

Enhancing Brassica Juncea Growth and Stress Resistance with Plant Growth-Promoting Rhizobacteria *Bacillus subtilis* (RS-10) Under Cadmium Stress

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Abstract

Pollution by industries and households has also led to increased cadmium (Cd) concentration in agricultural soils thus posing a major risk to plant growth and production. The over use of chemical fertilizers and pesticides further increases environmental degradation. *Brassica juncea* is a vital oil seed crop, and it is highly vulnerable to cadmium toxicity that interferes with normal physiological and metabolic processes. Rhizobacteria (PGPR) have been shown to promote plant tolerance to stress under such conditions providing a sustainable solution. This research assessed the cadmium-ameliorating effects of *Bacillus subtilis* RS-10, originally isolated from the rhizosphere of *Cynodon dactylon*, against cadmium-induced toxicity on *Brassica juncea*. Plants were subjected to graded levels of CdCl₂ (0, 50, 75, and 100 mM), followed by metabolomic characterization through gas chromatography-mass spectrometry (GC-MS). Metabolomic profiling revealed significant modulation of pyruvate metabolism, nitrogen metabolism, the tricarboxylic acid (TCA) cycle, and glutathione metabolism, indicating enhanced energy production and nutrient assimilation. Elevated levels of amino acids, fatty acids, and organic acids further contributed to cadmium tolerance. Overall, *B. subtilis* RS-10 exhibits strong potential as a sustainable bioinoculant for improving crop performance in Cd-contaminated soils.

1. Introduction

The environment comprises interconnected spheres of the lithosphere, hydrosphere, atmosphere and biosphere, where humans, plants, animals, and microorganisms coexist. Pollutants like chemical substances that exceed natural concentrations have increasingly disturbed this balance [1]. Among these, heavy metals pose severe ecological and agricultural threats. Anthropogenic activities such as mining, smelting, industrial waste discharge and excessive fertilizer and pesticide use are major contributors to heavy metal accumulation in soil [2]. These pollutants disrupt soil microbial populations and essential nutrient cycles, leading to reduced fertility and productivity [3]. Cadmium (Cd) is particularly harmful due to its high mobility and bioavailability, causing toxicity in both plants and humans. Cd exposure suppresses plant root and shoots development, interferes with nutrient uptake, and promotes oxidative damage. Its accumulation in edible crops such as *Brassica juncea* further threatens food security and public health [4].

Conventional agricultural practices such as intensive tillage, chemical fertilization, monoculture and flood irrigation have provided short-term productivity gains but caused long-term soil degradation [5]. These methods reduce biodiversity, emit greenhouse gases, and disrupt soil structure. Thus, sustainable and biologically driven strategies have become necessary to restore soil fertility and maintain crop yields [6, 7].

Plant growth-promoting rhizobacteria (PGPR) have emerged as a promising eco-friendly approach to sustainable agriculture. PGPR colonize plant roots and enhance nutrient uptake, growth, and resistance to biotic and abiotic stresses [8]. They stimulate plant growth through phytohormone production, nitrogen fixation, and heavy metal detoxification mechanisms, including exopolysaccharide and siderophore secretion [9]. For economically important crops like *Brassica juncea*, PGPR improve tolerance to salinity and heavy metal stress by modulating physiological pathways [10]. Among these microbes, *Bacillus subtilis* RS-10 has demonstrated strong potential for promoting growth and mitigating metal toxicity. RS-10 enhances nitrogen metabolism, pyruvate metabolism, and citrate cycle activity, resulting in improved nutrient uptake and stress tolerance.[11]

Given the environmental and agricultural challenges posed by Cd contamination, this research focuses on the use of *B. subtilis* RS-10 to enhance the growth and Cd tolerance of *B. juncea*. Through greenhouse experiments and GC-MS-based metabolomic analysis, this research investigates how RS-10 modulates key metabolic pathways under Cd stress. Understanding the metabolic shifts associated with RS-10 inoculation provides insights into its potential as a bioinoculant for sustainable agriculture. The findings aim to contribute to eco-friendly strategies that reduce chemical dependency, restore soil health, and improve crop productivity in heavy metal-contaminated environments.

2. Materials and Methods

To evaluate the influence of *B. subtilis* strain RS-10 on *B. juncea* growth and its tolerance to Cd stress, a series of interrelated experiments were conducted. These included extraction of bacterial metabolites, Cd sensitivity tests, greenhouse assays, and phytochemical profiling using gas chromatography-mass spectrometry (GC-MS).

2.1. Bacterial Isolation and Inoculum Preparation

Glycerol stock of *B. subtilis* strain RS-10 was obtained from the microbial culture collection of the cellular factories/Nanobiotechnology Laboratory, NUST. The inoculum was prepared by streaking RS-10 onto LB agar plates and incubating at 37°C for 24 h. After 3–4 streaking's, a single colony was aseptically inoculated into 100 mL LB broth and incubated at 37°C, 180 rpm for 24 h. The culture was centrifuged at 5,000 rpm for 15 min, and the pellet was washed twice with sterile water or phosphate-buffered saline (PBS). The optical density (OD₆₀₀) of the bacterial suspension was adjusted to 0.4–0.8 using a spectrophotometer.

2.2. Extraction of Secondary Metabolites of RS-10

Extracellular metabolites were extracted by culturing RS-10 in 200 mL LB broth at 37 °C, 180 rpm for 24 h. Culture was centrifuged at 5,000 rpm for 15 min at 4 °C, and the

supernatant was extracted with ethyl acetate (1:1 v/v). Organic phase was evaporated under reduced pressure using a rotary evaporator and the resulting solid extract was re-dissolved in 2 mL ethyl acetate. GC-MS analysis was performed using a Shimadzu GCMS QP2020 system fitted with an SH-Rxi-5Sil MS column (30 m × 0.25 mm × 0.25 µm). The injection volume was 1 µL (split-less mode). Electron ionization was carried out at 70 eV, with injector temperature at 250 °C and ion source at 200 °C. The oven program started at 110 °C, increased at 10 °C min⁻¹ to 200 °C, then at 5 °C min⁻¹ to 280 °C, held for 9 min. Metabolites were identified by comparison with spectra in the NIST Mass Spectral Library.

2.3. RS-10 Cadmium Tolerance Screening

To assess Cd tolerance, RS-10 was streaked on LB agar plates containing CdCl₂ (0–3 mM) followed by incubation at 37°C for 24 h. Growth was visually evaluated to determine sensitivity and establish the optimal Cd concentration for subsequent plant experiments.

2.4. Seed Inoculation and Germination Assay

B. juncea seeds were surface sterilized with 70 % ethanol (2 min) and 1 % sodium hypochlorite (10 min), then rinsed five times with sterile distilled water. Sterilized seeds were soaked in 15 mL RS-10 suspension (OD₆₀₀ = 0.5) for 2 h; control seeds were treated with sterile water. Seeds were sown on water agar plates, sealed, and incubated at 4 °C in darkness for 72–96 h. Germination rate was recorded to assess the effect of RS-10 inoculation.

2.5. Plant Material and Growth Conditions

Sterilized *B. juncea* seeds were germinated on deionized water agar plates at 25 °C in darkness for 48 h. Seven-day-old seedlings were transferred to pots containing 400 g autoclaved soil. Each pot held six seedlings. Plants were grown in the NUST greenhouse under controlled temperature (25 °C) and 16 h light / 8 h dark photoperiod. Each treatment comprised 3 biological replicates (pots) with 6 seedlings per pot (n = 3). Irrigation was performed every two days; bacterial suspension was reapplied every 20 days for two months to maintain effective rhizosphere colonization, as described in previous PGPR inoculation studies. For biomass measurements, all of the three replicates were harvested and pooled to calculate fresh and dry weights.

2.6. Application of Cadmium on *B. juncea*

Four-week-old plants were treated with CdCl₂ at concentrations of 0, 50, 75, and 100 mM. RS-10 inoculated plants and control plants were both exposed to these concentrations in small volumes to the soil around the root zone. Cd solutions were applied every three days throughout the experiment. Growth parameters (fresh and dry weights) were measured to

assess Cd tolerance. Enrichment analysis was used to compare Cd tolerance in inoculated versus control plants.

2.7. Leaf Sample Preparation and Metabolite Extraction

Leaves from two-month-old plants were collected, rinsed, and oven-dried at 40°C to constant weight. Dried leaves were powdered and extracted with 50 mL methanol for 3–5 h. Extracts were filtered and evaporated to dryness using a rotary evaporator. Dried residues were reconstituted in methanol for GC–MS analysis (Shimadzu GCMS QP2020; SH-Rxi-5Sil MS column, 30 m × 0.25 mm × 0.25 µm). Helium was used as a carrier gas. The temperature program was: 70 °C (6 min hold), ramped to 250 °C, then to 300 °C at 10 °C min⁻¹ (10 min hold). Injection temperature: 280 °C; ion source: 250 °C; interface: 290 °C.

2.8. Statistical Analysis

Fresh and dry weight data were analyzed using independent t-tests and one-way ANOVA to determine the effects of RS-10 inoculation and Cd concentration. Descriptive statistics (mean ± SD, SE) were computed, and Levene's test verified variance homogeneity. Interaction effects were assessed using partial eta-squared values to estimate effect size. Analyses were performed using IBM SPSS Statistics, ensuring robust evaluation of treatment impacts.

3. Results

3.1. RS-10 Cadmium Screening Test

The RS-10 inoculated strain exhibited greater tolerance to Cd stress compared with the non-inoculated control at all Cd concentrations tested. Both the control and RS-10 cultures showed robust growth at 0 mM Cd, forming compact, healthy colonies under optimal conditions. However, with increasing Cd concentrations, growth declined in both groups but remained markedly higher for RS-10. At 1.5 mM Cd, RS-10 maintained several colonies, whereas the control showed almost complete inhibition. At 2 mM Cd, RS-10 growth was minimal but still visible, while the control strain failed to grow. At 3 mM Cd, RS-10 formed only a few small colonies, indicating its tolerance threshold. The control strain displayed negligible growth, confirming its high Cd sensitivity (Fig. 1).

3.2. Untargeted Metabolic Profiling of *B. subtilis* RS-10

GC–MS analysis identified a diverse array of secondary metabolites secreted by RS-10 that potentially contributed to plant growth and stress tolerance. The metabolites included organic acids, amino acids, volatile organic compounds (VOCs), nitrogenous compounds, and other bioactive molecules. Key compounds identified were acetic acid, butanoic acid,

alanine, proline, acetaldehyde, and 2,3-butanediol (Fig. 2). These metabolites play critical roles in nutrient cycling, osmotic adjustment, and inter-species signaling, thereby enhancing plant-microbe interactions. Organic acids such as acetic and malic acid promote nutrient uptake [12], while amino acids (e.g., alanine, proline) improve microbial and plant resilience under stress [13]. VOCs like acetone and ethanol stimulate plant vigor through volatile signaling which induce systemic plant growth responses and abiotic stress tolerance [14]. These metabolites have direct effects on plant growth through the facilitation of nutrient uptake, the promotion of osmotic balance, and the triggering of plant signaling pathways. The full list of identified metabolites and enriched pathways supporting these interpretations is provided in Supplementary Table S1

3.3. Enrichment Analysis of Metabolic Pathways Underlying RS-10

Metabolites identified through GC-MS were analyzed using the enrichment module of MetaboAnalyst 6.0 to identify key metabolic pathways in RS-10 (Fig. 3) where is the list of the metabolites it should be included as a table of Supplementary file. The most enriched pathways were pyruvate metabolism (enrichment ratio = 12.4), glyoxylate and dicarboxylate metabolism (9.2), and glycolysis/gluconeogenesis (8.8), indicating enhanced energy generation and carbon utilization.

Alanine, aspartate, and glutamate metabolism (fold change = 8.0) and the citrate (TCA) cycle (8.6) were also prominent, reflecting the bacterium's efficiency in nutrient cycling and energy production [15]. Beta-alanine, arginine, and proline metabolism contributed to stress tolerance through osmotic regulation and reactive oxygen species (ROS) scavenging [16, 17]. Nitrogen metabolism (9.5) improved nitrogen availability to plants, while unsaturated fatty acid and phenylalanine metabolism supported membrane integrity and systemic resistance [18]. The enriched pathways are critical to the maintenance of plant development during stress. ATP production can be boosted by pyruvate metabolism and TCA cycle sufficient to keep the energy-dependent processes in plants alive throughout exposure to cadmium. Nitrogen metabolism assists in the provision of amino acids and nucleotides that are used to make proteins and repair the cells. The antioxidant defenses are enhanced because of the synthesis of amino acids based on the pathways to produce compounds like glutamate, a precursor to glutathione. Together, they enhance metabolic resilience, nutrient uptake and growth performance [19, 20].

3.4. RS-10-Induced Enhanced Germination and Seedling Growth

Germination assays indicated that RS-10 inoculation significantly enhanced seed germination and seedling emergence when compared to the untreated control. Inoculated seeds exhibited higher germination rates and longer root lengths, suggesting improved nutrient uptake and early growth promotion (Fig 4) indicating that RS-10 positively

influences early developmental stages, potentially leading to increased yield under field conditions.

3.5. Effect of Cd Stress on *B. juncea* Biomass

Cd stress significantly reduced *B. juncea* biomass however RS-10 inoculation mitigated these negative effects ($p < 0.05$). Inoculated plants maintained higher fresh and dry weights across all Cd concentrations compared with the control (Fig. 5). Fresh weight decreased from 4.1 g at 0 mM Cd to 2.53 g at 100 mM in RS-10 plants, whereas control plants dropped from 3.49 g to 1.07 g. Dry weight followed similar patterns, decreasing from 0.49 g to 0.30 g in RS-10 plants and from 0.42 g to 0.14 g in controls. The above-mentioned data confirm that RS-10 inoculation enhances Cd tolerance by preserving plant biomass under stress conditions. RS-10 leads to increased Cd tolerance by inhibiting Cd absorption, improving antioxidant capacity, and homeostasis of cells. The bacterium produces organic acids and siderophores to fix Cd in the rhizosphere making it unavailable to be absorbed by plants. It also activates glutathione metabolism, which allows effective ROS detoxification. RS-10 inoculation improves nutrient and water uptake due to better root structure that offsets growth-inhibitory effects of Cd [21, 22].

3.6. Comparative Metabolomic Enrichment Analysis of Leaf Metabolites: Response to Cadmium Stress

MetaboAnalyst 6.0 analysis of leaf metabolites revealed that RS-10 inoculation significantly altered key metabolic pathways under both normal and Cd-stressed conditions. In unstressed plants, enrichment was highest for pyruvate metabolism, glycolysis, nitrogen metabolism, and TCA cycle, confirming enhanced energy and nutrient utilization (Fig. 6).

Under Cd stress, RS-10 inoculated plants showed pronounced activation of nitrogen assimilation, amino acid biosynthesis, and glutathione metabolism (Fig. 7). Arginine biosynthesis was also up-regulated, supporting polyamine and nitric oxide production for stress signaling collectively reducing ROS accumulation and maintained cellular homeostasis.

4. Discussion

The current research offers an overall evaluation of the prospects of the *B. subtilis* strain RS-10 as a PGPR in alleviating Cd toxicity in *B. juncea*. The findings substantiate that RS-10 contributed significantly to the growth and survival of plants when exposed to the Cd stress by regulating the activity of important metabolic pathways as well as by promoting physiological functions that relate to its growth and defense. The results are in harmony with previous studies on the stress alleviation by means of PGPR, supporting the

hypothesis that useful microorganisms can cushion plants by adjusting their metabolism and decreasing uptake of heavy metals. RS-10 inoculation enhanced the germination percentage, root and shoot growth, and fresh and dry biomass under Cd stress, respectively, compared to non-inoculated controls [9]. Metabolomics profiling indicated the up-regulation of stress-associated pathways, including glutathione metabolism, pyruvate metabolism, and the TCA cycle, which were involved in helping the body to produce more energy, antioxidative defense, and nutrient assimilation. These outcomes reinforce the earlier studies that have shown the potential of PGPR in enhancing tolerance of plants to metal-oxidative damage [23] [24, 25].

As Fig. 6 and Fig. 7 demonstrate, RS-10 inoculation triggers important metabolic pathways that are critical in growth and tolerance to stress. Figure 6 shows that under normal conditions, the enrichment of pyruvate metabolism, glycolysis, and nitrogen metabolism are higher in RS-10-treated plants, which is an indicator of better primary metabolism. Glutathione metabolism, alanine/aspartate/glutamate metabolism, and arginine biosynthesis are highly induced in response to cadmium stress (Fig. 7) indicating improved antioxidant capacity and stress perception.

Increased plant biomass in RS-10 inoculated *B. juncea* is an indicator of direct and indirect bacteria in alleviating Cd toxicity [26]. PGPR strains are known to induce plant growth by enhancing nutrient cycling, producing growth-promoting compounds, and stabilizing physiological processes under stress [27]. In general, Cd toxicity decreases the biomass by disrupting nutrient uptake, photosynthesis, and water homeostasis [28], whereas RS-10 has been found to combine with these effects by preserving root integrity and sustaining the rhizospheric environment. The bacteria probably generated metabolites like organic acids and siderophores that increased nutrient solubility besides causing the antioxidant enzymes and hormonal regulation to keep plants operational. Similarly reported, the activation of glutathione metabolism, through the effect of PGPR, helps plants to resist oxidative stress by eliminating reactive oxygen species [29]. Cd resistance observed in RS-10-treated plants is consistent with published studies showing PGPR-mediated detoxification mechanisms. Similar to findings by Kamran et al. (2020) and Daraz et al. (2023), RS-10 reduces Cd bioavailability through organic acid and siderophore production and enhances antioxidant pathways, especially glutathione metabolism. This aligns with previous reports demonstrating that PGPR increase TCA cycle intermediates (citrate/malate), which chelate Cadmium and reduce its transport to shoots [30].

The biomass increment recorded in the Cd treatments indicates that RS-10 is capable of using a variety of protective mechanisms similar to other useful rhizobacteria. Improved growth under metal stress is also observed in Brassica species that are treated with PGPR that immobilize metals or generate antioxidants [31]. The release of exopolysaccharides, siderophores, and organic acids of RS-10 probably decreased the bioavailability of Cd by

entrapping metal ions within the rhizosphere, which reduced the uptake of plant roots [32]. The subsequent improvement in root development has facilitated nutrient and water absorption, which is in line with the previous findings [33]. Metabolomic analysis revealed that RS-10 induced a glutathione metabolism and nitrogen assimilation-type mechanism as reported with other PGPR strains [34, 35]. These overlapping data confirm the fact that RS-10 is capable of causing conserved molecular networks that facilitate stress resistance and growth promotion. Plants possess well-established molecular mechanisms for Cd resistance, including heavy-metal ATPases (HMAs), CAX transporters, metallothioneins (MTs), and phytochelatin synthase (PCS), which mediate Cd sequestration, vacuolar transport, and chelation. Antioxidant enzymes such as SOD, CAT, APX, and GSTs further detoxify ROS generated during Cd stress. PGPR have been shown to stimulate several of these plant defense routes by enhancing nitrogen assimilation, glutathione biosynthesis, and carbon metabolism, thereby supporting detoxification and metal homeostasis [24]

Metabolite analysis quantitatively showed the high-level enrichment of pyruvate metabolism, the TCA cycle, and nitrogen metabolism, highlighting their key role in stress adaptation and energy generation [36,37] The most enriched pathway (12.4-fold) is pyruvate metabolism, which connected glycolysis to the TCA cycle, as it is necessary to maintain the generation of ATP and produce defense-related metabolites [38]. Increased TCA cycle (8.6-fold enrichment) facilitated chelation of Cd by production of citrate and malate that lowered metal accumulation and toxicity in plant tissues[39]. Similarly, nitrogen metabolic (9.5 enrichment) supported the synthesis of amino acids and nucleotides, which are essential to repair cells and maintain the osmotic equilibrium [40]. Nitrogen-containing compounds, including proline and glutamate, accumulated acted as osmoprotectants and antioxidants mitigating Cd-induced oxidative stress [41, 42].

The GC-MS profiling of the RS-10 inoculated plants established the high levels of stress-related metabolic products such as alanine and proline that stabilize proteins and membranes during stress conditions [43]. Organic acids including acetic and malic acids enhanced the absorption of the nutrient and the fixation of metals, which decreased further Cd concentration in plant tissues [44]. Systemic signaling and enhanced resistance to abiotic and biotic stresses were supported by volatile organic compounds (VOCs) including acetaldehyde and 2, 3-butanediol. Glutathione is among the major antioxidant metabolites that were critical in the prevention of oxidative damage caused by Cd, which demonstrates its critical role in detoxification processes [45, 46]. Should refer to the table as well through which you identified different pathways.

RS-10 by itself showed the ability to withstand Cd concentrations in the range of 2 mM highlighting its possible application in contaminated soils. This inherent defense is probably due to exopolysaccharide synthesis, efflux recovery, and siderophore release that decreases intracellular levels of Cd [47]. This is because of temperature and metal-stress

effects, which will be tolerated by RS-10 as a bioinoculant and thereby the survival and functionality of RS-10. Similar tolerance responses have been documented in other strains of *Bacillus* and *Pseudomonas* that can proliferate in polluted locations [48]. The capability of RS-10 to work in high Cd conditions gives it a stronger argument toward large-scale bioremediation and sustainable crop management programs [49].

Altogether, the research shows the promising nature of *B. subtilis* RS-10 as an effective bioinoculant, which can be used to increase crop productivity and overcome the toxicity of heavy metals by reprogramming metabolism, biochemical rehabilitation, and physiological enhancement. RS-10 fosters growth and stress resilience, thereby decreasing the reliance on chemical inputs and promoting environmentally sustainable agriculture. The results are in line with the international attempts to devise a biological approach to the problem of heavy metal contamination and food security [50]. However, additional field experiments are required to confirm the RS-10 activity in the natural environment as well as to assess its ecological effect in the long-term. Differential mechanisms of metal tolerance and plant growth promotion would be clarified by comparative analysis of other species of PGPR including *Pseudomonas fluorescens*, *Azospirillum brasilense*, and *Rhizobium* spp. Moreover, RS-10 molecular characterization would help identify genetic determinants of metal resistance and metal stress signaling, which would provide more information about microbial-assisted phytoremediation.

4. Conclusion

The findings of this study demonstrate that *Bacillus subtilis* RS-10 is a promising plant growth-promoting rhizobacterium with significant potential for agricultural applications, particularly in cadmium-contaminated soils. RS-10 consistently improved seed germination, biomass accumulation, and overall physiological stability in *Brassica juncea* under both normal and cadmium-stressed conditions. Metabolomic profiling further revealed that RS-10 enhances the regulation of critical pathways—including pyruvate metabolism, nitrogen metabolism, the TCA cycle, and glutathione metabolism—that are directly associated with improved nutrient assimilation, energy production, and oxidative stress mitigation.

The presence of bioactive metabolites such as amino acids, fatty acids, organic acids, and stress-related compounds indicates that RS-10 contributes to the reinforcement of plant defense systems while supporting growth under heavy metal toxicity. These attributes highlight RS-10 as a strong candidate for development into bioinoculants for sustainable crop production, offering a biological alternative to chemical-based remediation methods. Integrating RS-10 into agricultural practices may help reduce cadmium uptake, enhance soil health, and improve crop productivity in contaminated environments.

Future work should focus on field-scale validation across different agro-ecological zones, long-term environmental impact assessment, and deeper molecular characterization to identify genes and regulatory networks associated with metal tolerance. Such advancements will support the commercialization of RS-10 as a reliable microbial product for modern agriculture and phytoremediation industries.

Abbreviations

Abbreviation	Full Form
PGPR	Plant Growth-Promoting Rhizobacteria
Cd	Cadmium
GC-MS	Gas Chromatography–Mass Spectrometry
VOCs	Volatile Organic Compounds
TCA	Tricarboxylic Acid Cycle
ROS	Reactive Oxygen Species
OD	Optical Density

Declarations

Data Availability

All data generated or analysed during this study are included in this published article and its Supplementary Information files (Supplementary_Files_RS10.zip). Raw GC-MS spectra, MetaboAnalyst project files and original biomass raw data are available from the corresponding author upon reasonable request.

Ethical Approval

This study did not involve human participants or animals. The experimental work conducted on *Brassica juncea* complied with institutional, national, and international guidelines for plant research. No specific permissions were required for the collection, propagation, or use of plant material.

Competing Interests

The authors declare that they have no competing financial or non-financial interests related to this work.

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Author Contributions: CRediT

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Dr. Muhammad Tahir & Dr. Muhammad Arshad: Resources, Review and Editing

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Figures

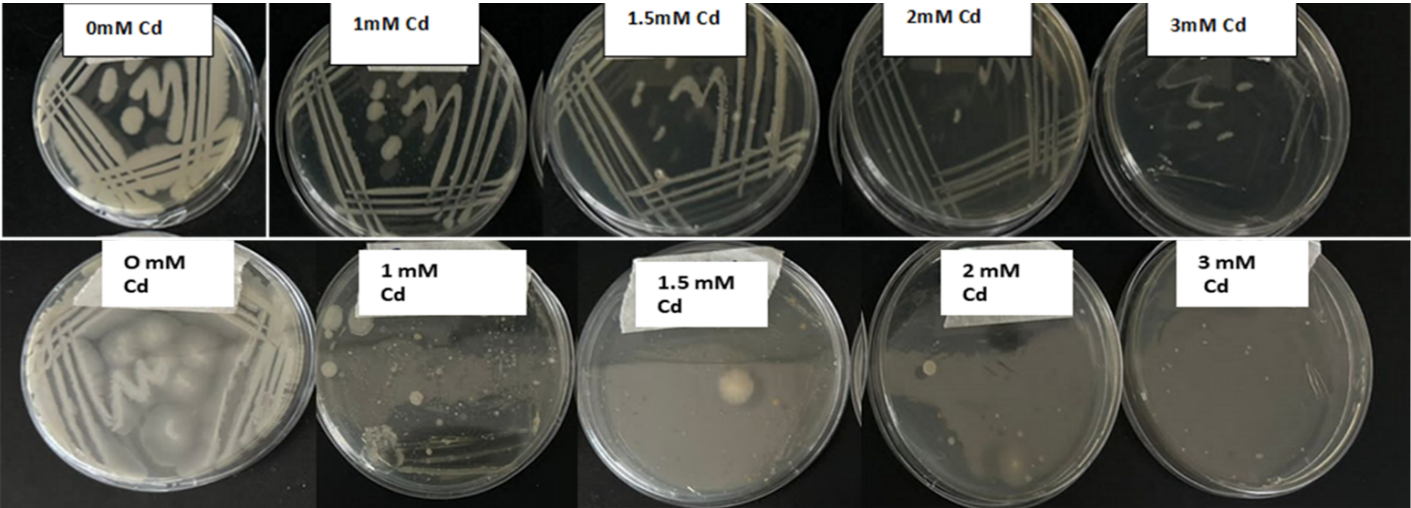


Figure 1

Cadmium tolerance of RS-10. Colony growth on LB agar supplemented with increasing Cd concentrations (0–3 mM). RS-10 maintains visible growth up to 2–3 mM Cd, indicating high tolerance.

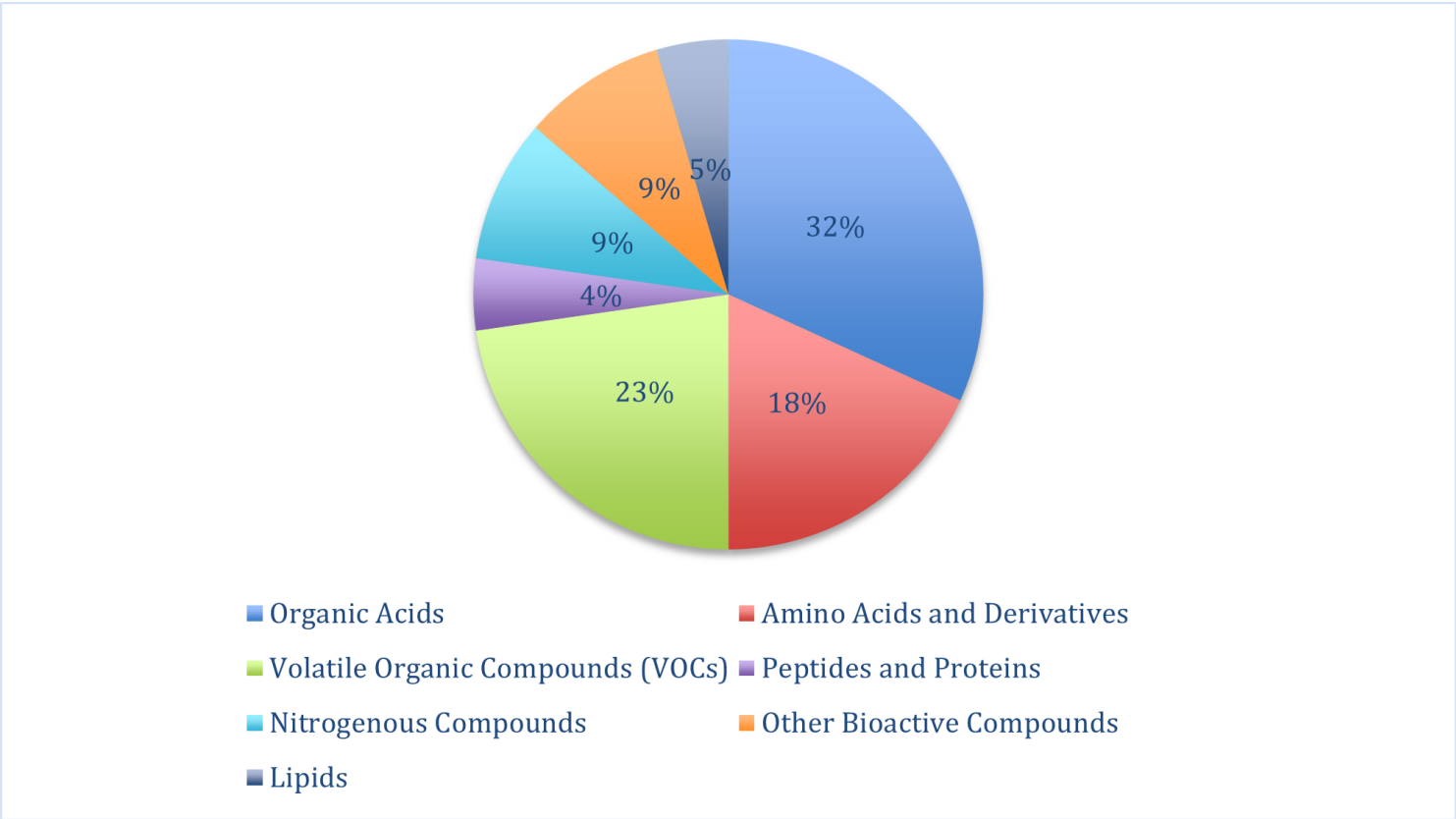


Figure 2

Distribution of RS-10 metabolites identified through GC-MS. Organic acids, amino acids, and VOCs are shown as percentage contributions to total metabolites.

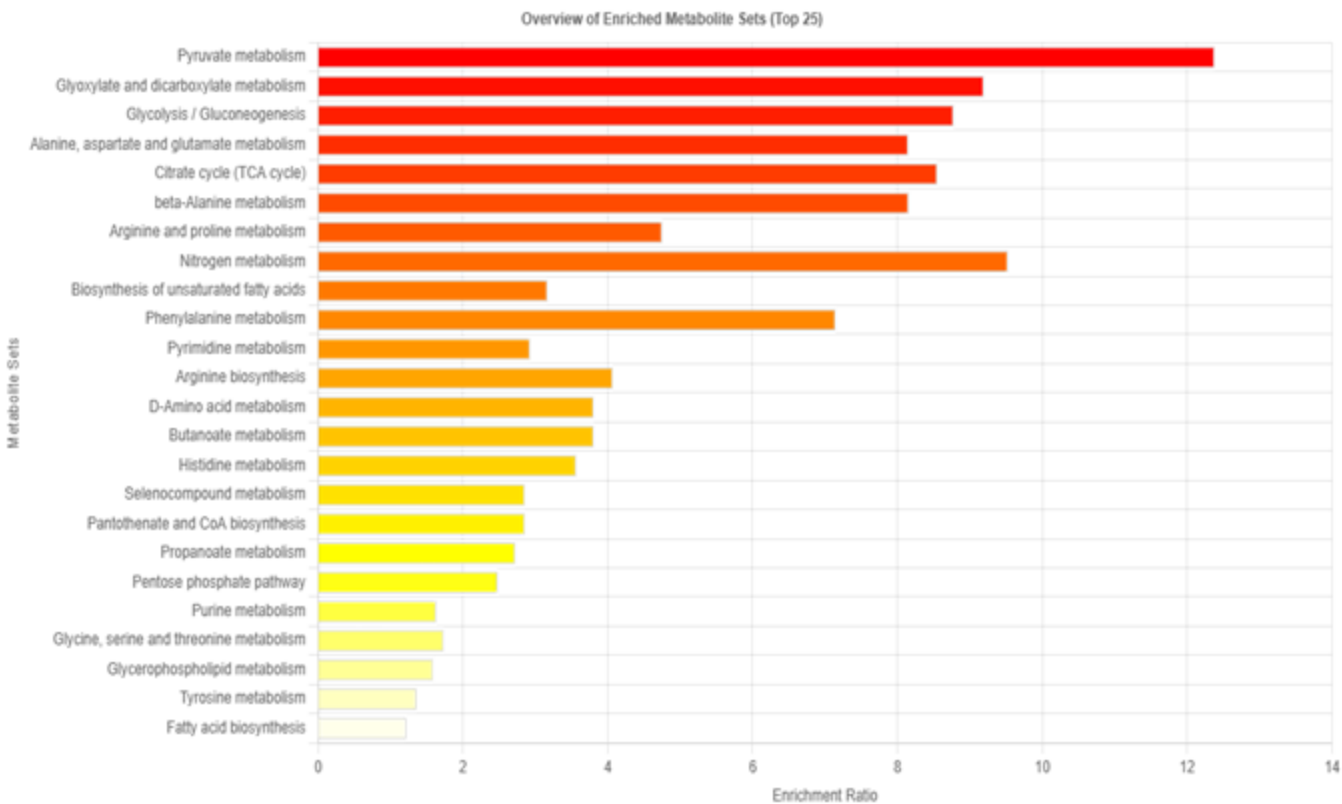


Figure 3

Pathway enrichment analysis of RS-10 metabolites. Key pathways include pyruvate metabolism, nitrogen metabolism, and the TCA cycle.

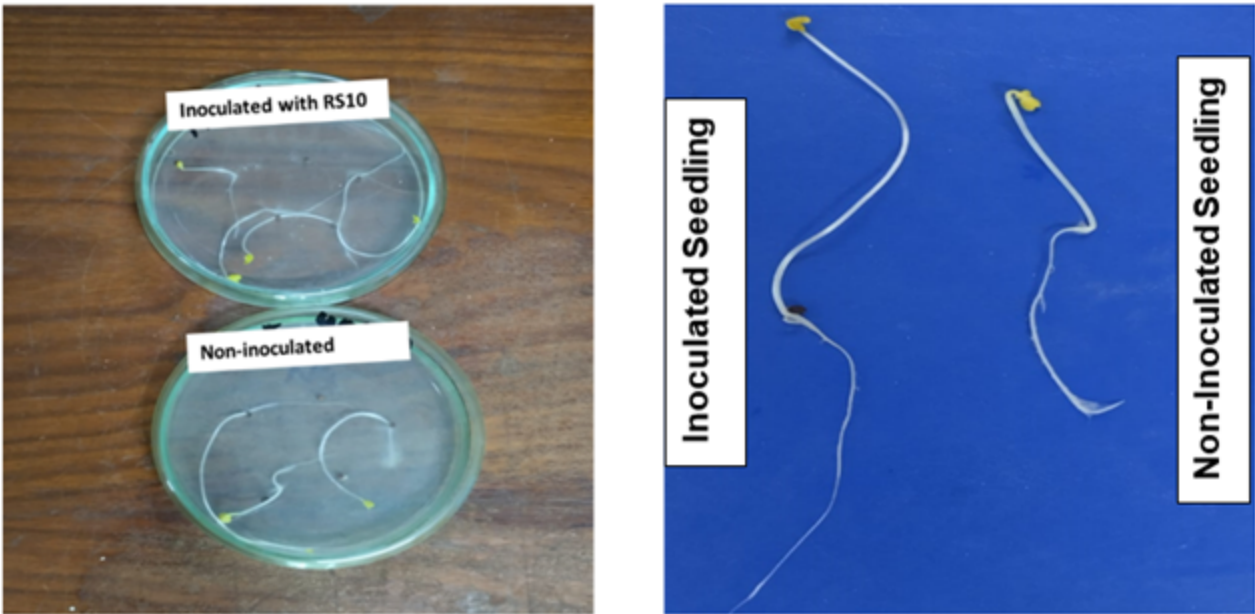


Figure 4

Effect of RS-10 on seed germination of *B. juncea*. Inoculation improved root elongation and seedling vigor (left) compared to control (right).

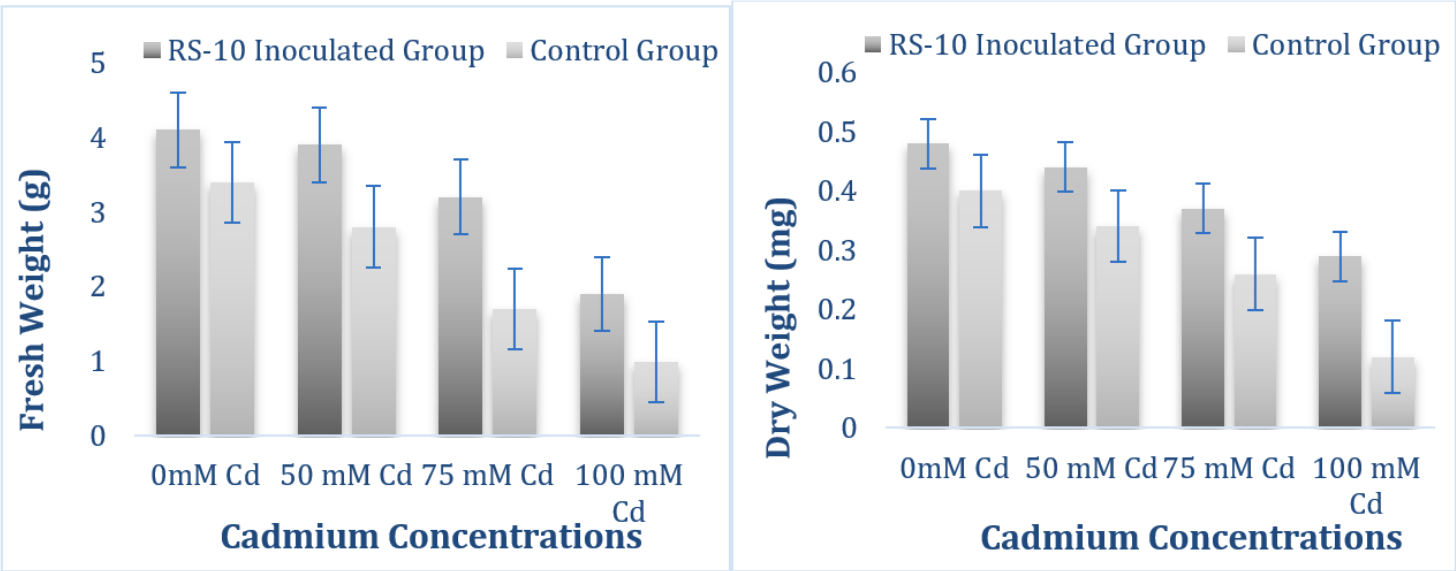


Figure 5

Effect of Cd stress on biomass of *B. juncea*. RS-10 inoculated plants maintained higher fresh and dry weights across all Cd treatments.

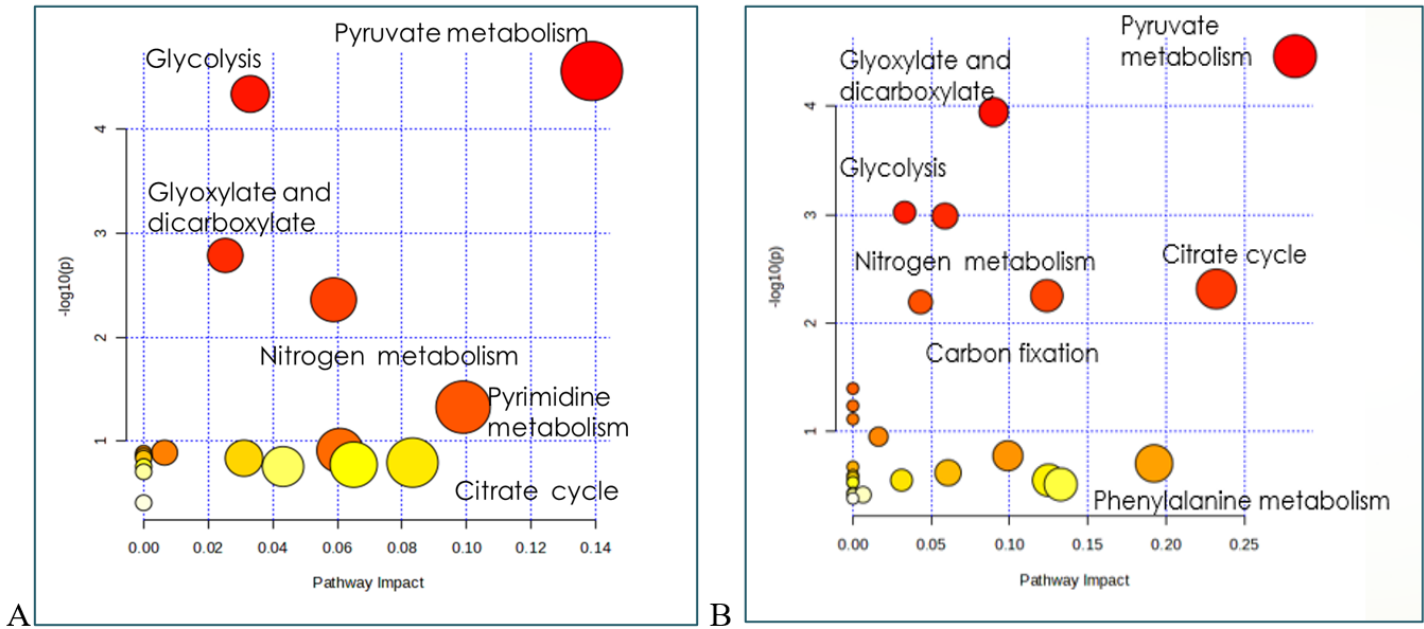


Figure 6

Metabolic enrichment in RS-10 inoculated plants under normal conditions. Pathways related to energy generation and nitrogen metabolism are up-regulated.

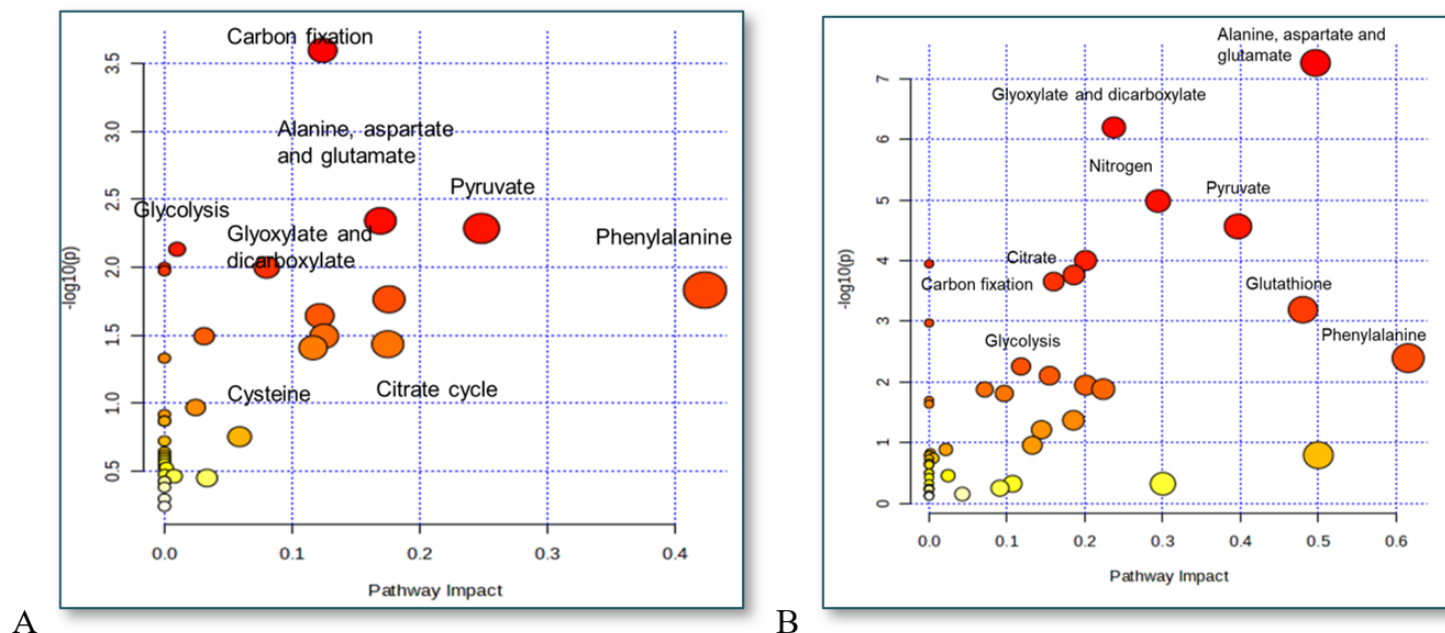


Figure 7

Metabolic enrichment under cadmium stress. RS-10 enhances glutathione metabolism, amino acid metabolism, and stress-related pathways.

Supplementary Files

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- [TableS1Metabolites.docx](#)
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