bacterial microbial community assessment during bioremediation assays in an aged creosote-polluted soil," *Soil Biol. Biochem*., vol. 67, pp. 114-123, Aug. 2013.
[46] Z. -Y. Wang, Z. -F. Ye, and M. -H, Zhang, "Bioremediation of 2,4-dinitrotoluene (2,4-DNT) in immobilized micro-organism biological filter," *J. Appl. Microbiol*., vol. 110, no. 6, pp. 1476-1484, Jun. 2011.
[47] J. -J. Jeong, H. Park, B. H. Park, M. Mannaa, M. K. Sang, I. -G. Choi, and K.D. Kim, "Draft genome sequence of a biocontrol rhizobacterium, *Chryseobacterium kwangjuense* strain KJ1R5, isolated from pepper (*Capsicum annuum*)," *Genome Announc*., vol.4, no. 2, e00301-16, Apr. 2016.
[48] J. -J. Jeong, B. Park, J. Y. Oh, M. Mannaa, Y. J. Kim, J. K. Hong, I. -G. Choi, and K. D. Kim, "Draft genome sequences of *Chryseobacterium artocarpi* UTM-3^T and *Chryseobacterium contaminas* C26^T, isolated from rhizospheres, and *Chryseobacterium arthrosphaerae* CC-VM-7^T, isolated from eh feces of a Pill Millipede," *Genome Announc*., vol. 4, no. 5, e01168-16, Oct. 2016.
[49] M. Kumar, K. Charishma, K. P. Sahu, N. Sheoran, A. Patel, A. Kundu, and A. Kumar, "Rice leaf associated *Chryseobacterium* species: An untapped antagonistic flavobacterium displays volatile mediated suppression of rice blast disease," *Biol. Control*, vol. 161, 104703, Jul. 2021.
[50] S, Luo, L. Chen, J. Chen, X. Xiao, T. Xu, Y. Wan, C. Rao, C. Liu, Y. Liu, C. Lai, and G. Zeng, "Analysis and characterization of cultivable heavy metal-resistant bacterial endophytes isolated from Cd-hyperaccumulator *Solanum nigrum* L. and their potential use for phytoremediation," *Chemosphere*, vol. 85, no. 7, pp. 1130-1138, Nov. 2011.
[51] G. B. Kougan, T. Tabopda, V. Kuete, and R. Verpoorte, "*Simple phenols, phenolic acids, and related esters from the medicinal plants of Africa*," in *Medicinal Plant Research in Africa*, V. Kuete, Ed. London: Elsevier, 2013, ch. 6, pp. 225-249.
[52] S. Sundaram, A. Tripathi, D. K. Gupta, "Metabolic modeling of rosmarinic acid biosynthesis pathway," *Bioinformation*, vol. 5, no. 4, pp. 168-172.
[53] J. Wang, J. Xu, X. Gong, M. Yang, C. Zhang, and M. Li, "Biosynthesis, chemistry, and pharmacology of polyphenols from Chinese *Salvia* species: A review," *Molecules*, vol. 244, 155, Jan. 2019.
[54] J. Yu, J. X, M. Sun, S. Xiong, C. Xu, Z. Zhang, M. Li, C. Li, and L. Lin, "Plant-derived caffeic acid and its derivatives: An overview of their NMR data and biosynthetic pathways," *Molecules*, vol. 29, 1625, Apr. 2024.
[55] R. Kataoka, M. Akashi, T. Taniguchi, Y. Kinose, A. E. Yaprak, and O. C. Turgay, "Metabolomics analyses reveal metabolites affected by plant growth-promoting endophytic bacteria in roots of the halophyte *Mesembryanthemum crystallinum*," *Int. J. Mol. Sci.*, vol. 22, no. 21, 11813, Oct. 2021.