MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology

QUALIFICATION THESIS

on the topic **Study of cadmium absorption inhibition in lettuce by phosphorus-dissolving bacteria for agricultural biotechnology applications**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-21
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KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN

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First (Bachelor's) level of higher education

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Educational and professional program Biotechnology

APPROVE
Head of Biotechnology, Leather and Fur Department, Professor,
Dr. Sc., Prof.
_____Olena MOKROUSOVA
«___»_____2025

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Nie Rongjiao

1. Thesis topic **Study of cadmium absorption inhibition in lettuce by phosphorus-dissolving bacteria for agricultural biotechnology applications**

Scientific supervisor Ph.D., Assoc. Prof. Olena Okhmat

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2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

4. Date of issuance of the assignments 05.03.2025

WORK CALENDAR

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	until 11 April 2025	
2	Chapter 1. Literature review	until 20 April 2025	
3	Chapter 2. Object, purpose, and methods of the study	until 30 April 2025	
4	Chapter 3. Experimental part	until 11 May 2025	
5	Conclusions	until 15 May 2025	
6	Draw up a bachelor's thesis (final version)	until 25 May 2025	
7	Submission of qualification work to the supervisor for feedback	until 27 May 2025	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)		
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)		Similarity coefficient ____% Citation rate ____%
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)		

I am familiar with the task:

Student _____ Nie Rongjiao

Scientific supervisor _____ Olena OKHMAT

ABSTRACT

Nie Rongjiao. Study of cadmium absorption inhibition in lettuce by phosphorus-dissolving bacteria for agricultural biotechnology applications. – Manuscript.

Qualification thesis on the specialty 162 «Biotechnology and Bioengineering». – Kyiv National University of Technologies and Design, Kyiv, 2025.

The rapid advancement of industrialization has led to an increasingly severe situation of soil cadmium pollution, posing a significant challenge to the stability of the agricultural production system and the safety of human health. This experimental design focuses on the phosphorus-solubilizing bacterium *Bacillus thuringiensis J16*, aiming to systematically investigate its inhibitory effect on the absorption process of cadmium elements by lettuce plants. The experiment was carried out using the pot cultivation method. The variables set include three gradients of soil cadmium addition concentrations (0, 1, 2 mg/kg) and two levels of J16 inoculation amounts (0, 1%). The results show that under different degrees of cadmium pollution, compared with the non-inoculated control group, the inoculation of strain J16 significantly increased the biomass of lettuce roots and leaves by 68.81%; and significantly reduced the cadmium content in the roots and leaves of the plants by 38.86%. In addition, the inoculation of strain J16 enhanced the antioxidant capacity of lettuce. For example, the content of malondialdehyde in the leaves decreased, and the activities of peroxidase and superoxide dismutase increased. This study enriches the resources of phosphorus-solubilizing and cadmium-fixing strains, providing a theoretical basis and technical support for the remediation of heavy metal-contaminated soil.

Keywords: Phosphate - solubilizing bacteria, Lettuce, Cadmium, Growth promotion, Absorption inhibition

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INTRODUCTION

Soil cadmium (Cd) pollution, driven by industrial and agricultural activities, poses significant threats to ecosystem stability and human health, including soil degradation, reduced crop yields, and health risks from Cd bioaccumulation in food chains. Conventional remediation methods are often unsustainable, prompting the need to address the research question: Can phosphate-solubilizing bacteria (PSB) like *Bacillus thuringiensis* J16 inhibit Cd absorption in lettuce while promoting growth, offering a viable bioremediation strategy? This study employs pot experiments with three Cd levels (0, 1, 2 mg/kg) to investigate J16's effects, using atomic absorption spectrophotometry to measure Cd content and biochemical assays to analyze antioxidant enzymes (MDA, POD, SOD).

Results show J16 inoculation significantly increases lettuce biomass by 67.51–70.1%, reduces root/leaf Cd content by 17.14–21.72%, and enhances antioxidant capacity (MDA ↓20.62%, POD/SOD ↑35.23–38.81%), indicating its dual role in stress alleviation and metal immobilization. The thesis structure supports this inquiry: Chapter I reviews Cd pollution and PSB functions; Chapter II details methods; Chapter III presents results on growth, Cd distribution, and enzyme activities; and the Conclusion discusses implications for agricultural bioremediation.

The relevance of the study lies in addressing sustainable solutions for Cd-contaminated soils to ensure food safety.

The purpose is to evaluate J16's efficacy in inhibiting Cd absorption and promoting lettuce growth under Cd stress.

The objectives include assessing biomass changes, quantifying Cd reduction, and analyzing enzyme activity modifications.

The object of the study is Roman lettuce and *Bacillus thuringiensis* J16 in pot experiments with sandy loam soil.

The subject focuses on PSB-mediated Cd uptake inhibition mechanisms in lettuce.

Research methods involve pot trials, Cd quantification, and enzyme assays.

The scientific novelty reveals J16’s dual function in growth promotion and Cd reduction.

The practical significance offers an eco-friendly strategy for safe vegetable production in Cd-contaminated environments.

CHAPTER I
LITERATURE REVIEW

1.1 OVERVIEW OF CADMIUM CONTAMINATION IN SOIL

The rapid development of industrialization and agricultural modernization has accelerated the unprecedented spread and severity of soil cadmium pollution, posing a growing threat to ecological environments and human health. Industrial activities are one of the primary sources of soil cadmium contamination [1]. During mining operations, cadmium often occurs as an associated metal with high concentrations in ores. Mining waste residues containing cadmium are often indiscriminately dumped nearby. When exposed to rainfall, these residues are washed by rainwater, allowing cadmium to leach into the soil. Additionally, wastewater generated from ore processing, if discharged without proper treatment, introduces large quantities of cadmium into the surrounding soil environment. In the smelting industry, cadmium-containing exhaust gases released during metal refining settle into the soil through atmospheric deposition. Furthermore, improperly disposed smelting waste residues can lead to secondary pollution. Industries such as electroplating and battery manufacturing extensively use cadmium-containing raw materials, generating significant amounts of cadmium-laden wastewater and waste residues. Inadequate handling of these byproducts allows cadmium to infiltrate and contaminate the soil.

Agricultural production also contributes significantly to pollution. Phosphate fertilizers often contain cadmium impurities, and prolonged, excessive application leads to cadmium accumulation in the soil. Moreover, wastewater irrigation and the agricultural use of sludge act as "accomplices" in soil cadmium contamination. Cadmium present in untreated wastewater and sludge enters the soil during irrigation and fertilization, gradually accumulating over time [2-3].

Soil cadmium pollution is a widespread global issue. Many regions in China, including the Pearl River Delta and Yangtze River Delta—areas with dense

industrial activity and economic development—as well as farmlands subjected to long-term wastewater irrigation, frequently exhibit cadmium levels exceeding safety standards. Similarly, in Europe, industrialized nations face varying degrees of soil cadmium contamination. This pollution severely disrupts the balance of soil ecosystems, impairing microbial activity and soil fertility, hindering crop growth, and reducing yields. Excessive cadmium levels in agricultural products directly threaten food safety and human health. Therefore, addressing soil cadmium pollution has become a critical global environmental challenge, requiring coordinated efforts across all sectors to develop effective solutions [4].

1.2 CADMIUM POLLUTION HAZARDS

In terms of ecological environment, cadmium's impact on the soil ecosystem is the most immediate. Once cadmium content in soil exceeds the standard, it hinders the growth and reproduction of soil microorganisms and alters the structure of microbial populations. For instance, it reduces the activity of beneficial microorganisms such as nitrogen-fixing bacteria and nitrifying bacteria, thereby affecting the material cycle and energy transformation processes in soil, leading to increasingly barren soil and hindering plant nutrient uptake [7]. When plants are exposed to cadmium-contaminated soil for prolonged periods, their growth and development are significantly constrained, manifesting as reduced seed germination rates, stunted root development, dwarfed plants, yellowing leaves, and other symptoms. Cadmium disrupts the absorption and transportation of essential nutrients like iron, zinc, and calcium in plants, causing nutritional imbalances. This not only affects crop yield but also compromises crop quality. Moreover, cadmium can be transmitted through the food chain, harming animals that consume these plants, impairing their growth, reproduction, and immune function, and disrupting the balance and stability of the entire ecosystem [11-12].

For human health, the threat posed by cadmium pollution is more direct and severe. People are often exposed to cadmium through food consumption, drinking water, and air inhalation. Prolonged consumption of agricultural products such as rice and vegetables grown in cadmium-contaminated soil leads to gradual accumulation of cadmium in the human body. Cadmium has strong bioaccumulation properties, primarily accumulating in organs like the kidneys and liver. Once cadmium levels in the body exceed a certain threshold, a series of health issues arise. The kidneys are the primary target organs for cadmium poisoning, which can impair renal tubular function, leading to conditions such as proteinuria and glycosuria. In severe cases, it may even cause kidney failure. Cadmium also harms bone health by disrupting calcium metabolism, leading to bone softening and osteoporosis, resulting in conditions like "itai-itai" disease, which causes extreme pain and mobility limitations. Research indicates that cadmium is carcinogenic, and prolonged exposure increases the risk of various cancers, including lung and prostate cancer. Additionally, it damages the immune and reproductive systems, reducing immunity and affecting reproductive function and offspring development [14].

1.3 REMEDIATION METHODS FOR CADMIUM-CONTAMINATED SOIL

When addressing the challenging issue of soil cadmium pollution, physical remediation stands as one of the primary approaches. The soil replacement method involves covering cadmium-contaminated soil with uncontaminated soil to dilute the cadmium concentration in the polluted soil and reduce its bioavailability to plants. For small-scale contaminated areas, this method can immediately mitigate the adverse effects of cadmium pollution on plant growth [15].

The soil excavation method is more straightforward, directly removing the contaminated soil and replacing it with clean soil. This approach effectively

reduces cadmium levels in the soil. However, it entails substantial labor, material, and financial costs and significantly disrupts the original soil ecosystem. Therefore, it is only suitable for severely contaminated areas of limited size.

Electrokinetic remediation relies on an electric field to drive cadmium ions in the soil toward electrodes, where they are collected and removed. This method is particularly effective for low-permeability soils but consumes considerable electricity and demands high equipment standards, posing challenges for large-scale implementation [16].

Chemical remediation also plays a significant role in managing soil cadmium pollution. This method focuses on using chemical reagents to alter the chemical forms of cadmium in the soil, thereby reducing its bioavailability. For instance, applying alkaline substances such as lime or calcium carbonate can increase soil pH, converting cadmium into less soluble precipitates and decreasing its uptake by plants. The addition of phosphates or silicates can react with cadmium to form insoluble compounds, further limiting its mobility and bioavailability in the soil.

However, prolonged use of chemical remediation agents may lead to adverse consequences, such as soil compaction, which damages soil structure and impairs aeration and water permeability. Additionally, soil fertility may decline due to inadequate nutrient supply for plant growth, and secondary pollution risks may arise. Therefore, when employing chemical remediation, it is crucial to strictly control the dosage and frequency of chemical reagent application to minimize environmental harm [18].

1.4 INTRODUCTION TO PHOSPHATE-SOLUBILIZING BACTYERIA

Phosphate-solubilizing bacteria (PSB) represent a functionally significant group of microorganisms within soil ecosystems, possessing the capability to convert insoluble phosphorus in soil into plant-available forms. This enhances soil phosphorus utilization efficiency and promotes plant growth [5-6].

Taxonomically, PSB encompass diverse genera, including commonly observed Bacillus, Pseudomonas, Rhizobium, among others. These bacteria are widely distributed in soil and plant rhizospheres, particularly forming unique rhizospheric microbial communities with close associations to plant roots. They solubilize phosphorus through multiple mechanisms [10]: First, PSB secrete organic acids such as citric acid, malic acid, and oxalic acid during their metabolic processes. These acids lower the ambient pH, chelate metal ions bound to insoluble phosphates, and thereby liberate phosphorus. Second, they produce phosphatases that hydrolyze organic phosphorus compounds, converting them into inorganic phosphorus accessible to plants [17].

Beyond phosphorus solubilization, PSB exhibit additional beneficial traits. They synthesize phytohormones like indole-3-acetic acid (IAA), gibberellins (GA), and cytokinins (CTK), which stimulate root development and enhance nutrient and water uptake. Certain PSB strains also demonstrate nitrogen-fixing capacity, converting atmospheric nitrogen into plant-assimilable ammonium nitrogen, thereby improving nitrogen nutrition. Furthermore, upon colonizing the rhizosphere, PSB competitively inhibit pathogenic microbes by occupying ecological niches, secreting antibiotics, and bolstering plant disease resistance [19-23].

Given these multifunctional attributes, PSB hold substantial potential in agricultural production and ecological restoration. They serve as core components of biofertilizers and play pivotal roles in remediating contaminated soils, showcasing broad application prospects [24-26]. Author Qin Shanmei previously demonstrated that inoculation with strains M2 and M8 elevated soil phosphorus content while reducing cadmium (Cd) and lead (Pb) concentrations. These strains simultaneously promoted crop growth and restricted Cd/Pd uptake, validating PSB's feasibility and efficacy in heavy metal immobilization and mitigation of crop-toxic metals [27-30].

1.5 THE PURPOSE AND SIGNIFICANCE OF THIS STUDY

This study investigated the effects of phosphate-solubilizing bacteria *Bacillus thuringiensis J16* on lettuce growth and cadmium uptake under cadmium-contaminated conditions through pot experiments, providing theoretical basis and technical approaches for the remediation of cadmium-polluted soils.

Conclusions to chapter 1

1. Soil cadmium pollution arises from industrial activities and agricultural practices, threatening ecosystems and human health (kidney damage, bone diseases). Physical/chemical remediation methods exist but have drawbacks.

2. Phosphate-solubilizing bacteria (PSB) enhance phosphorus uptake, promote plant growth, and reduce heavy metal absorption (e.g., Cd) via organic acid secretion and enzyme activity.

3. The study aims to evaluate *Bacillus thuringiensis* J16's effects on lettuce growth and Cd absorption in cadmium-contaminated soil through pot experiments.

CHAPTER 2
OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 TEST MATERIALS

Test plant: Roman lettuce.

Test soil: Uncontaminated farmland surface soil (0–20 cm), which is sandy loam with loose structure and good air permeability. The soil was air-dried naturally, weeds and stones were removed, and then sieved through a 2-mm aperture sieve for later use.

Test strain: Phosphorus-solubilizing bacterium *Bacillus thuringiensis J16* preserved in the laboratory.

The chemical reagents and main equipment used in the experiment are listed, such as LB medium, centrifuge, light incubator, etc (Tab. 2.1, 2.2).

Table 2.1 – **Experiment reagent**

Name of chemical reagent	Purity	Applicationn
cadmium chloride ($CdCl_2 \cdot 2.5H_2O$)	analytically pure	To simulate soil cadmium pollution, precisely prepare cadmium solutions of different concentrations and add them to the soil.
KDP(KH_2PO_4)	analytically pure	It is used to prepare bacterial culture media and nutrient solutions to meet the nutritional requirements during the cultivation of phosphorus-solubilizing bacteria and the growth of lettuce.
magnesium sulfate $MgSO_4 \cdot 7H_2O$	analytically pure	
ammonium nitrate (NH_4NO_3)	analytically pure	
beef extract	-	It is used to prepare solid culture media and carry out the cultivation and
peptone	-	

Name of chemical reagent	Purity	Applicationn
agar	-	purification of phosphorus-solubilizing bacteria.
EDTA （EDTA - Na_2）	guarantee reagent	It is used for digestion treatment before the determination of cadmium content in soil and plant samples to ensure that all cadmium elements in the samples are completely released, facilitating accurate subsequent measurement.
nitric acid (HNO_3)	guarantee reagent	
perchloric acid ($HClO_4$)	guarantee reagent	

Table 2.2 – **Main equipment**

Name of instrument	Characteristic	Application
illumination incubator	The temperature and light can be precisely controlled	Create a temperature of 25℃ during the day and 18℃ at night for the pot experiment, as well as an environment of 16 hours of light and 8 hours of darkness to imitate the natural climate for the growth of lettuce.
electronic scales	The accuracy reaches 0.0001g	Accurately measure soil, reagents, lettuce samples, etc., to ensure the accuracy of experimental data.
constant temperature shaker	nothing	Carry out liquid culture of phosphorus-solubilizing bacteria, providing oscillatory culture conditions with a rotational speed of 180 revolutions per

Name of instrument	Characteristic	Application
		minute and a temperature of 30℃ to promote the growth and reproduction of bacteria.
autoclave	nothing	Sterilization operations should be carried out on the culture medium, experimental vessels, etc., to eliminate the interference of miscellaneous bacteria and ensure the reliability of the experimental results.
atomic absorption spectrophotometer	High sensitivity and good accuracy	Measure the cadmium content in soil and lettuce samples.
pH meter	nothing	Measure the pH values of the soil and the culture medium, and observe the soil's acidity and alkalinity at any time to provide a reference for analyzing the experimental results.
electrothermal blowing dry box	nothing	Bake the lettuce samples to a constant weight to accurately calculate their dry matter content.

2.2 ACTIVATION OF BACTERIAL STRAINS AND PREPARATION OF BACTERIAL SUSPENSIONS

The strain J16 stored in the glycerol tube was dipped in a small amount of the bacterial liquid with an inoculation loop in the aseptic operation table and inoculated onto the LB solid medium plate. Then, it was placed in a constant

temperature incubator at 37℃ and inverted for 24-48 hours. After the colonies grew, individual colonies were picked and inoculated onto new LB solid medium plates. Reactivate it 1 to 2 times according to the previous steps to ensure the vitality of the strain. Take a single colony after activation, vaccination to 100 mL LB liquid medium, 180 r/min, 37 ℃ shock cultivation for 24 h. Centrifuge the fermentation broth at 8000 r/min for 10 minutes, collect the bacteria, and resuspend them with sterile physiological saline to dilute the bacteria, so that the concentration of the bacterial suspension reaches 1×10^{8} CFU/mL.

2.3 POT EXPERIMENT SETUP

Weigh a certain mass of $CdCl_2 \cdot 2.5H_2O$, deionize water to prepare a cadmium solution with a higher concentration, evenly sprinkle it on the soil, and let it stand for equilibrium for 7 to 10 days. The cadmium content in the soil was 0 (CK), 1 mg/kg (T1), and 2 mg/kg (T2). Put 2 kg of treated soil in each plastic basin, then water it with an appropriate amount of deionized water to maintain the soil moisture content between 60% and 70% of the field capacity. Pick out plump and uniformly sized lettuce seeds, and then evenly sow 10 seeds in each basin. Cover them with about 0.5 cm thick fine soil. Put these potted plants in the light incubator, set the cultivation conditions as a daytime temperature of 25℃ with continuous light for 16 hours, a nighttime temperature of 18℃ with continuous darkness for 8 hours, and replenish water on time to ensure stable soil moisture. One week after the lettuce seedlings grew, a 1% (V/W) inoculation treatment was carried out, and no inoculation was used as the control.

2.4 SAMPLE COLLECTION AND PROCESSING

Collect the lettuce and rinse the roots and leaves with deionized water. Some plant samples were blanched at 105℃ for 30 minutes and then dried at 70℃ until

they reached a constant weight. Grind the dried samples and pass them through a 60-mesh sieve for dry storage. Another part of the plant samples were stored at 20℃

2.5 DETERMINATION OF MALONDIALDEHYDE (MDA) CONTENT

The content of malondialdehyde (MDA) in lettuce was determined by the thiobarbituric acid (TBA) colorimetric method. Weigh 0.5g of fresh lettuce sample, add 5mL of 10% trichloroacetic acid (TCA) and a small amount of quartz sand, grind into a homogenate in an ice bath, and then use.5 ml.10%.TCA. Rinse the mortar and combine the rinsing solution with the homogenizer. Centrifuge at 4℃ and 10,000 r/min for 20 minutes. Take 2 mL of the supernatant and add 2 mL of 0.6%TBA solution prepared with 10% TCA, mix well and then boil in a boiling water bath for 15 minutes. After cooling, centrifugation was performed again. The absorbance was measured at wavelengths of 450 nm, 532 nm, and 600 nm respectively using a spectrophotometer. The MDA content was calculated by the formula MDA content (μ mol/gFW) =6.45 × (A532-A600)-0.56 × A450 to evaluate the degree of oxidative damage of lettuce.

2.6 DETERMINATION OF PEROXIDASE (POD) ACTIVITY

The determination was carried out by guaiacol method. 0.5 g of fresh lettuce sample was weighed, 5 mL of pre-cooled phosphate buffer (pH7.0) was added and a little quartz sand was added. Homogenization was performed in an ice bath. Then, the mortar was rinsed with 5 mL of phosphate buffer. The homogenization solution was combined with the rinsing solution and centrifuged for 20 minutes at 4 ° C and 10,000 r/min. The supernatant is taken as the enzyme solution. The reaction system contains 3 mL of phosphate buffer solution (pH6.0), 0.5 mL of 2% guaiacol solution, 0.5 mL of 1% hydrogen peroxide solution and 0.1 mL of enzyme solution.

It is placed at 37℃ for 5 minutes, and then 1 mL of 2 mol/L sulfuric acid is added to stop the reaction. The absorbance value was measured at a wavelength of 470 nm using a spectrophotometer. Taking a 0.01 change in absorbance value per minute as one enzyme activity unit (U), the POD activity (U/mg) was calculated by calculating the relationship between the change in absorbance value and parameters such as reaction time and enzyme liquid volume.

2.7 PHOSPHATE BUFFER SOLUTION

The NBT photochemical reduction method was adopted. Also, 0.5g of fresh lettuce sample was weighed and added to 5 mL of pre-cooled phosphate buffer (pH7.8), a small amount of quartz sand, and ground into a homogenate in an ice bath. The subsequent centrifugation and the process of taking the supernatant were the same as those for POD activity determination. The reaction system contained 1.5 mL of phosphate buffer (pH7.8). 0.3 mL of 130 mmol/L methionine solution, 0.3 mL of 750 μmol/L NBT solution, 0.3 mL of 100 μmol/L EDTA-Na2 solution, 0.3 mL of 20 μmol/L riboflavin solution and 0.1 mL of enzyme solution. Place the reaction system in a light incubator and let it react under 4000 lx of light for 20 minutes. Then cover it with a black cloth to stop the reaction. Use the reaction tube that has not been exposed to light as a blank control and measure the absorbance at a wavelength of 560 nm with a spectrophotometer. SOD activity is based on the amount of enzyme required to inhibit 50% of the photochemical reduction of NBT as one unit of enzyme activity (U). By comparing the difference in absorbance under light and without light and using relevant formulas, the SOD activity (U/mg) was calculated.

2.8 DETERMINATION OF CD CONTENT IN LETTUCE

By using the atomic absorption spectrophotometer method, approximately 0.5g of the dried and crushed lettuce root or leaf samples were accurately measured and placed in the digestion tube. Then, 5 mL of a mixed acid of nitric acid and perchloric acid (with a volume ratio of 4:1) was added, and the digestion was carried out on an electric heating plate. At the beginning, heat at a low temperature to avoid the sample boiling over. After the reaction stabilizes, gradually increase the temperature until the digestion solution becomes clear and transparent and emits white smoke. When there is about 1 mL left, stop heating. After the digestion is completed, let it cool to room temperature and finally make up to 25 mL with deionized water. Subsequently, the absorbance of the solution was measured using an atomic absorption spectrophotometer at the specific absorption wavelength of the Cd element. The obtained values were compared with those of the Cd standard solution, and the content of Cd in the sample (mg/kg) was calculated according to the standard curve. The so-called standard curve is a series of Cd standard solutions of known concentrations. It is depicted under the same determination conditions to accurately convert the Cd content in the sample.

Conclusions to chapter 2

1. Materials: Roman lettuce, uncontaminated sandy loam soil, and *Bacillus thuringiensis* J16 strain.

2. Design: Cadmium concentrations (0, 1, 2 mg/kg) and inoculation levels (0, 1%) in pot experiments with 2 kg soil/pot and 10 seeds/pot.

3. Conditions: Cultivated at 25°C/18°C (day/night), 16 h light, and 60–70% soil moisture.

4. Measurements: Root/leaf dry weight, MDA/POD/SOD activity (antioxidant indicators), and Cd content via atomic absorption spectroscopy after acid digestion.

CHAPTER 3
EXPERIMENTAL PART

3.1 THE INFLUENCE OF INOCULATION ON THE DRY WEIGHT OF LETTUCE LEAVES

As can be seen from Fig. 3.1, under the condition of the same cadmium addition amount, compared with the non-inoculated control, inoculation with phosphorus-soluble bacteria J16 could significantly increase the dry weight of lettuce leaves by 70.1%. The results show that the phosphorus-solubilizing bacteria J16 can promote the growth of lettuce and alleviate the toxicity of cadmium pollution to plants.

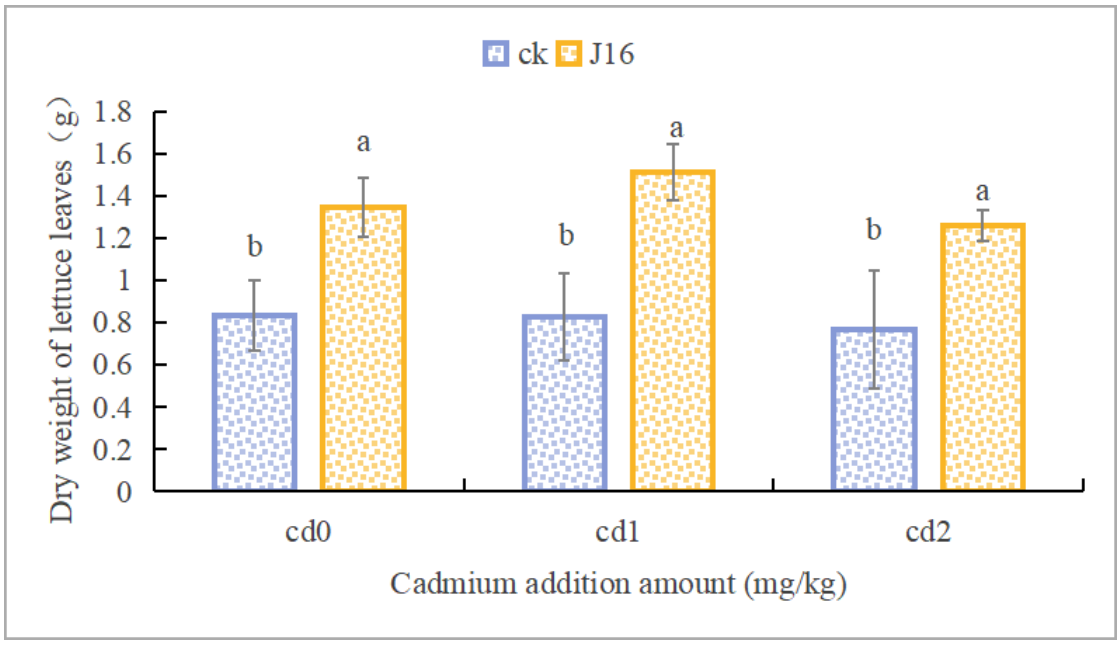


Figure 3.1 – Impact of Various Treatments on Lettuce Leaf Dry Weight

3.2 THE INFLUENCE OF INOCULATION ON THE DRY WEIGHT OF LETTUCE ROOTS

As can be seen from Fig. 3.2, under the same cadmium addition amount, compared with the non-inoculated control, inoculation with phosphorus-solubilizing bacteria J16 could significantly increase the dry weight of lettuce roots by 67.51%. The results show that the phosphorus-solubilizing bacteria J16 can promote the growth of lettuce and alleviate the toxicity of cadmium pollution to plants.

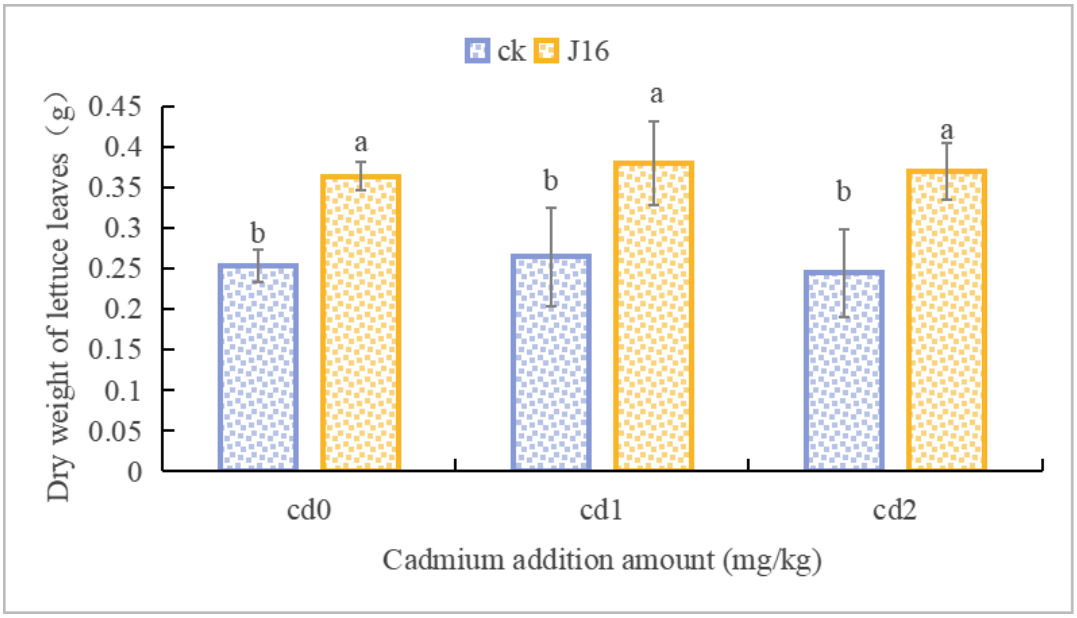


Figure 3.2 – Impact of Various Treatments on Lettuce Root Dry Weight

3.3 THE INFLUENCE OF BACTERIAL INOCULATION ON THE MDA ACTIVITY IN LETTUCE

It can be known from Fig.3.3 that in the absence of cadmium addition, inoculation with phosphorus-solubilizing bacteria J16 has little effect on the content of MDA in lettuce. Under the condition of the same cadmium addition amount, compared with the non-inoculated control, inoculation with phosphorus-solubilizing bacteria J16 could reduce the MDA content in lettuce by

20.62%. The results show that the phosphorus-solubilizing bacteria J16 can reduce the membrane lipid peroxidation level of lettuce cells and alleviate the toxicity of cadmium pollution to plants.

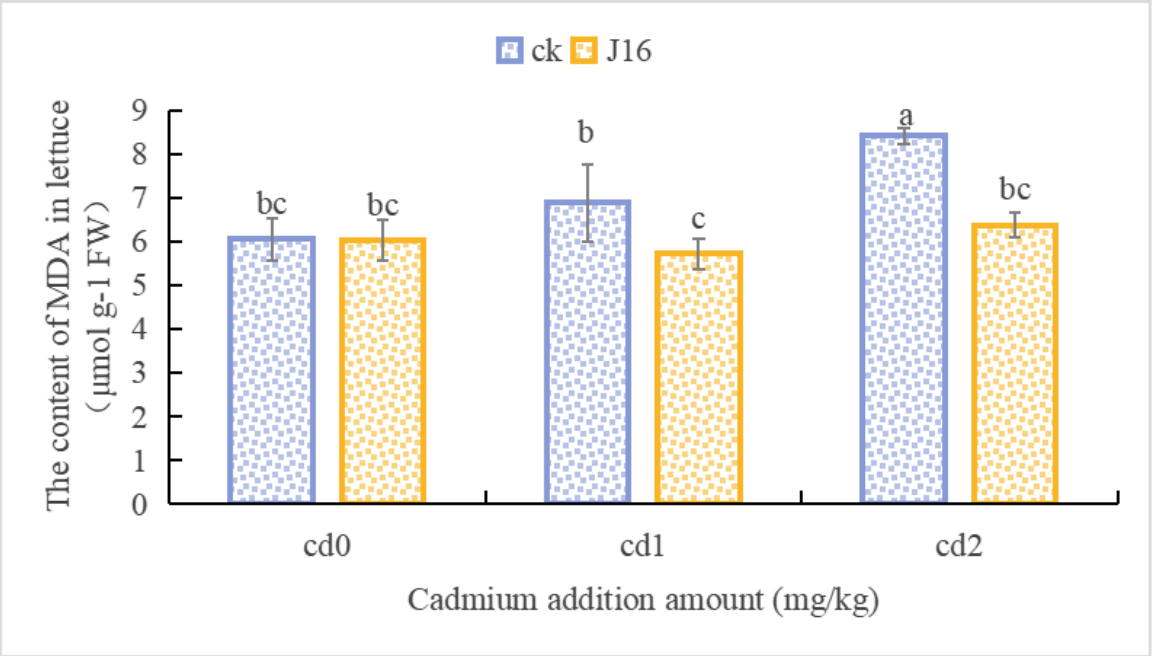


Figure 3.3 – Impact of various treatments on MDA levels in lettuce

3.4 THE INFLUENCE OF INOCULATION ON THE ACTIVITY OF POD IN LETTUCE

As can be seen from Figure 3-4, under the condition of the same cadmium addition amount, compared with the non-inoculated control, inoculation with phosphorus-soluble bacteria J16 could significantly increase the content of POD in lettuce by 35.23%. The results show that the phosphorus-solubilizing bacteria J16 can enhance the dereactive oxygen species capacity of lettuce and alleviate the toxicity of cadmium pollution to plants.

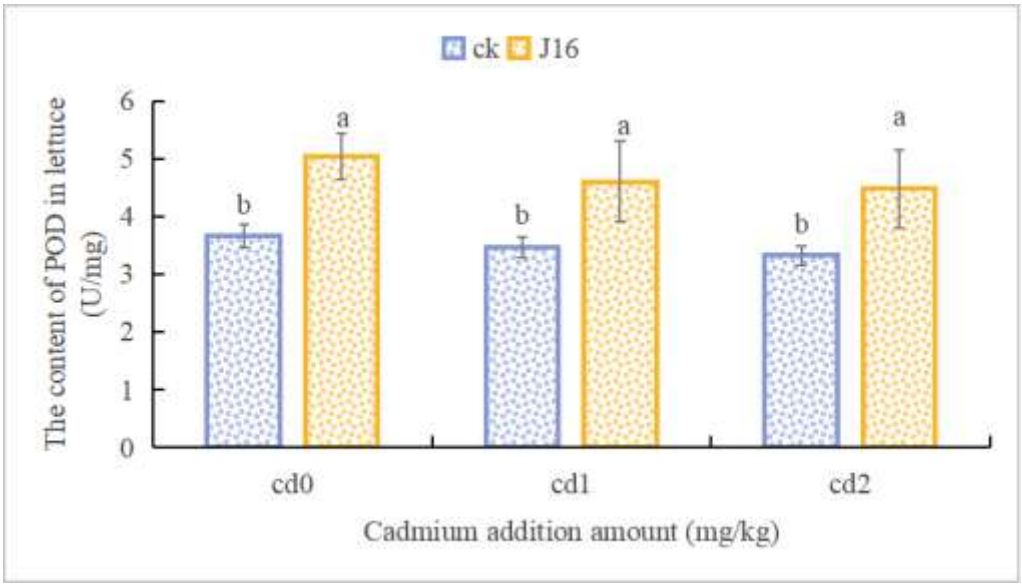


Figure 3.4 – Impact of various treatment methods on POD levels in lettuce

3.5 THE INFLUENCE OF BACTERIAL INOCULATION ON THE SOD CONTENT IN LETTUCE

As can be seen from Figure 3-5, under the condition of the same cadmium addition amount, compared with the non-inoculated control, inoculation with phosphorus-soluble bacteria J16 could significantly increase the content of SOD in lettuce by 38.81%. The results show that the phosphorus-solubilizing bacteria J16 enhances the coping ability of lettuce to oxidative stress caused by cadmium pollution and alleviates the toxicity of cadmium pollution to plants.

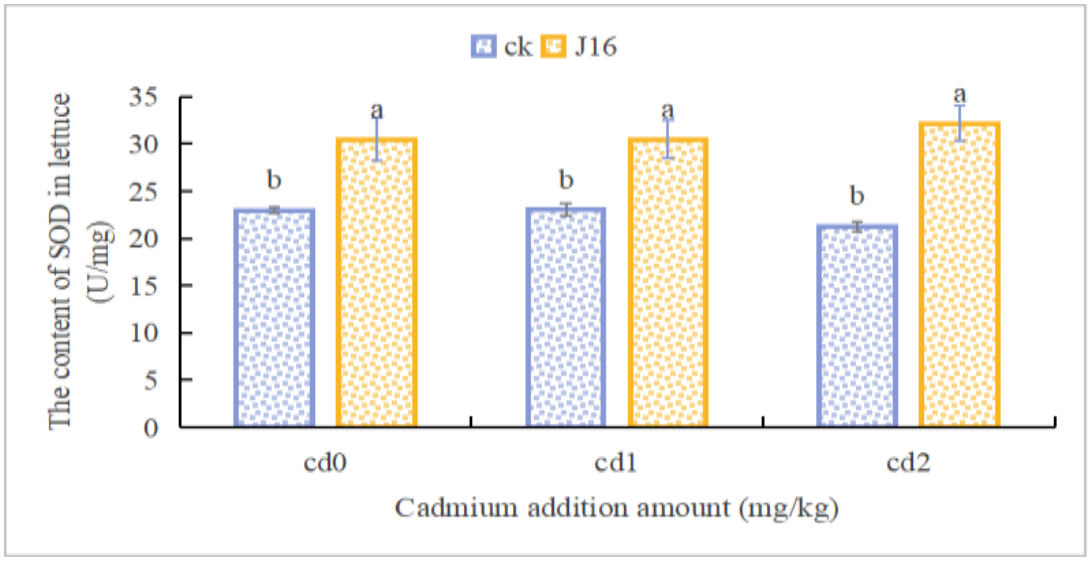
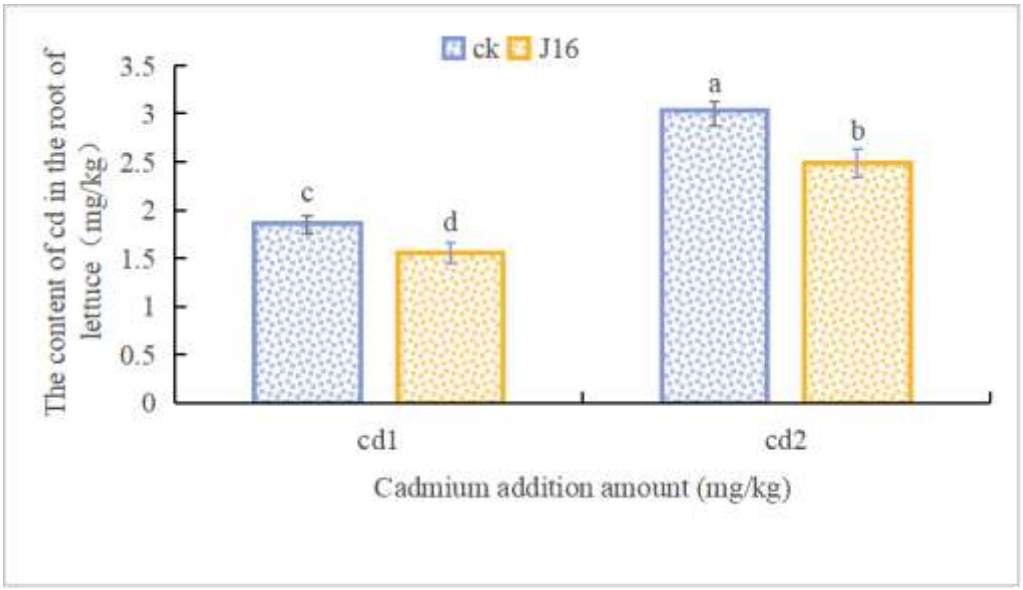


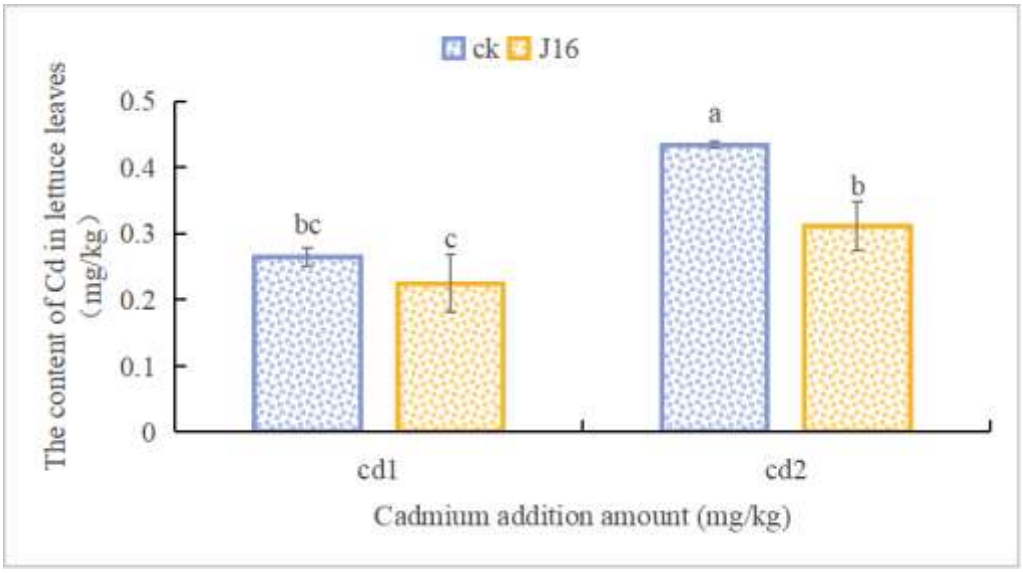
Figure 3.5 – Impact of various treatment methods on SOD levels in lettuce

3.6 THE INFLUENCE OF INOCULATION ON THE CD CONTENT OF LETTUCE

As can be seen from Fig. 3.6, under the same cadmium addition amount, compared with the non-inoculated control, inoculation with phosphorus-solubilizing bacteria J16 can significantly reduce the Cd content in lettuce leaves and roots by 17.14% and 21.72% respectively. Phosphorus-soluble bacteria can absorb Cd at the root of a lettuce plant while also blocking the transport of Cd in the lettuce, alleviating the toxicity of cadmium pollution to plants.



（a）



（b）

Figure 3.6 – Impact of various treatments on cadmium levels in green-stemmed lettuce

Conclusions to chapter 3

1. Under cadmium-contaminated conditions, inoculation with J16 can reduce the MDA content of lettuce by 20.62%, decrease membrane lipid peroxidation, and alleviate cadmium poisoning.
2. Inoculation with J16 significantly increased the POD content of lettuce by 35.23%, enhanced the antioxidant capacity of lettuce and resisted cadmium pollution.
3. Inoculation with J16 significantly increased the SOD content of lettuce by 38.81%, enhanced the coping ability of lettuce to oxidative stress, and alleviated cadmium pollution.
4. Inoculation with J16 significantly reduced the Cd content in lettuce leaves and roots by 17.14% and 21.72% respectively, indicating that J16 can reduce the absorption of cadmium by lettuce and block its transport within the plant.

CONCLUSIONS

1. After inoculation with phosphorus-solubilizing bacteria J16, both the dry weight of leaves and roots of lettuce were increased, indicating that it has a promoting effect on the growth of lettuce.

2. Strain J16 significantly reduced the cadmium content in the roots and leaves of lettuce, indicating that it plays a positive role in reducing the absorption of heavy metal cadmium by plants.

3. Strain J16 can alleviate the damage of cadmium to the cell membrane of lettuce and increase the activity of antioxidant enzymes such as POD and SOD, thereby enhancing the antioxidant capacity of lettuce.

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