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

QUALIFICATION THESIS

on the topic **Effects of polysaccharide-producing bacteria RB2 on the quality of pakchoi under lead-contaminated conditions**

First (Bachelor's) level of higher education

Specialty 162 "Biotechnology and Bioengineering"

Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
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APPROVE
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«___»_______________2024

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Jingqi Wang

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Scientific supervisor Olga Iungin, Ph.D., Assoc. Prof.

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2	Chapter 1. Literature review	From 06 April 2024 to 20 April 2024	
3	Chapter 2. Object, purpose, and methods of the study	From 21 April 2024 to 30 April 2024	
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I am familiar with the task:

Student ____________ Jingqi WANG

Scientific supervisor ____________ Olga IUNGIN

SUMMARY

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With the rapid development of industry, agriculture and social economy, the problem of heavy metal lead pollution (Pb) in cultivated land in China has become more and more prominent. This kind of pollution not only comes from traditional pollution sources such as industrial waste discharge and pesticide use, but also is affected by factors such as urbanization, industrialization and agricultural modernization. Lead pollution not only destroys the balance of soil ecosystem to a certain extent, but also has a great impact on agricultural production. Lead in soil will be absorbed with the growth of crops and spread to the human body through the food chain, posing a serious threat to human health. In this study, the polysaccharide-producing bacteria *Micrococcus antarcticus* RB2 was used for pot experiment. *Brassica chinensis* L. was planted in artificially prepared lead-contaminated soils with different pollution levels (0,25,50 mg kg^{-1}). The effects of *Micrococcus antarcticus* RB2 on the quality of pakchoi under lead pollution were studied by using the dry weight, soluble protein, vitamin C and nitrite content of pakchoi as indicators. The experimental results showed that the application of strain RB2 could reduce the harm of heavy metal lead to the quality of pakchoi. Compared with the non-inoculation treatment, the dry matter quality of the edible parts of pakchoi increased by 9.3%-14.7%, the vitamin C content increased by 9.7%-14.7%, the soluble sugar content increased by 14.1%-45.7%, and the nitrite content decreased by 12.9%-26.4%. Based on the above experimental results, strain RB2 was selected to prepare microbial agents for agricultural production, which is expected to be used for in-situ remediation of heavy metal lead-contaminated soil, reduce the impact of lead stress on crops, and promote the improvement of crop quality and yield.

Keywords: Polysaccharide-producing bacteria; heavy metals; lead; Brassica chinensis L.; quality.

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INTRODUCTION

The prevention and control of soil heavy metal pollution is related to the health of the people and the sustainable development of the economy. It plays an irreplaceable role in the long-term development of China in the future. Lead (Pb) is one of the main pollutants of heavy metals in farmland soil. Lead and lead compounds are a stable, non-degradable environmental pollutant. Soil heavy metal Pb pollution not only affects crop yield and quality, but also involves the quality of the atmosphere and water environment, and can endanger human life and health through the food chain. It will cause harm to the respiratory system, digestive system, central nervous system, reproductive system and so on. Therefore, the problem of soil heavy metal Pb pollution needs to be solved urgently.

At present, there are few examples of actual soil pollution remediation in China, and most of them are in the stage of research and development. Finding new cost-effective and environmentally friendly remediation measures is a new research hotspot. Compared with conventional physical and chemical remediation technologies, bioremediation has the characteristics of low risk of secondary pollution and low cost of remediation, and is one of the most potential means for soil heavy metal pollution remediation. Extracellular polysaccharides are secondary metabolites of microorganisms, which are linked by monosaccharides through glycosidic bonds. It is widely used in the study of enhancing plant stress resistance because of its rich species and various forms. As a regulator of plant growth, extracellular polysaccharides can promote plant growth, alleviate the stress of plants in heavy metal soil environment through EPS-mediated heavy metal adsorption, enhance the viability of plants, and significantly improve the effects of stress conditions on plants.

Pakchoi is a widely cultivated leaf and stem vegetable in China, and its planting area is all over the region. It has the advantages of short growth cycle, fresh and tender taste, low calorie, wide planting and eating range, strong tolerance to lead and certain enrichment ability, which makes pakchoi not only play a role in diet and nutrition, but also have certain application value in environmental protection and soil remediation.

In this study, we selected a marine bacterium *Micrococcus antarcticus* RB2 isolated from the surface of abalone seedlings, which can degrade a variety of organic pollutants and has the ability to produce high extracellular polysaccharides. A pot experiment was carried out to investigate the effect of strain *Micrococcus antarcticus* RB2 on the quality of pakchoi under lead pollution by measuring and analyzing the dry weight of pakchoi and the contents of vitamin C, total protein and nitrite. It is of great significance to ensure agricultural production and improve the biomass of crops. It is expected to benefit the society and maintain the green and sustainable development of agriculture in the future.

The relevance of the topic is application of polysaccharide-producing bacteria *Micrococcus antarcticus* RB2.

The purpose of the study is to study the effect of polysaccharide-producing bacteria *Micrococcus antarcticus* RB2 on improving crop quality, and to further explore the feasibility of using this strain for crop quality.

The objectives of the study to was to investigate the effect of polysaccharide-producing bacteria *Micrococcus antarcticus* RB2 on the quality of pakchoi under lead pollution by measuring the dry weight, vitamin C content, soluble protein content and nitrite content of pakchoi.

The object of the study is pakchoi.

The subject of the study is *Micrococcus antarcticus* RB2.

Research methods are pot experiment and determination of each index content.

The scientific novelty is exploring the effects of marine bacteria on crops under heavy metal pollution in soil.

The practical significance of obtained results is that the research has positive significance for protecting cultivated land resources and improving the safety level of agricultural products. It provides a certain reference value for the development of bioremediation technology, and can provide effective technical support for agricultural production and promote the sustainable development of agriculture.

CHAPTER 1
LITERATURE REVIEW

1.1 Overview of soil heavy metal lead pollution

Heavy metals are a class of metal elements with a high atomic density of more than 4 g/cm^3. Among the numerous metal elements, including lead (Pb), cadmium (Cd), arsenic (As), nickel (Ni), cobalt (Co), zinc (Zn), chromium (Cr), iron (Fe), silver (Ag) and platinum group elements, all show different biological activities1. Although some heavy metals such as zinc and copper can promote plant growth at appropriate concentrations and play a role in cofactors and enzyme catalysis, heavy metals such as cadmium, mercury and arsenic may have strong toxicity to biological enzymes, thereby inhibiting the growth of organisms and may lead to the death of living organisms in severe cases. Soil is very important to the survival and development of human beings. It plays an indispensable role in the ecosystem and is closely related to many environmental factors. However, with the rapid increase of social production capacity and the excessive use of chemical fertilizers in agriculture, as well as the continuous impact of human activities, the distribution of heavy metals in the biosphere has gradually expanded, especially in the soil environment. According to the data, more than 10% of the farmland in China has been polluted by heavy metals2, and lead is one of the most polluted elements3.

1.1.1 Status of heavy metal lead pollution in soil

With the rapid development of China 's industrialization and agricultural intensification, the problem of heavy metal pollution in soil is becoming more and more serious, and the situation of domestic soil environment has attracted wide attention. Lead (Pb) is one of the most common metal elements in cultivated land pollution in the world. Studies have shown that the degree of lead pollution varies across China. This pollution is distributed in soil, water and atmosphere, especially in soil, where lead pollution is more common. According to the 2014 national soil pollution survey bulletin data4, China's heavy metal contaminated soil area accounts for 21.7% of the total area, of which 82% of the excessive points are caused by inorganic substances, which is the main form of soil pollution. Among the eight major heavy metal pollutants, including copper, chromium, cadmium, nickel, lead, zinc, arsenic and mercury, the exceeding rate of lead was 2.1%5. The study of Chen Wenxuan et al.6 used the conventional Kriging interpolation method to evaluate the content of heavy metals in farmland soils in China. It was found that the lead content in cultivated land was 1.28 times higher than that in local soil on average, and the proportion of exceeding the standard reached 80.0 %, indicating the seriousness of lead pollution in soil in China. The problem of heavy metal pollution in water and soil has attracted wide attention from the society and the country. In May 2016, the " Action Plan for Soil Pollution Prevention and Control " announced by the State Council emphasized the urgent need to take measures to limit the five major heavy metal pollutants of lead, mercury, chromium, cadmium and arsenic7.

1.1.2 Sources of heavy metal lead pollution in soil

The sources of heavy metals can be divided into two categories: natural sources and anthropogenic sources. Natural sources include weathering, atmospheric deposition and crustal movement. These natural processes have a certain effect on the migration and distribution of heavy metals, but human activities have a more prominent impact on the flow of heavy metals. In fact, the rate of heavy metal cycling caused by human activities far exceeds the rate of natural processes, making heavy metals widely distributed in the environment8,. In recent years, with the increase of population and the rapid development of industrialization, the widespread use of pesticides and fertilizers and the exploitation of mineral resources have led to an increase in the emission of heavy metal pollutants. These pollutants enter the agricultural soil ecosystem through dust and precipitation10-.

Man-made sources mainly include agriculture, industry and daily life.

In agricultural activities, the use of sewage for irrigation and the application of pesticides and fertilizers are common practices. Since China is a country with a shortage of water resources, it is a common practice to use sewage to solve irrigation problems. The rapid development of industrialization has led to a large number of untreated industrial wastewater and domestic sewage mixed together into the sewer. When this mixed sewage eventually seeps into farmland soil, it can cause soil pollution of multiple heavy metals in different areas. As early as the 1980 s, the area of farmland contaminated by sewage irrigation has reached 629,000 hectares13. Long-term use of sewage irrigation will lead to the accumulation of heavy metals such as lead and cadmium in farmland soil. For example, long-term sewage irrigation has led to a significant increase in soil lead and cadmium concentrations in an area of agriculture in Beijing, China, increasing by 18 and 84 times, respectively, due to long-term sewage irrigation14. Improper use of inorganic and organic fertilizers containing heavy metals is also an important reason for the increase of heavy metal content in farmland soil. These fertilizers, including lime, sewage sludge and pesticides, have a significant negative impact on soil structure. The concentrations of heavy metals such as cadmium, chromium, nickel, lead and zinc in various fertilizers are different15. In order to improve soil quality, people usually add manganese, zinc, copper, cobalt and other elements to farm manure16. In addition, in the process of agricultural production, commonly used ground cover such as plastic film may also cause heavy metal pollution in soil. This is because the mulch usually adds heavy metal elements that are not easily decomposed, such as cadmium, zinc, lead and barium, in order to enhance the thermal stability of the mulch. Once the plastic film breaks and fails to be removed and treated in time, these residual metal elements will accumulate in the soil, causing significant harm to agricultural production activities and the ecological environment17.

In the industrial manufacturing process, if the heavy metal pollutants associated with product production are not treated in the first place, or the tailings and waste generated in the mining production and metallurgical environment are artificially stacked in the open air, then these toxic substances may penetrate into the soil through direct or indirect ways, and may further spread to the surrounding environment. In the early stage of metal refining, high-temperature treated metals will release heavy metals including arsenic, cadmium, copper, lead, tin and zinc. These heavy metals exist in the form of particles and steam, and combine with water vapor in the atmosphere to form aerosols, which eventually fall into farmland soil by wet deposition and dry deposition. Various types of industrial refineries and energy supply power stations, including coal-fired power plants, nuclear power plants, high-voltage lines and oil burning facilities, also release large amounts of heavy metals, including arsenic, cadmium, copper, zinc and nickel, into the environment. According to the relevant survey data, during the period from 1980 to 1992, the area of cultivated land pollution caused by industrial activities in China increased from 2,667,000 hectares to 1,000,000 hectares. Since the reform and opening up in 1979, the national heavy industry has developed rapidly, and various large factories such as steel plants and chemical plants have emerged. With the increase of industrial activities, soil pollution incidents have gradually increased. Typical events include the 'Chongchang incident' in Shacheng, Hebei Province and the 'three wastes' pollution incident caused by Harbin Pharmaceutical Factory18,. These events not only have a serious impact on the local environment, but also pose a threat to the health of the surrounding residents. The government and relevant departments have also begun to strengthen supervision and control measures, but there is still a long way to go to completely solve the problem of soil pollution. It is estimated that the global annual emissions of heavy metals, Mn emissions reached 15 million tons, Pb is about 5 million tons20.

Domestic pollution sources include incineration of garbage and random disposal of untreated heavy metal-containing items, such as batteries and thermometers, which will directly lead to heavy metal pollution in the surrounding soil.

1.1.3 Soil heavy metal lead hazards

Heavy metals show the characteristics of accumulation, concealment, non-volatility and irreversibility in contaminated soil. In a specific ecosystem, heavy metal pollution can enter the ecosystem through air, water and soil, and then be absorbed by plants and transmitted to other organisms on the food chain. All organisms in the food chain will be affected by the cycle to varying degrees, that is, by different degrees of heavy metal pollution21. Soil heavy metal pollution is a complex multi-dimensional problem, which mainly involves three aspects: first, the impact on the soil environment; the second is the impact on plants; finally, the impact on human health22. Among them, the harm of heavy metals to human body is most directly related to us. Once heavy metal ions enter the soil, most of them cannot be decomposed by microorganisms, but accumulate in the soil, which has a negative impact on the yield and quality of crops. Through the concentration of biological processes, these heavy metals will continue to enrich and accumulate along the food chain, and ultimately pose a potential risk to human health23. During the growth of vegetables, they can absorb heavy metals through roots, and the excessive accumulation of lead poses a serious threat to human health24. When the content of heavy metals in human body exceeds the biological recommended limit, it will produce biological toxic effects of heavy metals on the body. Lead ions can cause damage to the body 's normal physiological function after being ingested by the human body, leading to the emergence of various diseases, such as anemia, depressive brain disorders, and even paralysis25. Excessive accumulation of lead may cause irreversible damage and have a negative impact on the respiratory system, cardiovascular system, nervous system, digestive system and urinary system26.

1.1.4 Remediation technology of lead pollution in farmland soil

At present, the remediation technologies applied to heavy metal contaminated soil can be divided into physical, chemical and biological remediation technologies, and different combined remediation technologies need to be used according to different heavy metal pollution situations.

Physical remediation techniques cover a variety of methods such as soil stripping, excavation and replacement, aiming to reverse or stop soil damage caused by heavy metal pollution27. Soil replacement technology reduces the concentration of pollutants and enhances and enhances the carrying capacity of the environment by mixing or covering clean soil with contaminated soil. This method is suitable for areas with higher pollution and less pollution, but because of its large workload and high cost, it is usually only implemented in these areas. In order to prevent the further diffusion of pollutants, barrier wall technology can be used. These barrier walls are usually made of impermeable materials (such as steel, cement and bentonite), which are used to isolate and control pollutants. Heat treatment relies on high frequency voltage to evaporate heavy metals from the soil28.However, this method may destroy the original surface structure and even bring the risk of secondary pollution29

Chemical remediation technology changes the morphology, mobility and biological activity of heavy metals in soil by using special chemical methods to deal with heavy metal pollution in soil, including chemical decomposition or fixation reactions. This technology mainly uses chemical modifiers, solvent leaching treatment and specific reducing agents to implement reduction and other means to repair, involving the addition of harmless or low-toxic chemical components to the soil. Commonly used chemical modifiers include phosphate, lime, zeolite, and bio-composting to reduce the content of mobile heavy metals in the soil. The chemical leaching method dissolves the heavy metals by adding a solvent that can dissolve the heavy metals and discharges them with the solvent, and then processes and recovers the heavy metal ions in the solution, thereby further reducing the pollution in the soil. This method is simple and effective, but in the process of treatment, a large amount of wastewater and waste residue may be produced. If not properly treated or discharged, it may destroy the micro-aggregate structure of the soil, resulting in poor soil texture, decreased aeration and other problems, and ultimately lead to nutrient loss, which has a serious impact on the surrounding environment.

In order to minimize the accumulation of heavy metals in vegetables, it is urgent to develop environmentally friendly, economical and energy-saving treatment technologies. Physical and chemical methods have some limitations in the remediation of heavy metal contaminated soils. For example, they may change soil properties by affecting soil microbial communities, require a lot of time and capital investment, and may cause secondary pollution problems. These methods can change the pollution problem to a certain extent, but cannot completely eradicate pollutants30. As an emerging technology, bioremediation can repair the soil ecosystem contaminated by heavy metals to a certain extent. This method uses plant roots to absorb heavy metals in soil or microbial decomposition of organic pollutants and other natural biological pathways, as well as microbial-mediated redox reactions and methylation reactions and other biochemical processes to remove, destroy or fix harmful heavy metals, so as to achieve the purpose of cleaning the polluted environment31. There are many kinds of microorganisms involved in this process, including bacteria, fungi, yeasts and algae. Compared with traditional chemical and physical remediation technologies, the materials and equipment required for bioremediation are relatively simple and easy to obtain, and do not require a large number of expensive chemicals or high-end physical equipment. In addition, the bioremediation process also reduces environmental costs such as energy consumption and waste treatment, and has more advantages in resource utilization efficiency, which further reduces its cost and is more economical and affordable overall. In general, bioremediation has obvious advantages in terms of cost, and has also shown a positive impact on environmental protection and sustainable development, and is suitable for low concentrations of heavy metals in the environment.

1.2 Marine bacteria and extracellular polysaccharides

1.2.1 Overview of Marine Bacteria and Extracellular Polysaccharides

Marine bacteria are widely distributed prokaryotic single-celled organisms in the ocean. They play a key role in the marine microbial community and occupy an important position in the marine microbial community. These microorganisms are extremely small, usually no more than 1 micron in diameter, and their morphology is diverse, including spherical, rod-shaped, spiral, and branched filaments. Marine bacteria have adapted to extreme environmental conditions, such as low temperature, high salinity, high pressure and lack of light. Through long-term evolution, they have gained a rich diversity32. This not only shows that marine bacteria have strong viability and adaptability, but also shows that marine bacteria have the potential to produce different metabolic pathways compared with terrestrial microorganisms, and have strong resistance and biodegradability33.

Exopolysaccharides (EPS) are one of the significant differences in metabolites between marine bacteria and terrestrial bacteria. Extracellular polysaccharides play an important role in microorganisms, which can not only enhance the stress resistance of plants, but also affect the proliferation and attachment process of microorganisms. These polysaccharides are usually composed of monosaccharides connected by glycosidic bonds. There are many kinds and different forms, including two types of homologous polysaccharides and mixed polysaccharides, which are of great significance to the metabolic activities of microorganisms34,.

With the extensive development and research of microbial resources, microbial polysaccharides have gradually attracted people 's attention due to their advantages of high yield and short growth cycle. Recently, microbial polysaccharides have been widely used in food, pharmaceutical and chemical industries 36. Compared with other additives, polysaccharides have excellent environmental protection characteristics. They are derived from natural plants or animals, do not contain chemical synthetic components, and have no pollution to the environment. In the production process, low energy consumption and low emission technology are usually used to reduce the impact on the environment. In addition, polysaccharides can be naturally degraded or recycled after use, and will not impose a long-term burden on the environment. At the same time, the application of polysaccharides in enhancing plant resistance to adversity is gradually being valued. Due to the diversity of its structure and intramolecular active groups, polysaccharides can promote the reproduction and activity of soil microorganisms, increase soil organic matter content, and improve soil aeration and water retention. At the same time, it can also improve the resistance of crops to adverse environments, such as drought, salinity, etc.; and has the function of adsorbing heavy metal ions, to a certain extent, reduce the toxic effects of heavy metals on crops. These combined effects make polysaccharides considered to be one of the most promising choices for the application of agricultural biomaterials. Different from other sources, extracellular polysaccharides produced by microorganisms have the advantages of high yield, easy separation and short cycle. Microorganisms have gradually become the focus of attention in the field of microbial resource development due to their abundant polysaccharide resources. By fully utilizing and studying microbial polysaccharides, people can better explore their potential application value and bring more opportunities and benefits to agricultural production, environmental protection and human health. In the future development, microbial polysaccharides are expected to become an important sustainable resource to promote progress and development in various fields.

1.2.2 Effects of exopolysaccharides on plant growth and quality

In the metabolic process, some microorganisms have the ability to secrete extracellular polysaccharides. These polysaccharides show rich diversity through different composition and connection methods of monosaccharides, giving microorganisms unique biological activity and function, so that they can resist external damage37. These polysaccharides not only enhance the viability of microorganisms, but also are an efficient biological fertilizer that activates plant defense mechanisms and helps them cope with environmental changes and abiotic stresses such as drought, salinization, and heavy metal pollution38,. In agriculture, the pressure of environmental change on crops is an important factor affecting the sustainable development of agriculture. Adverse weather and soil conditions may limit crop growth and reduce crop quality and yield. Pollutants such as heavy metals can also enter plants through soil or water sources, posing risks to the quality and safety of food. Studies have shown that extracellular polysaccharides can significantly enhance the adaptability of crops to environmental stress, showing its great potential and development prospects in agricultural production.

Studies have found that extracellular polysaccharides can also improve the growth of crops under salt stress40, it shows the potential to improve plant salt tolerance by limiting sodium uptake and enhancing plant osmotic stress resistance. The application of extracellular polysaccharides can reduce the absorption of sodium and chloride ions by plants and increase the content of nitrogen, phosphorus and potassium, thereby improving the growth of rice, sunflower, corn and other crops, and enhancing the vitality of crops. In addition, extracellular polysaccharides also have a significant effect on photosynthetic pigments such as chlorophyll and carotenoids that promote plant photosynthesis and proline content in plants, which plays an important role in enhancing salt stress resistance of crops.

Studies have shown that extracellular polysaccharides can also regulate the growth characteristics of crops under drought stress41, it plays a key role in maintaining soil moisture and enhancing drought resistance of crops. The addition of microorganisms that can produce extracellular polysaccharides or the application of exogenous extracellular polysaccharides can promote the leaf area, bud growth, dry weight and other growth indicators of maize, millet and other crops, and can significantly increase the expression level of drought-related genes in plants, thereby significantly improving the drought resistance of plants. This finding confirms the important role of extracellular polysaccharides in inducing and improving the drought resistance of crops.

A large number of studies have shown that extracellular polysaccharides can also regulate the growth characteristics of crops under heavy metal stress42,. It has excellent heavy metal ion adsorption capacity, which can reduce the accumulation effect of heavy metals in crops and improve the safety and quality of crops. Under heavy metal stress conditions, extracellular polysaccharides can improve the growth and development of crops. The results showed that the root length, bud length, dry weight and fresh weight were significantly improved after the application of extracellular polysaccharides on common plants such as spinach. At the same time, the contents of photosynthetic pigments such as chlorophyll a and chlorophyll b increased, and the contents of phosphate and nitrogen also increased, which reflected the improvement of plant nutrition.

Therefore, mining exopolysaccharide-producing strains as biological inoculums is an effective strategy to enhance plant resistance to adversity. At present, many researchers have devoted themselves to discovering and utilizing these microbial resources to help agricultural production better cope with various pressures and environmental changes. According to statistics, researchers have found that 76 species, a total of 46 genera of microorganisms can secrete extracellular polysaccharides, but only a few species are widely adopted and used in practical applications. Therefore, it is of great significance to further explore and screen the resources of exopolysaccharide-producing strains and apply them to improve the quality of agricultural production44. Through in-depth study of different types of microorganisms and their mechanisms of action in plant stress resistance, efficient, safe and sustainable use of bio-fertilizers and plant protection products can be better developed. This will provide new technical means for the agricultural field and promote the improvement of crop quality and yield, so as to promote the whole agricultural system towards a healthier and sustainable development.

1.3 The basis and research content of the topic

1.3.1 Basis of the subject

As a country with a large population, relatively limited cultivated land resources and deeply troubled by lead pollution, the deterioration of soil quality has led to a series of problems such as the reduction of agricultural production and the decline of quality, which has brought huge economic losses to the country. What is more worrying is that the excessive heavy metal content in agricultural products poses a serious threat to human health. Lead is a physiological toxin and neurotoxin with high accumulation and affinity. When it enters the human body, it will form a ' reservoir ', which is transmitted to the whole body through blood circulation and deposited in bones and soft tissues, and gradually accumulates to dangerous levels, resulting in severe cumulative poisoning45. This cumulative poisoning may lead to damage to the nervous system, renal dysfunction, anemia and other serious health problems. In addition to directly affecting body organs, lead is also associated with an increased risk of cancer. Long-term exposure to low concentrations of lead can cause DNA damage and gene mutations, thereby increasing the possibility of cancer. In addition, due to the long half-life of lead in the human body, it may continue to have an impact on health after exposure stops. Therefore, remediation of heavy metal contaminated farmland soil is an urgent problem to be solved in current environmental protection and agricultural production, and it is also a necessary measure to ensure agricultural production, protect ecological security and people 's health and safety.

Pakchoi is a widely cultivated leafy vegetable in China, and its planting area is all over the country. According to statistics, pakchoi accounts for 19 % of the total yield of leafy vegetables in China and is one of the important crops. In addition to being an edible vegetable, pakchoi also has a strong adsorption capacity, especially for heavy metals such as lead. This makes pakchoi not only play a role in dietary nutrition, but also have certain application value in environmental protection and soil remediation.

As a bacterial fertilizer, microbial amendments can improve soil structure and increase nutrient content, thereby improving soil quality. At the same time, it can also degrade pesticide residues and heavy metal pollutants and reduce environmental pollution. In terms of plant growth, microbial passivators can also promote root development, enhance plant stress resistance, and help improve crop yield and quality. Therefore, in the process of sustainable agricultural development, microbial passivator is considered to be an environmentally friendly soil remediation strategy, which is of positive significance for protecting cultivated land resources and improving the safety level of agricultural products. Studying the effects of plant-microorganism interactions on plant quality and safety can provide effective technical support for agricultural production and promote the development of green agriculture. Through this study, we can not only better understand the effects of heavy metals in soil on plant growth, but also provide a certain reference value for the development of bioremediation technology.

1.3.2 Research content

In this study, we selected a marine bacterium *Micrococcus antarcticus* RB2 isolated from the surface of abalone seedlings, which can degrade a variety of organic pollutants and has the ability to produce high extracellular polysaccharides. A pot experiment was carried out to investigate the effect of strain *Micrococcus antarcticus* RB2 on the quality of pakchoi under lead pollution by measuring and analyzing the dry weight of pakchoi and the contents of vitamin C, total protein and nitrite. It is of great significance to ensure agricultural production and improve the biomass of crops. It is expected to benefit the society and maintain the green and sustainable development of agriculture in the future.

Conclusions to chapter 1

1. The distribution range of heavy metals in the biosphere has gradually expanded, particularly in the soil environment, due to the rapid improvement of social production capacity and excessive utilization of chemical fertilizers in agriculture, as well as continuous human activities.
2. The sources of heavy metals can be categorized into natural sources and anthropogenic sources. Natural sources encompass weathering, atmospheric deposition, and crustal movement. Anthropogenic sources primarily comprise agriculture, industry, and daily activities. The impact of human activities on the transport of heavy metals is particularly significant.
3. Excessive lead accumulation may cause irreversible damage to the respiratory, cardiovascular, nervous, digestive, and urinary systems of the body, leading to harmful effects.
4. At present, the remediation technologies applied to heavy metal contaminated soil can be divided into physical, chemical and biological remediation technologies. Among them, bioremediation is economical and environmentally friendly.
5. Microbial-produced extracellular polysaccharides possess advantages such as high yield, easy separation, and short production cycles. In future development, they are expected to become an important sustainable resource.
6. Extracellular polysaccharides can significantly enhance crop resilience to

environmental stress, thereby exhibiting substantial potential and promising prospects in agricultural production.

CHAPTER 2
OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 Object, purpose of study

The object of the study is pakchoi.

The purpose of the study is to study the effect of polysaccharide-producing bacteria *Micrococcus antarcticus* RB2 on improving crop quality, and to further explore the feasibility of using this strain for crop quality.

2.2 Experimental material

2.2.1 The strains tested

This study selected the Antarctic *Micrococcus antarcticus* RB2 strain stored in the laboratory.

2.2.2 Experimental soil

The soil samples in this study were derived from the experimental field of the Maize Research Institute of Shandong Academy of Agricultural Sciences, located at 36°44'N and 117°22'E. Five-point sampling method was used to collect samples from uncontaminated surface soil at a depth of 0-15 cm, and three groups of parallel samples were taken and stored in sterile sampling bags for subsequent experiments. The texture classification system of the United States Department of Agriculture divides the soil into different texture categories according to its particle size and proportion. These soils are classified as dark original soil, which refers to the soil type with high organic matter content and darker color in the soil, usually with good fertility and fertility.

Table 2-1 **Basic physical and chemical properties of test soil**

item	pH value	organic matter (g kg^{-1})	total nitrogen (g kg^{-1})	total phosphorus (g kg^{-1})
numerical	7.90	13.65	1.41	2.42

value				

2.2.3 Instrument and equipment

Table 2-2 **Main experimental equipment and manufacturers**

Instrument name	Model	Manufacturer
Refrigerator (4℃, -20℃)	BCD-539WT	Haier
Ultra clean workbench	FD-01	Suzhou Yuanda purification equipment factory
Enzyme calibration	EPOCH-2	BioTek
High pressure steam sterilization pot	MJ3760D	Jinan Deqiang Instrument Co., Ltd
High-speed freezing centrifuge	Centrifuge 5424R	Eppendorf
Electric-heated thermostatic waterbath	HH-3A	Changzhou Jintan Jingda Instrument Manufacturing Co., Ltd
Incubator shaker	DHZ-2001A	Suzhou Pei Ying Laboratory Equipment Co., LTD
pH meter	Ssrtourius PB-10	Sartorius
Ultracentrifuge	Centrifuge 5840R	Eppendorf
Waterproof constant temperature incubator	GRP-9160	Suzhou Pei Ying Laboratory Equipment Co., LTD
Electronic balance	JA2003	Sartorius
Electric constant temperature drying oven	DHG-9140A	Shanghai Jinghong Experimental Equipment Co., LTD
Inductively coupled plasma emission instrument	Optimal 7000DV	Perkin Elmer
Spectrophotometer	UV-1500 spectrometer	Shanghai Meisei Instrument Co., LTD

2.2.4 Experimental reagents and test kits

BCA method protein content determination kit, nitrite content determination kit

2.3 Experimental methods

2.3.1 Pb treatment of the tested soil

The collected soil was thoroughly mixed with the newly prepared $PbCl_2$ solution, and three different concentration gradients were set: 0, 25, and 50mg kg^{-1}. Six parallel samples were set up under each concentration gradient. Place the mixed soil in flower pots with a diameter of 28 centimeters and a height of 35 centimeters. Weigh and add 4.8 kilograms of test soil to each pot. To maintain the soil moisture content at around 70% of the field capacity, deionized water needs to be added. Loosen the soil once a week and maintain a balance for 45 days to ensure sufficient exchange of substances in the soil.

2.3.2 Culture of *Micrococcus antarcticus* RB2

The ratio of liquid culture medium is: 5 grams of peptone, 1 gram of yeast extract, 35 grams of sea salt, 1000 milliliters of distilled water, and the pH value is controlled between 7.6 and 7.8.

The strain *Micrococcus antarcticus* RB2 was inoculated into the above medium and cultured at 25°C for 10 hours. Subsequently, the bacterial suspension was collected, centrifuged (speed of 5000 rpm, time of 4 minutes), and washed. Finally, the bacterial suspension was resuspended to 1×10^8 cell mL^{-1} to ensure that the purity and number of bacteria were suitable for subsequent experiments.

2.3.3 Pot experiment

Pot experiment is a commonly used research method in the field of botany. The potted plant experiment followed the method of Han46 and made some modifications. The balanced and stabilized soil was placed in a greenhouse, the temperature in the greenhouse was controlled at 25±3°C, the relative humidity was maintained at 70%, and the average daily light time was 12 hours to ensure the stability and consistency of the test environment. Next, the cabbage seeds were screened by flotation to select the seeds that were not damaged, uniform in size, and full in particles. The seeds were disinfected by soaking in 5% sodium hypochlorite solution for 10 minutes, then thoroughly washed with sterile water, repeated three times to ensure that the seed surface was sterile, and finally fully blotted with clean filter paper after sterilization. Sow 30 seeds in each pot. The inoculated pots are placed in a greenhouse and diluted after waiting for seeds to germinate. After the seeds germinate, wear sterile gloves, select 15 seedlings with vigorous growth, good condition and similar maturity in each pot, dilute the seedlings to 15 plants per pot so that they are evenly distributed in the pots, and number each pot of seedlings. Subsequently, the pakchoi was continuously cultivated until the third leaf stage, a small groove 1-2 cm deep was dug in the root of the pakchoi, and 90 mL of bacterial resuspension was injected into it using a sterile syringe, and the sterile water was set as the control group, and each group was set up with three parallels. After the root irrigation treatment was completed, the original greenhouse conditions were maintained and the cabbage plants were cultivated for 45 days.

2.4 Methods of sample collection and determination

2.4.1 Sample collection and processing

After harvesting the pakchoi, thoroughly clean the roots and edible parts of the plant with distilled water, then rinse further with 0.01 M ethylenediaminetetraacetic acid (EDTA) solution and evenly divide it into two parts. One of the plants was subjected to a killing treatment at 105°C for 30 minutes to terminate their metabolism, and then dried to a constant weight at 65°C. The dried plants were weighed and their dry weight was recorded; The other part of fresh edible tissue is used to determine vitamin C (Vc), soluble protein, and nitrate content using standard methods. At the same time, other unnecessary parts are treated evenly and harmlessly.

2.4.2 Weigh the dry weight of pakchoi

The fresh weight samples were placed in an oven at 105°C for 30 min and then adjusted to 65°C to constant weight, and the dry weight of pakchoi was weighed. Each group set up three parallel, record data.

2.4.3 Determination of Vc content

The experimental principle: In acetic acid solution, ascorbic acid reacts with fast blue salt B to form a yellow oxalyl hydrazide-2-hydroxybutyrolactone derivative, and the absorbance is measured at the maximum absorption wavelength of 420 nm.

Experimental reagents: 2 mol L-1 acetic acid solution, 0.25 mol L^{-1} EDTA disodium salt solution, 0.5 mol L^{-1} acetic acid solution, 2 mol L^{-1} fast blue salt B solution (prepared before use and stored away from light), stored at 4°C.

Experimental steps: First, 0.1 g of fresh edible tissue of pakchoi was weighed and 1 mL of extract was added together for ice bath homogenization. Subsequently, they were centrifuged (8000 g, 20 min) at 4 °C. After centrifugation, 100 μL of the supernatant was placed on ice for testing. Then, 30 μL acetic acid solution, 50 μL LEDTA disodium salt solution, 120 μL solid blue salt B solution and 700 μL distilled water were added to the sample in turn. Preheat the microplate reader for 30 minutes in advance, adjust the wavelength to 420 nm, and adjust the distilled water to zero. At the same time, a blank group was set as a control. After fully mixing all the solutions, standing at 25°C for 20 minutes, 200 μL samples were taken and placed in 96-well plates, and the absorbance of each tube was measured at 420 nm.

Determine the regression equation under standard conditions as $y = 0.0088x - 0.018$， $R^2 = 0.9978$。

The Vc content is expressed in a formula calculation：

$$Vc(\mu g\ mL^{-1}) = 27.27 \times (\Delta A + 0.018)$$

$$\Delta A = A_{\text{assay}} - A_{\text{blank}}$$

2.4.4 Determination of soluble protein content

Experimental principle: In an alkaline environment, cysteine, tyrosine, tryptophan, cystine, and peptide bonds in proteins can reduce Cu^{2+} to Cu^{+}. Two molecules of BCA (basic copper protein reagent) reacted with Cu^{+} to form a purple complex, which had an absorption peak in the wavelength range of 540-595 nm, especially at 562 nm.

Experimental reagents: Reagent A (0.16% sodium tartrate, 1% BCA disodium salt, 0.4% sodium hydroxide, 2% anhydrous sodium carbonate and 0.95% sodium bicarbonate were mixed and adjusted to pH 11.25), Reagent B (4% copper sulfate solution), standard substance (1. mg mL^{-1} protein standard solution prepared from crystalline bovine serum albumin and normal saline), working solution (25mL Reagent A and 0.5mL Reagent B were mixed). All the above reagents need to be stored at 4°C.

Experimental steps: First, 0.1 g of fresh edible tissue samples of pakchoi were weighed, and 1 mL of extract was added for ice bath homogenization. Centrifuge at 4°C (10000 rpm, 10 min) and take the supernatant for testing. The microplate reader was preheated for 30 min in advance, the wavelength was adjusted to 562 nm, and the distilled water was adjusted to zero. Standard tube was prepared with standard substance. 4 μL of supernatant and 200 μL of working solution were added to the determination tube, and the mixture was placed in a water bath at 60°C for 30 min. The blank control group was set up, and 4 μL distilled water and 200 μL working solution were fully mixed for the same treatment. 200 μL samples were taken from each group to 96-well plates, and the absorbance value A was measured at 562 nm, which was recorded as A blank tube, A standard tube and A determination tube.

The soluble protein content is expressed in a formula calculation：

$$Cpr\ (mg\ g^{-1}\text{fresh weight}) = 0.5 \times (A_{\text{determination tube}} - A_{\text{blank tube}}) \div (A_{\text{standard tube}} - A_{\text{blank tube}}) \div W$$

W: Sample quality (g).

2.4.5 Determination of nitrite content

The experimental principle: In an acidic environment, nitrite reacts with p-aminobenzenesulfonic acid to form diazo compounds, which are further combined with N-1-naphthylethylenediamine to form purple-red azo compounds. This azo compound has a specific absorption peak at 540 nm.

Experimental reagents: 50 g L^{-1} saturated borax solution, 2 g L^{-1} naphthalene ethylenediamine hydrochloride solution, 106 g L^{-1} potassium ferrocyanide solution, 4 g L^{-1} p-aminobenzenesulfonic acid solution, 220 g L^{-1} zinc acetate solution.

Experimental procedure: 0.2 g fresh edible tissue samples of pakchoi were weighed and broken. Add 0.5 mL saturated borax solution, and put the mixture in boiling water bath for 15 min. After cooling to room temperature, 0.5 mL potassium ferrocyanide solution was added and shaken well. Then 0.5 mL zinc acetate solution was added and allowed to stand for 30 min. After centrifugation (8000 g, 15 min) at 25°C, 350 μL supernatant was collected. 325 μL of p-aminobenzene sulfonic acid solution and 325 μL of naphthalene ethylenediamine hydrochloride solution were added to the determination tube, and the mixture was fully mixed. The mixture was placed at 25°C for 15 min. Set the blank control group. The absorbance of 200 μL was measured at 540 nm on a 96-well plate.

Determine the regression equation under standard conditions as： $y = 0.234x + 0.0002$， $R^2 = 0.999$。

The nitrite content is expressed in the formula:

$$NO^{2-}(\mu g\ g^{-1}) = （\triangle A - 0.0002） \div 0.234 \times V_{\text{Sample}} \div （V_{\text{Sample}} \div V_{\text{gross sample}} \times W） \times 0.6668$$

$$= 4.27 \times (\triangle A - 0.0002) \div W$$

Among them, $\Delta A = A_{\text{determination}} - A_{\text{blank}}$ W: Sample quality (g)

2.5 Data analysis

In this study, SPSS 20.0 software (SPSS Inc., USA) was used to analyze the experimental data. Data measurement results are shown as mean ± standard deviation (SD). One-way analysis of variance and Tukey test (p > 0.05) were used to compare the significant differences of each treatment. After that, graphic prism software was used to analyze the quality of pakchoi.

Conclusions to chapter 2

1. The treatment of the soil was as follows: it was thoroughly mixed with a newly prepared $PbCl_2$ solution, and three different concentration gradients of 0, 25, and 50 mg kg^{-1} were set up, with six parallel samples at each concentration gradient, and the soil was continuously balanced in a greenhouse for 45 days.
2. The treatment of the strain *Micrococcus antarcticus* RB2 was to inoculate the strain into a medium and culture it at 25 °C for 10 h. Subsequently, the bacterial suspension was collected, centrifuged (5000 rpm, 4 min), washed, and finally re-suspended to 1×10^8 cells mL.
3. Pakchoi seeds were screened by flotation method, 30 seeds were sown in each pot, and cultivated in a greenhouse. After seed germination, the seedlings were diluted to 15 plants per pot, and pakchoi was continuously cultivated until the third leaf stage, and root irrigation treatment was carried out. Sterile water was set as the control group, each group is set up with three parallel runs. The original greenhouse conditions were maintained and the cabbage plants were cultured for 45 days.
4. The contents of vitamin C, soluble protein and nitrite in the samples were calculated by measuring the absorbance of each derivative at different wavelengths with enzyme labeling apparatus

CHAPTER 3
EXPERIMENTAL PART

3.1 Effect of strain RB2 on dry weight of pakchoi

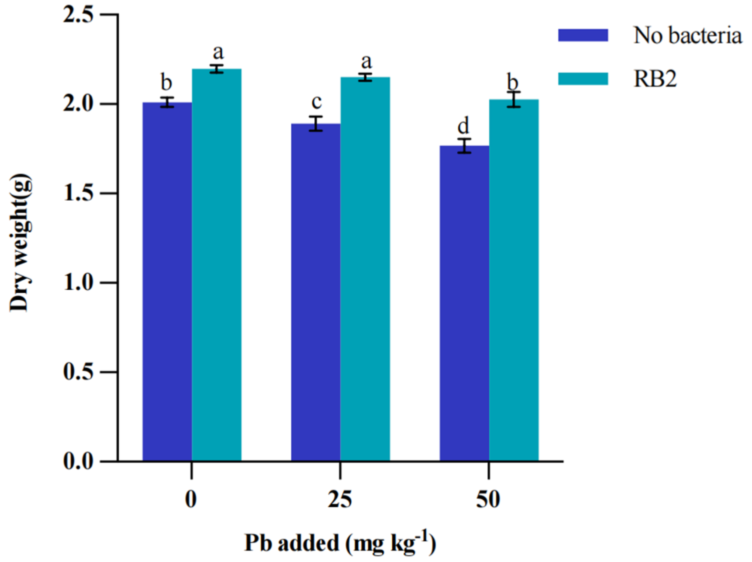

Fig.3-1 Effects of *Micrococcus antarcticus* RB2 on dry weight of edible parts of pakchoi

Note: The data are mean ± standard error (n = 3). According to the Tukey test, the same letter means that there is no significant difference between the two groups ($p > 0.05$).

The biomass of plants is due to the combined effects of different environmental conditions and their own characteristics. It is affected by environmental conditions including soil nutrients, water supply, light intensity and other environmental conditions, as well as the genetic characteristics and physiological status of plants. It is an important manifestation of plant health and environmental adaptability, and is of great significance for studying the growth of plants in different environments. When plants are inhibited by heavy metal stress, because heavy metals have an inhibitory effect on plant metabolic activities, they affect their nutrient absorption and utilization ability, thus affecting the accumulation of dry matter quality, which will lead to significant changes in the dry weight of their roots and leaves. By measuring and evaluating the dry weight of plant roots and leaves, we can more accurately understand its adaptability and growth status in different environments. Therefore, when assessing the growth status of plants under heavy metal stress, dry weight quality becomes a very ideal and objective indicator.

In the pot experiment of pakchoi, three strains of pakchoi were randomly selected from each flowerpot for data statistics on the dry matter quality of edible tissues. As shown in Fig.3-1, under Pb^{2+} stress conditions, regardless of whether the strain *Micrococcus antarcticus* RB2 was applied, the dry matter mass in the edible tissues of pakchoi gradually decreased while the Pb^{2+} concentration in the soil increased. Compared with the blank group, when the soil environment was 25mg L^{-1} Pb^{2+}, the dry weight of edible tissues of pakchoi decreased by 6.0%; when the soil environment was 50 mg L^{-1} Pb^{2+}, the dry weight of pakchoi decreased by 12.1%, and the presence of Pb^{2+} significantly inhibited the growth and development of pakchoi. Under the condition without Pb^{2+} stress, the application of *Micrococcus antarcticus* RB2 could significantly increase the edible tissue dry matter quality of pakchoi by 9.3%. The application of *Micrococcus antarcticus* RB2 to 25 mg L^{-1} Pb^{2+} and 50 mg L^{-1} Pb^{2+} soil also showed a positive effect, increasing the dry weight of pakchoi by 13.8% and 14.7%, respectively. The inoculation of *Micrococcus antarcticus* RB2 increased the dry weight of roots and leaves of pakchoi compared to the treatment group where the strain *Micrococcus antarcticus* RB2 was *Micrococcus antarcticus* RB2 not applied.

These data indicated that the strain *Micrococcus antarcticus* RB2 could alleviate the negative effect of Pb^{2+} on the growth of pakchoi, reduce the harm of Pb^{2+} to pakchoi, increase the dry matter quality of edible tissues of pakchoi, and promote the growth of pakchoi. This indicates that the strain has potential value for improving the growth performance and environmental adaptability of crops in soils contaminated by heavy metal lead, and helps to improve the quality of crops grown in soils polluted by heavy metal and improve the sustainable utilization of farmland.

3.2 Effect of strain RB2 on Vc content of pakchoi

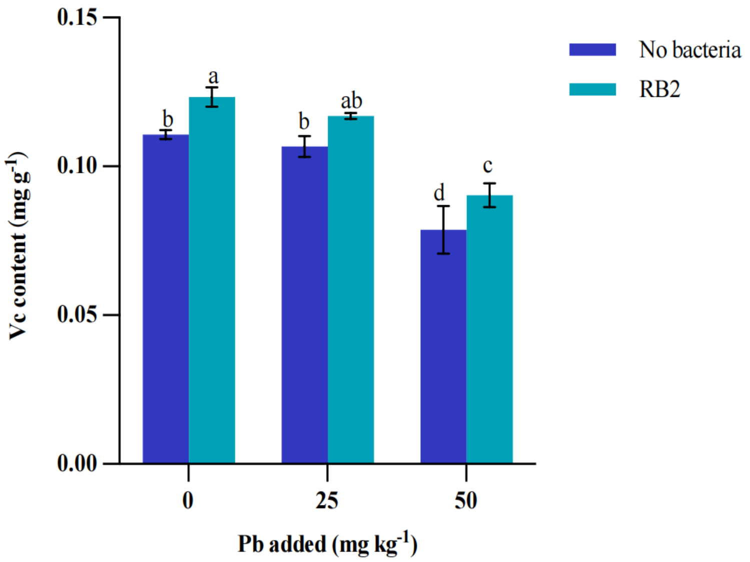

Fig.3-2 Effects of *Micrococcus antarcticus* RB2 on Vc content in edible tissues of pakchoi.

Note: The data are mean ± standard error (n = 3). According to the Tukey test, the same letter means that there is no significant difference between the two groups ($p > 0.05$).

Vc content is one of the important criteria for measuring plant quality. In plants, vitamin C plays a variety of important roles, including as a major redox buffer and a cofactor for various enzymes, as well as participating in signal transduction and cell growth regulation. Ascorbic acid (Vc) is an important substance that cannot be synthesized by the human body and must be taken from food. Many plants can synthesize this substance by themselves. The antioxidant properties of vitamin C are essential for delaying cell aging and promoting healthy aging. It helps protect cells from oxidative stress, reduces the production of free radicals, and maintains the normal function of cells. By consuming enough vitamin C, we can slow down skin aging, protect heart health, and reduce the risk of chronic diseases. Therefore, maintaining an adequate intake of vitamin C is essential for maintaining human health.

In the pot experiment, the Vc content of edible tissues of pakchoi in three pots of each treatment group was determined. The experimental results are shown in

Fig.3-2. Under Pb^{2+} stress conditions, regardless of whether the strain *Micrococcus antarcticus* RB2 was applied, the Vc content in the edible tissues of pakchoi gradually decreased while the Pb^{2+} concentration in the soil increased. Especially under the condition of high concentration of Pb^{2+} (50 mg kg^{-1}), the content of Vc in edible tissues of pakchoi was significantly inhibited. Compared with the control group without Pb^{2+} and the control group with low concentration of Pb^{2+} (25 mg L^{-1}), the content of Vc decreased by 28.9% and 26.2%, respectively. Under the condition of low concentration of Pb^{2+} (25 mg L^{-1}), although the Vc content decreased, the difference was not significant. Compared with the blank control group without Pb^{2+}, the Vc content decreased by 3.6%. However, the application of strain *Micrococcus antarcticus* RB2 could increase the Vc content in the edible tissues of Pakchoi to restore to the level without Pb^{2+} stress. In the absence of Pb^{2+} stress, the application of *Micrococcus antarcticus* RB2 can significantly increase the Vc content in the edible tissues of pakchoi, with an increase rate of 11.4%. Under the stress of 25 mg L^{-1} Pb^{2+} and 50 mg L^{-1} Pb^{2+}, the Vc content in Pakchoi increased by 9.7% and 14.7% respectively after adding the strain.

In summary, it can be seen from the experimental results that Pb^{2+} stress can significantly affect the content of Vc in plants, but the addition of *Micrococcus antarcticus* RB2 strain can effectively increase the Vc content in pakchoi and alleviate the effect of lead stress on Vc content. This finding is of great significance for improving the stress resistance and quality of plants. Increasing the content of ascorbic acid in plants not only helps to promote plant growth and development, but also improves the nutritional value of vegetables. With the improvement of people 's health awareness, intake of sufficient ascorbic acid has become one of the topics of concern in people 's daily life. Therefore, the results obtained in this study are of positive significance in meeting the health needs of humans for ascorbic acid intake.

3.3 Effects of strain RB2 on soluble protein content of pakchoi.

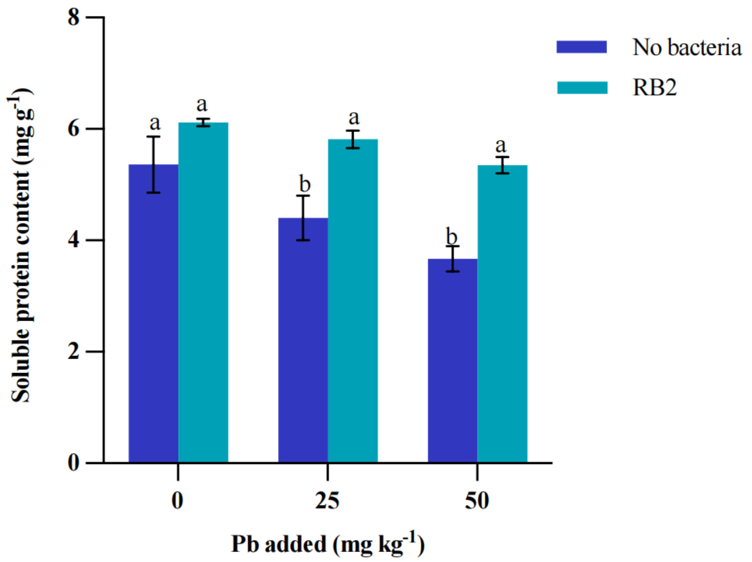

Fig.3-3 Effects of *Micrococcus antarcticus* RB2 on soluble protein content in edible tissues of pakchoi.

Note: The data are mean ± standard error (n = 3). According to the Tukey test, the same letter means that there is no significant difference between the two groups ($p > 0.05$).

Soluble protein content plays multiple important roles in plants. First of all, it improves the water-holding capacity of cells and helps maintain plant growth and development. Secondly, soluble protein is also one of the important osmotic adjustment substances and nutrients, which affects the efficiency of plant absorption, transmission and utilization of water and nutrients. In addition, the nitrogen in plants mainly exists in the form of soluble protein, and the change of its content will affect the metabolism and senescence process of plants. Therefore, by monitoring and studying the content of soluble protein, the resistance and growth status of plants can be better evaluated. It is often used to screen plant resistance indicators and is one of the important physiological and biochemical indicators for evaluating plant growth status and quality.

In the pot experiment, the content of soluble protein in edible tissues of pakchoi in three pots of each treatment group was determined. The experimental results are shown in Fig.3-3. Under Pb^{2+} stress conditions, regardless of whether the strain *Micrococcus antarcticus* RB2 was applied, the soluble protein content in the edible tissues of pakchoi gradually decreased while the Pb^{2+} concentration in the soil increased. Specifically, compared with the blank group, when the soil Pb^{2+} content was 25 mg L^{-1} Pb^{2+} and 50 mg L^{-1} Pb^{2+} without strain *Micrococcus antarcticus* RB2, the soluble protein content decreased by 17.9% and 31.5%, respectively. By applying the strain *Micrococcus antarcticus* RB2, the soluble protein content increased by 14.1%, 32.1%,45.7% respectively, compared with the blank control group.

The results showed that the soluble protein content of pakchoi was negatively affected under Pb^{2+} stress, and a series of complex biochemical reactions may occur in the cells. Lead stress may accelerate the decomposition of soluble proteins and strengthen the decomposition of proteins by promoting the increase of proteolytic enzyme activity. At the same time, lead ions may also affect the activity of enzymes related to protein synthesis and inhibit the synthesis of new proteins. These changes have an impact on the biochemical reactions such as protein synthesis, denaturation and degradation in the cells of pakchoi, thereby reducing the content of soluble protein in the edible tissues of pakchoi. However, the addition of *Micrococcus antarcticus* RB2 strain could partially alleviate this effect. After the application of *Micrococcus antarcticus* RB2, the toxic effect of lead on pakchoi was alleviated to a certain extent, which reduced the adverse physiological and biochemical reactions in the tissue cells of pakchoi and increased the content of soluble protein, thus improving the quality of pakchoi.

3.4 Effect of strain RB2 on nitrite content of pakchoi

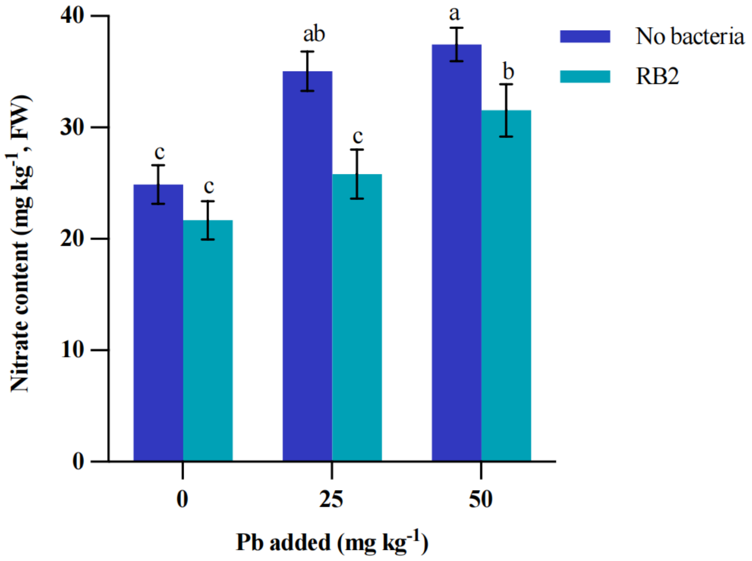

Fig. 3-4 *Effect of Micrococcus antarcticus RB2* on nitrite content in edible tissues of pakchoi

Note: The data are mean ± standard error (n = 3). According to the Tukey test, the same letter means that there is no significant difference between the two groups ($p > 0.05$).

Nitrogen is a key nutrient for plant growth and development, and nitrate is the main form of nitrogen that plants absorb and utilize from the environment. During the process of absorbing nitrogen from the environment and synthesizing amino acids, the generation of nitrate is an inevitable chemical reaction and an important step for plant growth and development. When there is a large amount of fertilizer or pollution in the soil, there will be a buildup of nitrates. These nitrates can be reduced to nitrite by certain reducing enzymes within the plant, and nitrite is a toxic compound. Despite the fact that nitrates are commonly found in plants, excessive nitrates can also be harmful to plants. When plants absorb too much nitrate and nitrite, it can affect their healthy growth. In addition to causing harm to plants themselves, nitrates can enter the human body through the food chain and cause health problems, even leading to acute poisoning and increasing the risk of cancer. Therefore, controlling nitrate intake is crucial for protecting both plants and human health. We need to be aware of this potential danger and take steps to reduce the amount of nitrite in the food chain to ensure that our ecosystems and health are not affected.

In the pot experiment, the content of nitrite in the edible tissues of pakchoi in three pots of each treatment group was determined. The experimental results are shown in Figure 3-4. Under Pb^{2+} stress conditions, regardless of whether the strain *Micrococcus antarcticus* RB2 is applied or not, the concentration of Pb^{2+} in the soil increases, and the nitrite content in the edible tissues of pakchoi also gradually increases. When *Micrococcus antarcticus* RB2 was not applied, lead stress significantly increased nitrite content compared with blank group and control group. Specifically, under the condition of 25 mg L^{-1} Pb^{2+} and 50 mg L^{-1} Pb^{2+}, the content of nitrite increased by 41.0% and 50.6% respectively. In the absence of lead stress, the application of *Micrococcus antarcticus* RB2 reduced the nitrite content of edible tissues of pakchoi by 12.9%, while the nitrite content of pakchoi grown in 25 mg L^{-1} Pb^{2+} and 50 mg L^{-1} Pb^{2+} soils decreased by 26.4% and 15.8%, respectively, due to the application of *Micrococcus antarcticus* RB2.

According to the experimental results, it can be concluded that Pb^{2+} stress will have an adverse effect on the nitrite content of pakchoi. The addition of RB2 strain can effectively reduce the nitrite content in pakchoi, which is helpful to alleviate the adverse effects of lead stress on plants. In agricultural production, effective control of the effect of lead stress on plant nitrite content can not only improve crop yield and quality, but also make a positive contribution to food safety and environmental protection, promote sustainable agricultural development, reduce environmental pollution and protect ecosystem health. This discovery provides important theoretical support and practical guidance for improving the stress resistance and quality of plants, which will have a positive impact on human life and health, and promote scientific research and technological innovation in related fields.

3.5 Outlook

In this study, pakchoi (*Brassica chinensis* L.) was selected as the test plant species, and the strain *Micrococcus antarcticus* was studied in the form of pot experiments. The improvement effect of RB2 on the quality of pakchoi under lead contamination showed the excellent bioremediation potential of this strain, and provided a new idea for solving the problem of soil heavy metal pollution. In view of the vast soil area in China, covering many types of soil and plants, a wide variety of plant species and complex and changeable soil environment will affect the experimental results. Therefore, in future experiments and research, other different plant species, such as wheat and other plants with long growth cycles, can be selected, and other types of soil heavy metal pollution can be considered, and different soil conditions, such as cadmium pollution, can be changed to carry out experiments with a larger area and scope. In this way, the effects of this study on different plants and different heavy metals can be more comprehensively evaluated and explored. At the same time, future research directions can further explore the specific influence mechanism of this strain on plant growth and heavy metal stress, provide a scientific basis for more effective use of microorganisms to remediate soil pollution, and further exert its potential in the field of environmental remediation, so as to better apply it in actual production, protect the environment, improve crop yield and quality, and promote the healthy and sustainable development of the ecological environment.

As a natural and bio-friendly method, microbial remediation technology has many advantages over traditional soil remediation methods. First of all, microbial remediation methods are cost-effective, which can not only effectively reduce the cost of treatment, but also reduce the risk caused by secondary environmental pollution. Secondly, microbial remediation has a certain degree of sustainability and can effectively remediate soil pollution problems in the long term. Therefore, the application of microbial remediation technology is very important for promoting environmental soil remediation, and has broad application prospects and important positions in the field of soil pollution control. It is of great significance to continue to promote and explore the development of microbial remediation technology to improve environmental quality and promote ecological construction. The application of microbial remediation technology can help better protect the ecological environment and people's health, and promote sustainable environmental development. Only by continuously strengthening technical research and practice and continuously improving microbial remediation technology can we better deal with environmental problems such as soil pollution and achieve the goal of sustainable development. Let us work together to make our own contribution to the cause of environmental protection

Conclusions to chapter 3

1. Compared with the blank group, the application of strain RB2 can improve the quality of Pakchoi, promote the growth of Pakchoi, and have a positive impact on agricultural production.
2. Data Analysis of Dry Weight of pakchoi, the dry matter quality of the edible parts of Pakchoi increased by 9.3%-14.7%.
3. Data Analysis of Vc content of pakchoi, the vitamin C content increased by 9.7%-14.7%.
4. Data Analysis of soluble protein content of pakchoi, the soluble sugar content increased by 14.1%-45.7%.
5. Data Analysis of nitrate content of pakchoi, the nitrite content decreased by 12.9%-26.4%.

CONCLUSIONS

1. Through the determination of the quality index of pakchoi, it was found that the quality of pakchoi could be improved by using the extracellular polysaccharide-producing marine bacteria *Micrococcus antarcticus* RB2. The experimental results showed that the dry matter quality, soluble protein content and Vc content of edible tissues of pakchoi were increased after treatment, and the nitrite content was decreased.

2. The use of *Micrococcus antarcticus* RB2 to treat pakchoi improved the quality of pakchoi, promoted the growth of pakchoi, and had a positive impact on agricultural production.

3. The exopolysaccharide-producing marine bacteria *Micrococcus antarcticus* RB2 showed great potential in repairing heavy metal lead pollution in farmland. The use of this strain to prepare microbial agents for agricultural production is not only expected to repair lead pollution in farmland and reduce lead stress in crops, but also to increase crop yield and improve its quality.

4. In summary, the polysaccharide-producing bacteria *Micrococcus antarcticus* RB2 shows great potential in repairing heavy metal lead pollution in farmland. The application of this strain in agricultural production can not only increase the yield of crops and improve their quality, but also repair lead pollution in farmland soil and reduce the impact of lead stress on crops. The use of *Micrococcus antarcticus* RB2 provides a new idea for the safe production of crops. This technology is not only environmentally friendly and ecologically friendly, but also of great significance for farmland ecological restoration. Therefore, on the road of sustainable development in the future, this method will have a profound impact on agricultural production and environmental protection, and provide us with an important and effective technical approach, which is worthy of further research and application.

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