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 **Isolation and Identification of Salt-Tolerant Rhizosphere Microorganisms**

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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Educational and professional program Biotechnology

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

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Deng Xinru

1. Thesis topic **Isolation and Identification of Salt-Tolerant Rhizosphere Microorganisms**
Scientific supervisor Dr.Sc., Prof. Olga Andreyeva

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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

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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	
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I am familiar with the task:

Student _____ Deng XINRU

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SUMMARY

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With the continuous expansion of saline-alkali land in China, this study focuses on the rhizosphere microorganisms of the halophyte «Suaeda salsa» to improve soil cultivation conditions and ensure food security. Using the dilution plating and streak plate methods, strain 1-9 was isolated and purified to investigate its salt tolerance and plant growth-promoting functions. Selective medium tests revealed that this strain can tolerate 15% salinity and exhibits growth-promoting traits, including IAA production, ammonia production, nitrogen fixation, and phosphate solubilization. Plant experiments demonstrated that under salt stress, maize plants treated with strain 1-9 showed significantly enhanced salt resistance, with better stem and leaf growth compared to the control group. Measurements of dry matter accumulation and antioxidant enzyme activity further confirmed that the strain promotes maize growth under both normal and salt-stressed conditions, likely by enhancing antioxidant enzyme activity to improve stress resistance. This study provides theoretical and practical insights into the mechanisms of salt-tolerant microorganisms in promoting plant growth and offers a novel microbial-based ecological strategy for saline-alkali soil remediation.

Key words: Salt-tolerant microorganisms, IAA-producing; strains; plant growth-promoting

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INTRODUCTION

With the continuous expansion of saline-alkali land in China, this study focuses on the rhizosphere microorganisms of the halophyte «Suaeda salsa» to improve soil cultivation conditions and ensure food security. This study provides theoretical and practical insights into the mechanisms of salt-tolerant microorganisms in promoting plant growth and offers a novel microbial-based ecological strategy for saline-alkali soil remediation.

The relevance of this topic lies in salt-tolerant plants and the microorganisms in their root zones.

The purpose of this study is to isolate salt-tolerant strains and investigate their functional characteristics such as the ability to produce IAA, nitrogen fixation, phosphorus solubilization, ammonia production, etc., and to determine whether they have a beneficial effect on plants.

The objectivity of this research lies in seeking solutions to improve saline-alkali land from the perspective of microorganisms, providing a completely new possibility for the restoration of saline-alkali land.

Object of the study – salt-tolerant strain 1-9.

Subject of the study – selection of salt-tolerant strains, evaluation of their biostimulating capacity and various functional characteristics.

Tasks and research methods:

1. Selection and isolation of salt-tolerant strains;

2. Determination of salt tolerance of salt-tolerant strains;

3. Analysis of characteristics that promote plant development (determination of nitrogen fixation ability, measurement of auxin production ability, measurement of ammonia production ability, analysis of phosphorus removal effect);

4. Effect of salt-tolerant plant-associated microorganisms on plant growth and salt tolerance.

Research methods used: gradient dilution method and plate streaking method.

The scientific novelty of the work consists in the isolation of salt-tolerant plants from the resosphere soil, purification and identification of a new strain of mycoorganisms 1-9, which has increased (15% NaCl) salt tolerance and the potential for stimulating plant growth. Improvement of the growth of corn stems and leaves under salt stress after treatment with the strain can be explained by an increase in the activity of antioxidant enzymes, established during the experiment.

The practical significance lies in providing a completely new perspective for the restoration of saline-alkali land and ensuring food security.

CHAPTER I
LITERATURE REVIEW

1.1 The current situation of soil salinization

Saline-alkali land is widely distributed on the earth. As of now, China is one of the countries with the most extensive distribution of saline-alkali land, with a total area of 99.13 million hectares. It is mainly distributed in the northeastern, northwestern, Huaihai and coastal regions, forming four typical saline-alkali areas, including Xinjiang, Gansu, Qinghai, Inner Mongolia, Ningxia and coastal areas [1]. The formation of saline-alkali land is the result of the combined effect of various factors, such as arid climate, seawater intrusion, excessive fertilization, vegetation destruction and unreasonable irrigation. These factors have jointly led to the dynamic imbalance of soil salinity, aggravating the degree of soil salinization. Saline-alkali land is a special type of land resource with great potential for development and utilization. The large areas of saline-alkali land in China have been abandoned for a long time, resulting in resource waste and environmental damage. The widespread and increasingly serious situation of soil salinization also has a huge impact on agricultural production. How to improve the utilization efficiency of saline-alkali land and achieve the goals of carbon sequestration and increase of sinks is an urgent problem to be solved [1].

Soil salinization is a global environmental problem that poses a serious threat to the sustainable development of agriculture in China and the health of the ecosystem [3] ·From the perspective of agricultural production, when the soil EC value exceeds 4 dS/m due to salinization, crops suffer from osmotic stress and ionic toxicity, resulting in a 30%-50% reduction in corn, rice and other food crops, while accelerating the decomposition of organic matter in the soil and seriously damaging soil fertility. [4]In terms of the ecological environment, the continuous expansion of saline-alkali land has led to a sharp decline in biodiversity[1], with

only a few salt-tolerant plants such as alkali grass and strange willow remaining, posing a huge threat to biodiversity. The structure of soil microbial communities has been significantly simplified, and ecosystem functions have deteriorated seriously.[5] From the social and economic perspective, soil salinization not only increases agricultural production costs and causes the loss of arable land resources [6], but also results in the loss of approximately 2 million hectares of arable land globally each year (FAO, 2023)[5], exacerbating the pressure on food security. Moreover, salinization and climate change form a vicious cycle[7]. Drought intensification leads to salinization accumulation, and vegetation degradation further changes the local climate. This cumulative and long-term harmful characteristic highlights the urgency of constructing a comprehensive prevention and control system including salt-tolerant plant restoration [8,] precise irrigation management and soil improvement technologies, which is of great strategic significance for ensuring China's food security and ecological security.

1.2 Current research achievements on the improvement of saline-alkali land

Saline-alkali land improvement requires human intervention and the use of various measures to improve soil salinization[6]. Traditional methods have the shortcomings of long duration, high cost and slow effect, which makes the selection of salt-tolerant plant root-associated microorganisms crucial for the improvement of saline-alkali land. The core value lies in breaking the limitations of single biological restoration through the synergistic interaction mechanism between plants and microorganisms [9], thereby achieving more efficient and comprehensive restoration effects. Relevant studies have shown that salt-tolerant symbiotic bacteria have a mitigating effect on plants subjected to salt stress [10], revealing the possibility and broad prospects of using salt-tolerant plant root-associated microorganisms to improve saline-alkali land. The functional microorganisms enriched in the root zone of salt-tolerant plants can enhance the system's stress resistance through multiple

pathways: on the one hand, microorganisms regulate ion homeostasis[11] and secrete extracellular polysaccharides [12]to promote soil aggregation and improve the permeability of saline soil; on the other hand, their metabolic activities can stimulate nutrient cycling such as nitrogen fixation[13] and phosphorus release[14], alleviating the nutrient imbalance caused by salt stress. Screening and constructing functional microbial communities can not only provide a resource basis for the development of efficient microbial agents, but also enhance plant adaptability by regulating the root micro-ecological network[15], achieving the sustainable development strategy of improving saline-alkali land and providing theoretical support and practical paths for the "microbial-plant joint restoration of saline-alkali land" technological innovation. Salt stress is an important abiotic factor affecting plant growth.

Current studies have shown that rhizobacteria (PGPR) that promote plant growth have excellent root anchoring and enzyme production capabilities. It is well known that salt-tolerant plants have a special root-associated microbial community, which is widely enriched with PGPRs [16]. Currently, some PGPRs have been proven to have the ability to alleviate the impact of salt stress on plant growth and promote plant adaptation to salt stress [17]. For instance, Yasmin et al. found that Pseudomonas halophilica and Bacillus subtilis significantly improved the growth of soybeans under salt stress through a series of physiological regulatory processes [18]. Currently, the known mechanisms by which microorganisms promote plant growth in saline soil[17-19] may include the following aspects: producing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase and indole-3-acetic acid (IAA) to promote plant growth and indirectly alleviate the salt stress on plants; synthesizing phosphatases and iron carriers to provide available nutrients for plants; activating the plant's antioxidant system to remove reactive oxygen species (ROS); producing extracellular polysaccharides to promote the formation of biological membranes and regulate ion balance. In addition, scholar Yan Li et al. revealed the interaction mechanism among soil conditions, plants, and microorganisms in their research on rhizobacteria of cannabis[20].

1.3 The research findings and significance of this article

This study screened salt-tolerant plant root-associated microorganisms and analyzed their diversity and functional characteristics. At the theoretical level, by constructing a three-dimensional correlation model of "screened strains - determination of their salt tolerance - validation of plant adaptability", the multi-dimensional mechanism of microbial-driven salt-alkali land remediation was revealed. At the practical level, this study based on isolated highly salt-tolerant symbiotic strains will create a foundation for the development of composite microbial agents [21] and reduce the cost of salt-alkali land remediation, which is of great strategic significance for ensuring food security and ecological strategic security.

The functional characteristics of the studied strains for promoting symbiosis include nitrogen fixation ability, ammonia production ability, IAA production ability, and phosphorus solubilization ability, etc. Taking the nitrogen fixation ability of leguminous plants as an example, leguminous plants can form root nodules and fix nitrogen through rhizobacteria [22]. Rhizobacteria differ in composition and quantity from the root secretions secreted by non-leguminous plants. These root secretions are usually used as signal molecules and nutrients for soil microorganisms, leading to leguminous plants being rich in nitrogen [23]. Leguminous plants such as soybeans, which live in saline-alkali land in the Yellow River Delta, can tolerate high concentrations of salt. Existing studies have shown that the number of rhizobia in the roots of soybeans is higher than that of cultivated leguminous plants Glycine max [24]. By studying the various biochemical mechanisms of plant root-associated microorganisms to achieve mutualistic symbiosis with plants, the significance of isolating plant root-associated microorganisms is revealed[25].

The total area of saline-alkali land in China is nearly 100 million hectares. Among them, the areas that can be improved are mostly distributed in major grain-producing regions such as Northeast and Northwest China. The efficient utilization of these areas is related to the national food security strategy.[26] The traditional management of saline-alkali land relies on physical flushing or chemical improvement,

which is costly and prone to damaging the soil microecology. There is an urgent need to develop green and sustainable remediation technologies.[27] The rhizosphere microorganisms of salt-tolerant plants, as the "biological regulators" for hosts to adapt to saline environments, their functional analysis and resource exploration not only provide new ideas for the governance of saline-alkali land, but also are of crucial significance for ensuring food security and promoting the green transformation of agriculture.[28]

By analyzing the functional mechanisms of rhizosphere microorganisms in salt-tolerant plants and developing their application potential, not only has the theoretical gap of biological interactions been filled[29], but also the transformation of saline-alkali land management from engineering models to biotechnology has been promoted. In the future, with the integration of interdisciplinary technologies, salt-tolerant microorganisms may become the core engine for the restoration of saline ecosystems, providing technological support for the sustainable development of agriculture. The promotion of salt-tolerant microbial technology will deeply align with the global demand for sustainable development. With the deepening of agricultural cooperation under the Belt and Road Initiative, the salt-tolerant microbial agents developed by China are expected to provide governance solutions for salinized regions such as Central Asia and North Africa, and build a global ecological restoration network for saline-alkali land. The isolation and identification of rhizosphere microorganisms in salt-tolerant plants is the core basis for developing low-cost and environmentally friendly saline-alkali land improvement technologies[30]. By screening the core functional strains with the capabilities of salt tolerance, nitrogen fixation, phosphorus dissolution or plant hormone production, and constructing the microbial-plant combined restoration system, an efficient solution can be provided for the ecological function restoration and agricultural utilization of saline-alkali land. [2] Saline-alkali land in China is mainly concentrated in major grain-producing areas such as the Songnen Plain in Northeast China and the arid zone in Northwest China. Its improvement and utilization have been incorporated into the national food security

strategy and ecological civilization construction plan[31]. Microbial remediation technology has advantages such as low cost, environmental friendliness and strong sustainability. Its promotion and application can reduce reliance on chemical fertilizers, lower carbon emissions from soil improvement, and promote the transformation of agriculture towards a resource-conserving model[32].

From the functional exploration of single strains in the past, to the current mechanism analysis driven by multiple omics, and then to the future integration of synthetic biology and ecological engineering, the research on rhizosphere microorganisms of salt-tolerant plants has always closely followed the core demands of sustainable agricultural development and ecological security. This field not only deepens the theoretical understanding of biological interactions in extreme environments, but also provides disruptive solutions for the governance of saline-alkali land, marginal land development and global ecological restoration through technological innovation, which has profound scientific value and socio-economic significance. In the management of saline-alkali land, traditional chemical and engineering remediation methods have limitations such as high costs and susceptibility to salt re-accumulation. This study identified the root-associated functional microorganisms of salt-tolerant plants, determined their salt tolerance thresholds and promoting characteristics, and could directly screen out strain resources suitable for saline habitats. The research results provide direct experimental basis for developing low-cost microbial agents and optimizing the plant-microbe combined remediation technology, improving the desalination efficiency, and have practical application value in enhancing the efficiency and sustainability of saline-alkali land improvement. At the same time, it provides key strain resources for analyzing the synergistic salt-tolerance mechanism of plant-microbe interaction, promoting the transformation of saline-alkali land restoration from empirical improvement to targeted regulation.

Summary of the chapter I

1. Saline-alkali land improvement requires human intervention and the use of various measures to improve soil salinization.

2. The functional microorganisms enriched in the root zone of salt-tolerant plants can enhance the system's stress resistance through multiple pathways.

3. At the theoretical level, by constructing a three-dimensional correlation model of «screened strains - determination of their salt tolerance – validation of plant adaptability», the multi-dimensional mechanism of microbial-driven salt-alkali land remediation was revealed. At the practical level, this study based on isolated highly salt-tolerant symbiotic strains will create a foundation for the development of composite microbial agents and reduce the cost of salt-alkali land remediation, which is of great strategic significance for ensuring food security and ecological strategic security.

Chapter II

OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 Required materials

2.1.1 Soil sample

In the saline-alkali areas of Ningxia and Dongying, salt-tolerant alkali grass Spartina was collected from the rhizosphere soil. It was placed in plastic sample collection bags, sealed, and sent to the laboratory on campus for refrigeration storage at 4 degrees Celsius. It was used as needed upon retrieval. During sampling, the rhizosphere soil was gently shaken off and collected and placed in a sterilized 10 mL centrifuge tube. Then, sterile water was poured in, shaken to resuspend, and left to stand for 10 minutes.

2.1.2 Strain

After the soil bacterial suspension has been statically re-suspended, a gradient dilution is performed on the bacterial suspension. 200 μ L of the bacterial suspension is spread onto LB solid medium using the streaking method and is placed in a 37-degree constant temperature incubator for about 24 hours. Several single colonies are picked and streaked onto the prepared 3%, 5%, and 10% NaCl culture media at 37 degrees Celsius for constant temperature incubation. After the colonies grow, the ones that grow on different concentrations of NaCl culture media are identified.

The colonies on the high-salt plates are picked and streaked onto the LB solid medium again for further cultivation. The single colonies are isolated and a single colony is picked and picked up with a pipette gun and repeatedly shaken to mix with 20 mL of LB liquid medium. It is then activated and cultivated in a 37-degree constant temperature shaker for 24 hours.

After the bacterial solution becomes turbid, 1 mL of the solution is mixed with 1 mL of glycerol and labeled and sealed for storage in a -20-degree Celsius refrigerator.

Then, 1 mL of the bacterial solution is taken for sequencing to determine the species of the strain.

2.1.3 Culture medium

Solid LB medium

Table 2.1 **Solid LB culture medium formula**

Peptone	1%
Yeast powder	0.5%
NaCl	1%
Agar	1.5%

Liquid LB medium

Table 2.2 **Liquid LB culture medium formula**

Peptone	1%
Yeast powder	0.5%
NaCl	1%

High-concentration NaCl solid medium

Table 2.3 **High-concentration NaCl solid medium formula**

Peptone	1%
Yeast powder	0.5%
NaCl	3%、5%、10%、13%、14%、15%
Agar	1.5%

NBRIP (National Botanical Research Institute's Phosphate Fluid Medium）

Table 1.4 **NBRIP formula**

Dextrose	10.0 g
Calcium Phosphate	5.0 g
Magnesium Chloride	5.0 g
Magnesium Sulfate	0.25 g
Potassium Chloride	0.2 g
Ammonium Sulfate	0.1 g

Weigh 20.55g of this product into 1L of distilled water or deionized water, stir to suspend it, add agar powder, and adjust the pH to 7.0±0.2 (at 25 ℃) using dilute hydrochloric acid or NaOH solution. Then, divide it into portions, sterilize at 121℃ under high pressure for 15 minutes, and store it for later use. This product contains insoluble substances, which can be dissolved by heating and stirring.

Ashby's glucose medium

Table1.5 **Ashby's glucose medium formula**

Glucose	20.0 g
$CaCO_3$	5.0 g
K_2HPO_4	0.2 g
$MgSO_4 \cdot 7H_2O$	0.2 g
NaCl	0.2 g
K_2SO_4	0.1 g
Agar	15.0 g
pH 7.4±0.2（25 ℃）	

Weigh 40.7 g of this product into 1 L of sterile water, heat and boil until completely dissolved. Sterilize at 116 ℃ under high pressure for 30 minutes. After sterilization, mix well and pour into a petri dish. Let it solidify before use.

2.1.4 Plants

Seeds of the maize inbred line KN5585

2.2 Instruments and equipment

EPOCH-2 microplate reader, constant temperature incubator, constant temperature shaker, refrigerator, centrifuge, autoclave, alcohol lamp, petri dishes, spreader, inoculation loop, conical flasks, centrifuge tubes, pipette gun, sterile workbench, vortexer, pH meter, spectrophotometer, etc.

2.3 Methods

2.3.1 Selection and isolation of salt-tolerant strains

Using the soil bacterial suspension prepared by static settling, perform seven concentration gradients of dilution in centrifuge tubes. Light the alcohol lamp and separately aspirate 200 μL of the diluted bacterial suspension from each centrifuge tube. Use a spreader to spread the diluted bacterial suspension onto solid LB medium. Pay attention to sterile operation during the process. Place the LB medium in a 37 ℃ constant temperature incubator for 12-24 hours of cultivation.

Light the alcohol lamp and use the outer flame of the alcohol lamp to sterilize the inoculation loop. Use the inoculation loop to pick the colonies on the plate and inoculate them onto NaCl culture media with concentrations of 3%, 5%, and 10% for streaking and cultivation for 24 hours. Pick the colonies grown on all three-culture media and spread them onto the LB medium for streaking and isolation of single colonies. Prepare a liquid LB medium, pick the single bacteria onto 20 mL of liquid medium, and cultivate it in a shaker at 37 ℃ for 180 r for 24 hours. Mix 1 mL of

bacterial liquid with 1 mL of glycerol at a 1:1 ratio and transfer it to a centrifuge tube. Place it in a -20 ℃ refrigerator for freezing storage. Take 1 mL of bacterial liquid and transfer it to a centrifuge tube to use molecular biology techniques such as 16S rRNA for sequencing to determine the species of this strain.

2.3.2 Determination of salt tolerance of salt-tolerant strains

Prepare 13%, 14%, and 15% solid NaCl culture media. Dilute the bacterial solution to different concentration gradients and transfer them to the 13%, 14%, and 15% NaCl culture media respectively. Cultivate at a constant temperature for 24 hours. Then, add 5 μL of the bacterial solution to the culture medium at each point and cultivate at 37 degrees Celsius for 24 hours. Observe the results and take photos.

2.3.3 Determination of the ability of salt-tolerant strains to produce IAA

Preparation of the required yeast maltitol agar (YMA) medium: 10 g of maltitol, 0.2 g of heptahydrate magnesium sulfate, 0.1 g of sodium chloride, 3 g of yeast powder, 0.25 g of disodium hydrogen phosphate, 0.25 g of dipotassium hydrogen phosphate, 3 g of calcium carbonate, 20 g of agar, 1000 mL of sterile water, pH around 7.0. Salkowski color solution: 1 mL of 0.5 mol/L $FeCl_3$ dissolved in 49 mL of 35% concentrated H_2SO_4.

Prepare the LB liquid medium for the determination of auxin (IAA) secretion by the test strain. Add 200 mg/L of L-tryptophan to the sterilized YMA liquid medium, inoculate the strain, and incubate it in a 37 ℃, 180 r/min constant temperature shaking incubator for 7 days. Take 50 μL of the supernatant of the centrifuged bacterial suspension and drop it onto a white ceramic colorimeter plate, and add 50 μL of Salkowski color solution. Leave it at room temperature in the dark for 30 minutes. If the color turns red, it indicates that the strain has the ability to secrete IAA [25]. A blank control should be set up to measure the OD value.

2.3.4 Determination of nitrogen fixation ability of salt-tolerant strains

Experimental principle: The nitrogenase activity of the strain is determined using the acetylene reduction method, and the nitrogen fixation ability of the strain is evaluated based on this. The core principle of this method lies in the fact that nitrogenase has the ability to catalyze the reduction of acetylene to ethylene. Even if the generated ethylene is at a low concentration, it can be effectively detected using the hydrogen flame ionization detector equipped with the gas chromatograph. Nitrogen fixation activity = actual ethylene peak area x standard acetylene content x reaction bottle volume x standard ethylene peak area x sample volume x injection volume x incubation time[26].

This research method refers to the above principle. The Asaebes' glucose medium was prepared for actual operation, and 5 μL of the bacterial solution was pipetted and placed on the prepared medium. It was spot-distributed four times in a 37 ℃ constant temperature incubator for 7 days. The results were observed and photographed for record.

2.3.5 Determination of the ammonia production capacity of the strain

Transfer the strain to a test tube containing 10 mL of peptone solution (10 g/L). Cultivate at 28 ℃ for 2 days. Add 0.5 mL of Nessler's reagent to each tube.

If the color changes from brown to yellow, it indicates that ammonia is produced in the bacterial solution. The solution in the centrifuge tube that changes from brown to yellow is ammonia-producing, while the one that remains unchanged is not ammonia-producing. The determination of ammonia-producing ability refers to the method of Kang Yijun et al. [27].

2.3.6 Detection of phosphorus solubilization ability of salt-tolerant strains

Take 5 μL of the bacterial solution and spread it onto the prepared NBRIP phosphorus removal culture medium. Place four drops (up, down, left, and right) on

the medium, then put it in a 37 ℃ constant temperature incubator for 24 hours. Observe the colony morphology and take photos.

2.3.7 Detection of the plant-promoting effect of salt-tolerant bacteria

The strain was inoculated onto 20 mL LB medium for activation, and then incubated in a shaker at 37°C, with a speed of 180 r/min for 24 hours. After the bacterial solution became turbid, 3 mL of the solution was transferred to 200 mL liquid LB medium for further cultivation. After 24 hours, the OD value was measured using a spectrophotometer. If the OD value was between 0.6 and 0.9 nm, the bacterial solution was placed in a large centrifuge and centrifuged at 8000 r for 5 minutes, then the supernatant was discarded, and the precipitate was rinsed with water until 200 mL was obtained. Four plants with consistent growth were selected, and the control group CK was set without watering salt or bacteria, the groups H1 to H9 were set with watering bacteria, the group N was set with watering salt only, and the group NH was set with both watering salt and bacteria. Before the treatment, photos were taken.

Variables were set, and salt stress treatment was applied to the corn plants.

This included the measurement of fresh weight of the leaves. After digging out the corn seedlings and thoroughly washing them, the surface moisture of the corn seedlings was dried with absorbent paper, and their fresh weight was measured in the above-ground and underground parts. The corn plants were placed in an oven preheated to 105 ℃ for 15 minutes to kill them, then the oven was adjusted to 60 ℃ and dried until a constant weight was reached to measure their dry weight. After measuring the fresh weight and dry weight, the results were recorded.

2.4 Measurement indicators and methods

2.4.1 Determination of sodium and potassium ion content

The leaves of corn were dried at 70℃, ground using a plant grinder and sieved. 1 g was placed in a 250 mL conical flask, and 30 mL of a mixed acid solution of nitric

acid and perchloric acid (5:1) was added. The bottle mouth was placed in a bent neck funnel, and it was left overnight. The next day, it was placed in an adjustable electric furnace in the fume hood to boil slightly, and a large amount of brown NO2 was released. Without brown gas, the temperature in the furnace was increased, and SiO2 was dehydrated into white smoke. After cooling, 20 mL of deionized water was added, and it was filtered into a 250 mL capacity bottle using quantitative filter paper. The conical flask and filter residue were washed with 1% hot hydrochloric acid solution until the reaction of Fe3+ ended. Finally, the solution was made up to the volume with deionized water and mixed evenly to form the test solution. Standard working solution series: Take 5 capacity bottles, add 0.1, 0.2, 0.4, 0.6, and 0.8 mL of 100 μg/mL sodium standard solution and 1.0,

2.0, 3.0, 4.0, and 5.0 mL of 100 μg/mL potassium standard solution respectively, then add 2 mL of 5% CsCl solution to each of the 5 bottles, and then make up with deionized water. Take 2-10 mL of the test solution and the blank, respectively, and place them in 100 mL capacity bottles, then add 2 mL of 5% CsCl solution and make up with deionized water. Use an atomic absorption spectrophotometer to measure sodium and potassium at 766.5 nm and 589.0 nm, input the standard concentrations into the equipment, and read the concentration values.

2.4.2 Detection of superoxide dismutase (SOD) activity in plants

Superoxide Dismutase (SOD) can catalyze the disproportionation reaction of superoxide anions, generating hydrogen peroxide (H_2O_2) and oxygen (O_2), and is an important antioxidant enzyme in biological systems.

The Cytochrome C method is also one of the commonly used means for SOD activity detection. However, cytochrome C itself has a high oxidation activity and is easily interfered by reducing agents in the sample. In addition, this method requires continuous determination of absorbance values, which not only has low sensitivity for SOD detection, but also has limitations in the detection of large sample quantities, making it difficult to meet the requirements of large-scale detection.

Currently, the more advanced methods for SOD activity measurement include the WST-1 method and the WST-8 method. Compared with the WST-1 method, the WST-8 method performs better in stability and sensitivity. This method can detect superoxide dismutase with a concentration as low as 0.5 Ug/mL.

WST-8 can react with the superoxide anion O2- produced by xanthine oxidase (XO) catalysis, generating water-soluble methafulvin dye (formazan dye). Since SOD can catalyze the disproportionation reaction of superoxide anions, the above reaction process can be inhibited by SOD. Therefore, the activity of SOD is negatively correlated with the amount of methafulvin dye generated. Based on this principle, the colorimetric analysis of the reaction product of WST-8 can accurately calculate the enzymatic activity of SOD.

The formula for calculating the enzymatic activity of SOD is as follows: the unit of SOD enzymatic activity in the sample = The unit of SOD enzymatic activity in the detection system = Inhibition percentage / (1 - inhibition percentage) units. For example, when the inhibition percentage is 50%, the unit of SOD enzymatic activity in the sample = 50% / (1 - 50%) units = 1 unit; when the inhibition percentage is 60%, the unit of SOD enzymatic activity in the sample = 60% / (1 - 60%) units = 1.5 units.

2.4.3 Detection of plant catalase (CAT) activity

The metabolites of flavin oxidase in plants usually contain hydrogen peroxide, such as the acetoacetic acid oxidase in photorespiration and the glucose oxidase in respiration. Excessive accumulation of hydrogen peroxide can lead to destructive oxidation of the cells. Catalase (CAT) and peroxidase (POD) are important protective enzymes that remove H_2O_2. They can break down H_2O_2 into O_2 and H2O, thereby protecting the human body from the harmful effects of H_2O_2. Their activity is closely related to stress resistance.

The principle of the experiment for determining catalase (CAT): (CAT) catalyzes the following reactions:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2 \qquad H_2O_2 \rightarrow 2\ H_2O + O_2.$$

In this experiment, the activity of CAT is determined by measuring the reduction of H_2O_2. In the presence of H_2O_2, peroxidase can oxidize croton lactone to produce brown 4-aminophenol. Activity can be determined by measuring the content of the product using a spectrophotometer.

Preparation of enzyme solution: Weigh 0.25 g of leaves, add 5 times the volume of pH 7.0 (M/V) PBS, and grind at 15,000 r/min on an ice bath. Centrifuge for 15 minutes to obtain a portion of the supernatant and appropriately dilute it for enzyme activity determination. Determination of CAT activity: In a 3 ml reaction system, add 0.3 mL of H_2O_2, 1 mL of H_2O_2, and finally 0.05 mL of enzyme solution. Start the reaction and measure the decrease rate of POD at a wavelength of 240 nm.

2.4.4 Detection of plant peroxidase (POD) activity

In a 3-milliliter reaction system, 1 milliliter of 0.3% H_2O_2, 0.95 milliliters of 0.2% Guaiacol, and 1 milliliter of pH 7.0 PBS were added. Finally, 0.05 milliliter of the enzyme solution was added to start the reaction, and the increase rate of POD at 470 nanometers was recorded. An increase of 0.01 per minute of POD is defined as 1 unit of activity.

2.4.5 Determination of Sodium and Potassium Ion Contents in Plant Leaves

This study employed atomic absorption spectroscopy to determine the content levels of Na^+ and K^+ in the leaf tissues of corn samples. During the specific operation, corn leaf samples from different treatment groups were obtained, including the control group CK (without any treatment), groups 1-9 with individual treatments, the group that only received NaCl treatment, and the group that received 1-9 treatments combined with sodium chloride. The collected corn leaf samples were placed in an 80 ℃ oven for drying for 3 days to remove moisture.

After drying, concentrated HNO3 was added to the samples, and a high-temperature of 550 ℃ was used to perform the ashing operation on the samples to

decompose organic substances and retain only inorganic components.

After the ashing process, the ashing products were transferred to a 25 mL volumetric flask and made up to the calibration line with distilled water. Finally, the sample solution after filling was measured again using atomic absorption spectroscopy to analyze the specific contents of Na^+ and K^+.

2.5 Technical roadmap

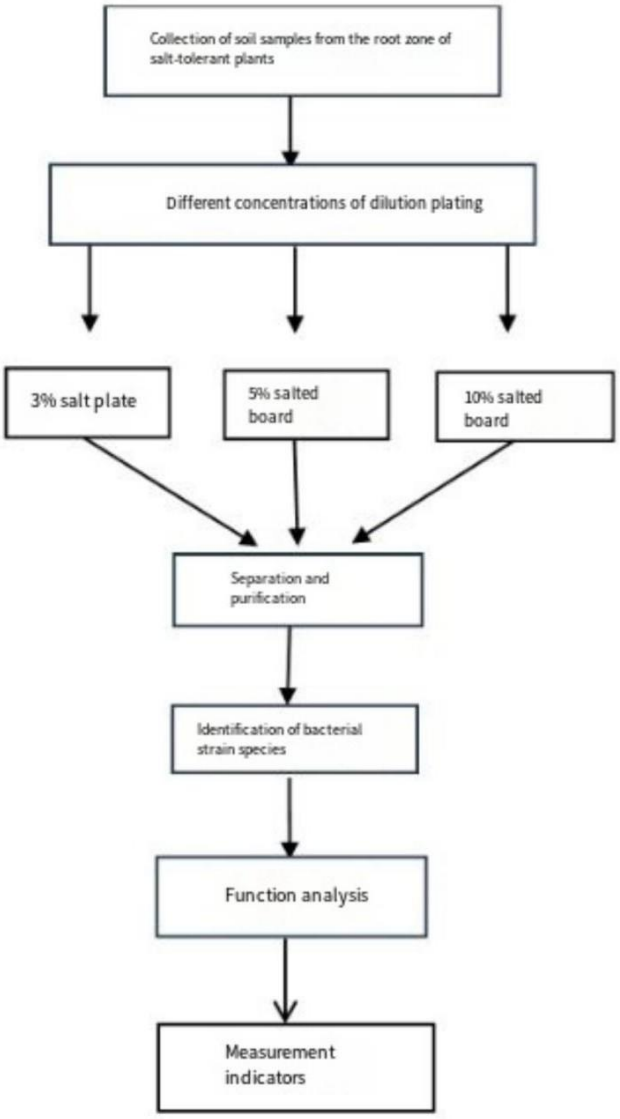

Figure 2.1 Technical Route

Summary of the chapter II

Clarify the materials methods required for the research:

1. Where to obtain the soil samples.

2. How to select the strains

3. The reagents needed for preparing the culture medium.

4. The strains of the plants required.

5. The instruments and equipment needed in the laboratory.

6. Use the plate streaking method to isolate and purify single colonies of salt-tolerant bacterial strains.

7. Prepare NaCl plates of different concentrations to determine the highest salt tolerance of the strains.

8. Determine whether the strains have the ability to produce IAA by preparing a chromogenic agent.

9. Determine whether the strains have the nitrogen fixation ability by culturing them on the Azospirillum medium.

10. Determine whether the strains have the ability to produce ammonia by using specific reagents.

11. Determine whether the strains have the phosphorus solubilization ability by culturing them on the NBRIP medium.

12. Conduct plant experiments to determine the promoting effect.

13. Measure the peroxide indicators.

14. Draw the technical flowchart.

Chapter III
EXPERIMENTAL PART

3.1 Selection of salt-tolerant strains

After selective cultivation in different concentration gradients of NaCl medium, 1-9 salt-tolerant bacterial strains that could withstand 10% NaCl were screened out. After isolating and purifying the single bacterial colonies by the plate streaking method, the colony morphology is shown in the following Figure 3.1. Based on this result, it can be preliminarily determined that the colony morphology is a white, large, viscous, semi-transparent circular colony.

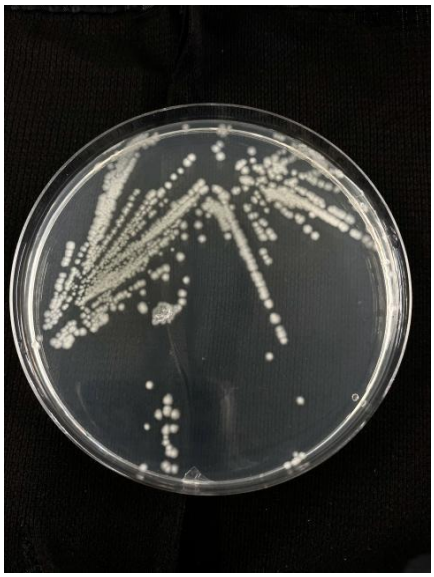

Figure 3.1 Colony morphology of strain 1-9

3.2 Determination of salt tolerance of strains 1-9

To determine whether the 1-9 strains are resistant to high concentrations of NaCl and can grow on high-concentration NaCl media, the 1-9 bacterial solutions were diluted at different concentrations (10-1, 10-2, 10-3, 10-4, 10-5, 10-6, 10-7) at gradient points and 5 μL was added to 13%, 14%, and 15% NaCl media. After incubation at a

constant temperature for 24 hours, only colonies grew on the 13% NaCl medium, as shown in Figure 3.2. This indicates that the 1-9 strains can tolerate up to 13% NaCl, and they cannot survive on 14% and 15% NaCl media.

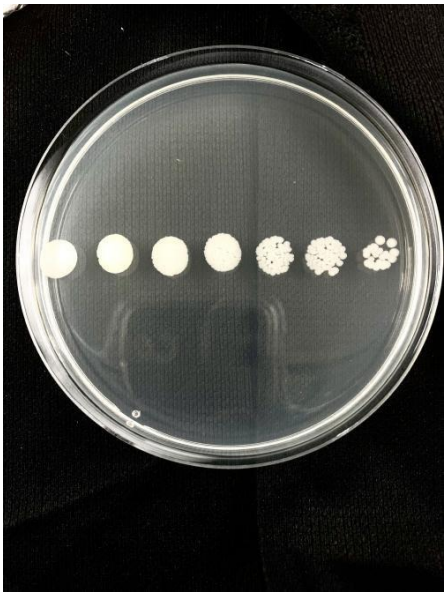

Figure 3.2 Growth conditions of bacteria 1-9 on a 13% NaCl medium

3.3 Determination of IAA Production Capacity by Strains 1-9

According to the above method, strains 1-9 were inoculated into LB medium (containing 100 mg/L L-tryptophan) and cultured in a shaking incubator at 37 ℃ with a speed of 180 r/min for 24 hours. 50 μL of the supernatant of the bacterial suspension after centrifugation at 8000 r/min was taken and 50 μL of Salkowski colorimetric solution was added. A white ceramic plate was placed at room

temperature and exposed to light for color development for 30 minutes. If a pink color appeared, it was positive, indicating that strains 1-9 could secrete IAA. The darker the color, the stronger the ability to secrete IAA. The results are shown in the Figure 3.3 below. This result indicates that strains 1-9 produce auxin and have a strong ability to secrete IAA. Theoretically, they have certain promoting growth capabilities.

Figure 3.3 Determination of IAA Production Capacity in 1-9 Strains

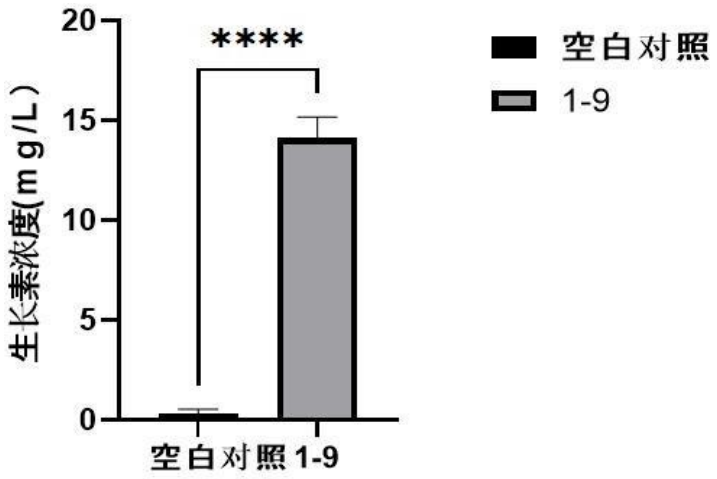

Figure 3.4 Determination of auxin production by bacteria 1-9

Note: Compared with the blank group, ** ** ** P < 0.0001

3.4 Determination of Nitrogen Fixation Capacity of Strains 1-9

The 1-9 strains were inoculated onto the Asubai glucose medium and cultured for 7 days. The results are shown in the Figure 3.5 below. On the Asubai medium, there appeared viscous, semi-transparent, and white-colored colonies. This result indicates that strains 1-9 have good nitrogen fixation capabilities, and theoretically, strains 1-9 may possess some plant-promoting effects

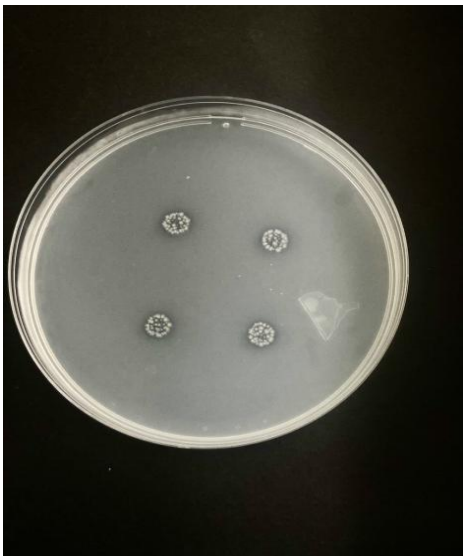

Figure 3.5 Growth chart of Aspergillus 1-9 in the culture medium

3.5 Determination of Ammonia Production Capacity of Strains 1-9

Transfer the 1-9 strains to 10 mL test tubes containing peptone solution (10 g/L), and incubate at 28 ℃ for 48 hours. Add 0.5 mL of Nessler's reagent to each tube. If the color changes from brown to yellow, it indicates the production of ammonia.

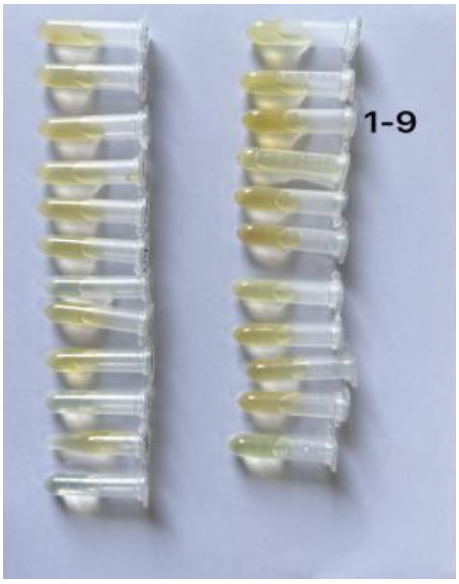

Figure 3.6 Ammonia production performance of bacteria 1-9

The results are shown in the figure below. The color in the 1-9 centrifuge tubes turned brown, which suggests that the 1-9 strains have a certain ability to produce ammonia and theoretically have a certain promoting effect.

3.6 Detection of phosphorus solubilization ability of salt-tolerant strains

Take 5 μL of the bacterial solution and spread it onto the prepared NBRIP phosphorus-removal culture medium. Place four drops, namely top, bottom, left and right, in a 37 ℃ constant temperature incubator for 24 hours. Observe the colony morphology and take photos. The results are shown in the figure below. Bacteria 1-9 can grow on the NBRIP medium and have certain phosphorus-removal capabilities. The experimental results indicate that bacteria 1-9 may have a certain degree of plant-promoting effect.

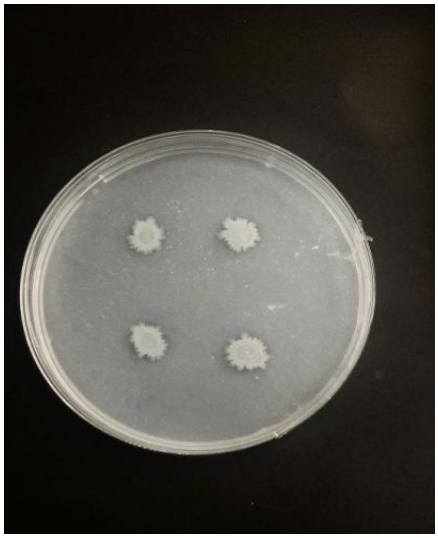

Figure 3.7 Phosphorus removal performance of bacteria 1-9

3.7 Comparison Results of 16S rDNA

The comparison results of 16S rDNA are shown in Figure 3.8.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Halomonas alkaliantarctica strain CRSS 16S ribosomal RNA, partial sequence	Halomonas alkaliantarctica	2510	2510	100%	0.0	98.94%	1518	NR_114902.1

Figure 3.8 Comparison results of 16S rDNA

3.8 Detection of Salt-Tolerant Promoting Effects of Plants

Based on the above findings, we can conclude that bacteria 1-9 may belong to a type of salt-tolerant plant-promoting bacteria. Through 16srDNA comparison, bacteria 1-9 belong to Halomonas alkaliantarctica. Therefore, we conducted plant-promoting and salt-tolerance experiments.

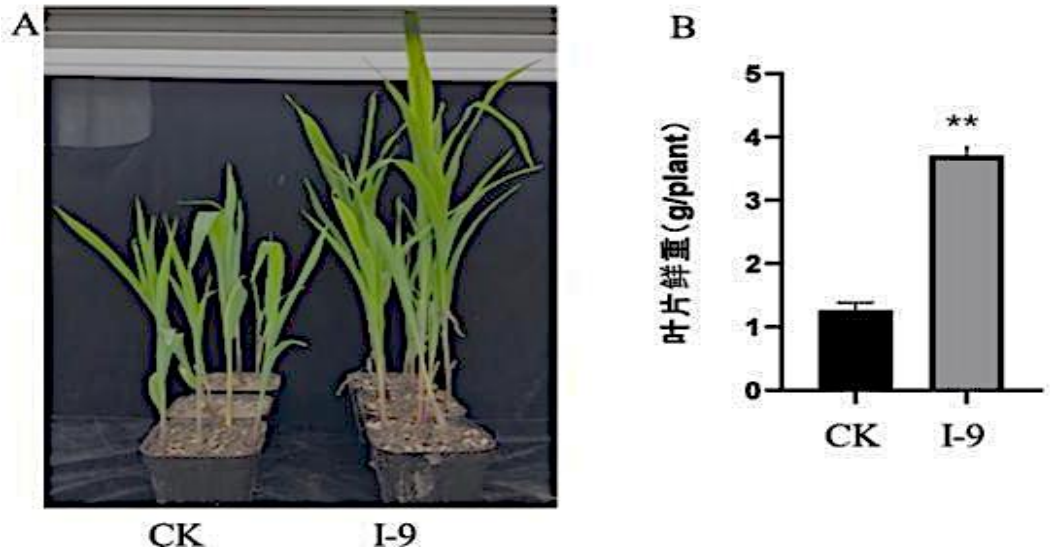

Figure 3.9 The effects of 1-9 treatment on the appearance of corn and the fresh weight of leaves

The experimental results show that under normal treatment, the growth of the treated group with bacteria was significantly better than that of the control group (CK

group). Under salt stress conditions, the plant type of the CK group became smaller, the stems became thinner, and the leaves wilted. The NH group treated with both bacteria and salt showed larger and thicker stems and leaves after 13 days of salt treatment, as shown in Figure 3.9. The growth condition was significantly better than that of the CK group. This indicates that the plants treated with bacteria 1-9 exhibited obvious salt-tolerance under salt stress, effectively reversing the restricted growth state of the plants after salt treatment.

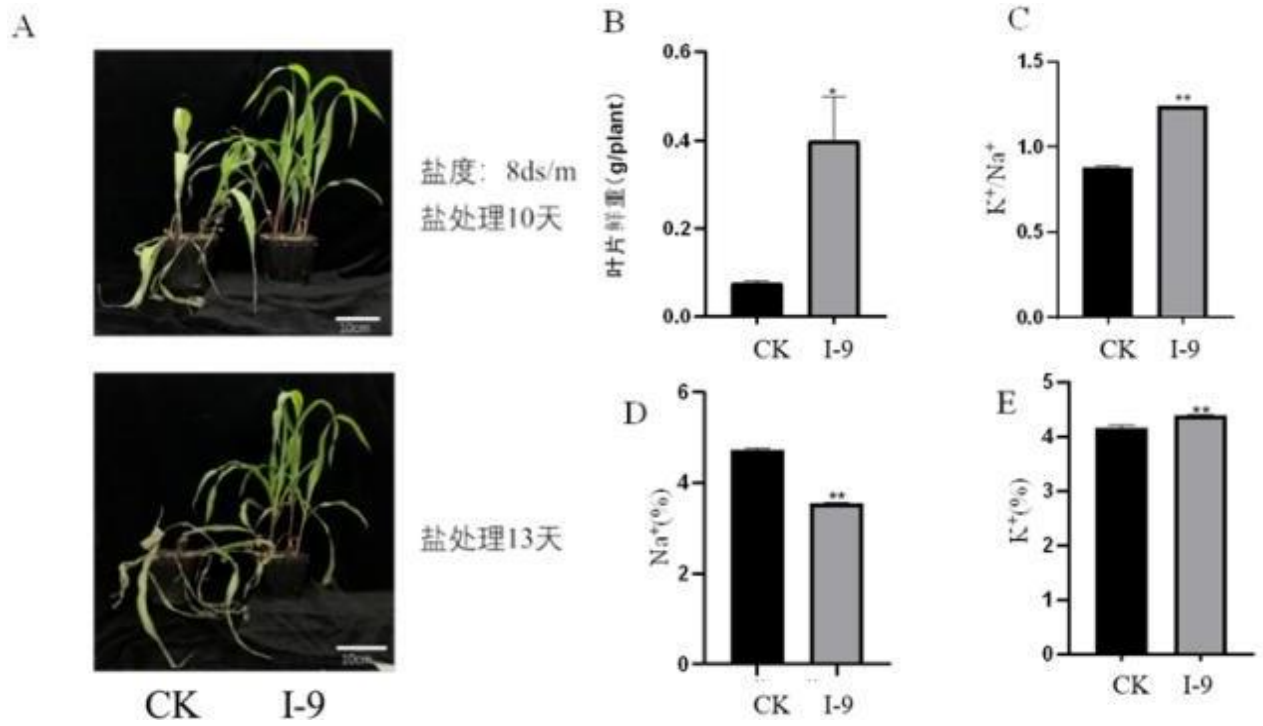

Figure 3.10 Growth status after salt stress treatment

Note: A represents the changes in plants before and after salt treatment, B represents the comparison of fresh weight of leaves after treatment, C represents the comparison of potassium-sodium ratio of leaves after treatment, D represents the comparison of sodium ion content of leaves after treatment, E represents the comparison of potassium ion content of leaves after treatment, * indicates $P < 0.05$, ** indicates $P < 0.01$.

From Figure 3.8, it can be concluded that salt stress significantly inhibited the accumulation of dry weight and fresh weight of corn. In the salt stress treatment group, the fresh weight of corn seedlings decreased by 83% compared to the blank group, and the dry weight decreased by 46% compared to the blank group. However, it is worth noting that the corn plants inoculated with strain 1-9 showed a significant promoting effect under salt stress. Under normal conditions, the fresh weight of the strain 1-9 treatment group significantly increased, compared to the blank control group and the salt treatment group, it increased by 53% and 93% respectively; Similarly, under salt stress conditions, the fresh weight of the corn plants treated with strain 1-9 was 56% higher than the salt treatment group. In terms of dry weight, under normal conditions, the aboveground dry weight of the strain 1-9 treatment group was 47% higher than the blank control group and 75% higher than the salt treatment group; and under salt stress conditions, the strain 1-9 treatment group was also able to reverse the insufficient dry weight accumulation of corn, which was 29% higher than the salt treatment group.

At the same time, it can also be seen that strain 1-9 reduced the Na+ content in plant leaves, increased the K^+ content, thereby increasing the potassium-to-sodium ratio and improving the damage of Na^+ to plants.

3.9 Measurement Indicators

3.9.1 Detection Results of Plant Superoxide Dismutase (SOD) Activity

As the results of the above experiments show, under salt stress conditions, the salt treatment group had significantly lower superoxide dismutase (SOD) activity compared to the blank group. Under normal treatment conditions, the SOD expression levels of strain 1-9 treatment groups were significantly lower than those of the CK group. After measurement, it was found that they were reduced by 65%. This might be because strain 1-9 reduced the generation of free radicals in the cells of the corn plants, thereby reducing the demand of the corn plants for SOD. Under salt stress conditions, the 1-9 treatment could reverse the decline in SOD expression levels under salt stress.

Compared to the salt stress treatment, the SOD expression level of the 1-9 treatment increased by 33%. This indicates that strain 1-9 may enhance the adaptability of the corn plants to salt stress through specific physiological mechanisms, such as reducing cellular oxidative damage or strengthening antioxidant defense mechanisms.

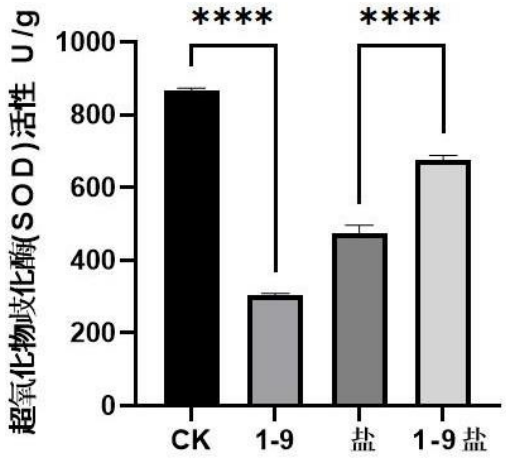

Figure 3.9 SOD Test Results

3.9.2 Detection Results of Plant Catalase (CAT) Activity

As shown in the above experimental results, under salt stress conditions, the salt treatment group had significantly lower catalase (CAT) activity compared to the blank group. Under normal treatment conditions, the SOD expression level of strain 1-9 treatment groups was significantly lower than that of the CK group, and the measurement showed a 17% decrease. This might be due to the fact that strain 1-9 reduced the generation of free radicals in the corn plant cells through corresponding mechanisms [28], thereby reducing the demand of the corn plant for CAT.

Under salt stress conditions, the 1-9 treatment could reverse the decline in CAT expression level caused by salt stress. Compared to the salt stress treatment, the SOD expression level of the 1-9 treatment increased by 25%. This indicates that strain 1-9 may enhance the adaptability of the corn plant to salt stress through specific physiological mechanisms, such as reducing cellular oxidative damage or strengthening antioxidant defense mechanisms.

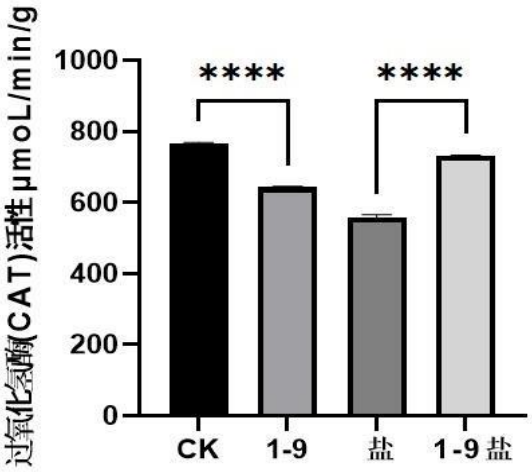

Figure 3.10 CAT test results

3.9.3 Detection Results of Plant Peroxidase (POD) Activity

As the results of the above experiments show, under salt stress conditions, the salt treatment group had significantly lower peroxidase (POD) activity compared to the blank group. Unlike the determination of SOD and CAT indicators, the expression level of POD in strain 1-9 treatment groups significantly increased.

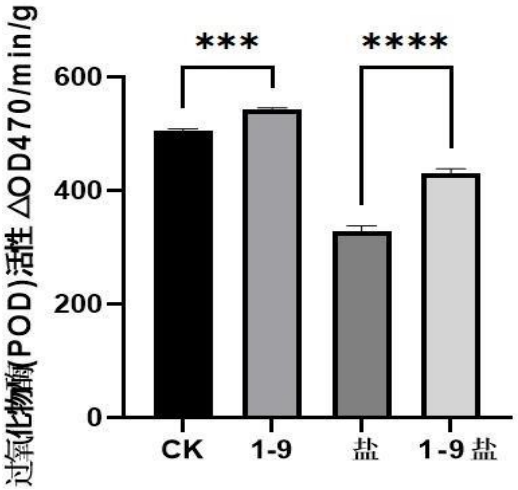

Figure 3.11 POD Test Results

After measurement, it was found that it increased by 8% compared to the salt stress treatment group, indicating that this strain may achieve the purpose of protecting cells from oxidative damage by effectively removing H_2O_2 within the cells. These results all indicate that strains 1-9 may enhance their adaptability to salt stress by regulating the plant's antioxidant enzyme system [29].

Summary of chapter III

It has been experimentally established that:

1. Strain 1-9 has the highest tolerance to 13% NaCl.
2. Strain 1-9 has the ability to produce IAA.
3. Strain 1-9 has the ability to produce ammonia.
4. Strain 1-9 has the ability to fix nitrogen.
5. Strain 1-9 has the ability to dissolve phosphorus.
6. Strain 1-9 has a beneficial effect on plants.

CONCLUSIONS

This study used the dilution plating plate method to isolate microorganisms from the rhizosphere soil of salt-tolerant plants. Through a high-concentration NaCl selective medium, 1-9 salt-tolerant strains were screened out.[41]Using the plate streaking method, the strains 1-9 were isolated and purified into single colonies[42], which were then preserved and sequenced for 16S rRNA[43] to determine the species of the bacteria[44]. After that, different concentrations of high-concentration NaCl media were used to determine the highest salt tolerance of strains 1-9. By preparing various functional media for selection and screening, it was determined whether strains 1-9 had the ability to produce IAA, fix nitrogen, dissolve phosphorus, or produce ammonia. This theoretically proved that they had a promoting effect.

When verifying the plant promoting effect, this study used the soil culture method to divide the uniformly growing corn seedlings into the CK group and the 1-9 group for bacterial inoculation to verify the promoting effect of strains 1-9. The results showed that strains 1-9 exhibited significant promoting growth effects on corn plants under normal conditions. At the same time, the promoting effect of strains 1-9 under salt stress conditions was tested[45]. The experimental results showed that the corn plants irrigated with the 1-9 bacterial solution exhibited good promoting effects. Under salt stress conditions, it significantly promoted the growth of corn plants.

Through the detection of plant salt-tolerant promoting effects, the fresh weight of the leaves of corn plants inoculated with strains 1-9 was significantly increased compared to the CK group. Under salt stress conditions, the plant type of the CK group became smaller, the stem became thinner, and the leaves wilted. The NH in the group irrigated with 1-9 bacteria and salt showed larger and thicker stems and leaves after 13 days of salt treatment, and the growth condition was significantly better than that of the CK group. This indicates that the plants treated with strains 1-9 showed obvious salt tolerance under salt stress, effectively reversing the growth restriction state of the

plants after salt stress.

Analysis of antioxidant enzyme activity showed that the treatment of strains 1-9 could reverse the decline in antioxidant enzyme activity under salt stress, indicating that strains 1-9 may enhance the stress resistance of corn by regulating the antioxidant enzyme system.[46]

In conclusion, strains 1-9 have significant plant promoting effects, and under salt stress, they show obvious salt tolerance[47,] which has a certain promoting effect on the growth and stress resistance of corn under salt stress, which is beneficial to the growth of plants under salt stress[48].This conclusion indicates that this study provides a theoretical basis for studying the promoting ability of strains under various environmental conditions limitations such as salt stress, and provides a new and efficient way to solve salinized land through the next step of developing microbial agents and improving saline-alkali land[49].

In addition, most of the current research still remains at the theoretical level and lacks the practical application effect of crops in saline-alkali land.20This study plans to further explore the application of strains 1-9 in certain-scale saline-alkali land crop production under different environmental conditions[50], obtain the actual data for increasing yield, and thereby more deeply solve the problem of crop yield reduction caused by soil salinization, laying a foundation for large-scale production and safeguarding national food security.

1. Where to obtain the soil samples
2. How to select the strains
3. The reagents needed for preparing the culture medium
4. The strains of the plants required
5. The instruments and equipment needed in the laboratory
6. Use the plate streaking method to isolate and purify single colonies of salt-tolerant bacterial strains.

7. Prepare NaCl plates of different concentrations to determine the highest salt tolerance of the strains.
8. Determine whether the strains have the ability to produce IAA by preparing a chromogenic agent.
9. Determine whether the strains have the nitrogen fixation ability by culturing them on the Azospirillum medium.
10. Determine whether the strains have the ability to produce ammonia by using specific reagents.
11. Determine whether the strains have the phosphorus solubilization ability by culturing them on the NBRIP medium.
12. Conduct plant experiments to determine the promoting effect.
13. Measure the peroxide indicators.
14. Draw the technical flowchart.
15. Strain 1-9 has the highest tolerance to 13% NaCl.
16. Strain 1-9 has the ability to produce IAA.
17. Strain 1-9 has the ability to produce ammonia.
18. Strain 1-9 has the ability to fix nitrogen.
19. Strain 1-9 has the ability to dissolve phosphorus.
20. Strain 1-9 has a beneficial effect on plants.
21. The selected strains have a significant promoting effect.
22. Salt stress enables corn plants to acquire adaptability.
23. By regulating the antioxidant enzyme system, the resistance of corn is enhanced.
24. This research still lacks the practical application effect of crops in saline-alkali land and remains at the theoretical level.

REFERENCES

1 Bhardwaj S, Badiyal A, Dhiman S, et al. Exploring Halophiles for Reclamation of Saline Soils: Biotechnological Interventions for Sustainable Agriculture [J]. J Basic Microbiol, 2025: e70048.

2 Meng W, Zhou z, Tan M, et al. Integrated Analysis of Meta transcriptome and Amplicon Sequencing to Reveal Distinctive Rhizospheric Microorganisms of Salt-Tolerant Rice [J]. Plants (Basel), 2024, 14(1).

3 Liang M, Wu Y, Jiang Y, et al. Microbial functional genes play crucial roles in enhancing soil nutrient availability of halophyte rhizospheres in salinized grasslands [J]. Sci Total Environ, 2025, 958: 178160.

4 Alswat A S, Alharthy O M, Alzahrani S S, et al. Halophilic Pectinase-Producing Bacteria from Arthrocnemum macrostachyum Rhizosphere: Potential for Fruit-Vegetable Juice Processing [J]. Microorganisms, 2024, 12(11).

5 Zhang Y, Wang H, Zhang X, et al. Effects of salt stress on the rhizosphere soil microbial communities of Suaeda salsa (L.) Pall. in the Yellow River Delta [J]. Ecol Evol, 2024, 14(9): e70315.

6 Khoso M A, Wang M, Zhou Z, et al. Bacillus altitudinous AD13-4 Enhances Saline-Alkali Stress Tolerance of Alfalfa and Affects Composition of Rhizosphere Soil Microbial Community [J]. Int J Mol Sci, 2024, 25(11).

7 Gao X, Wang S, Kong W, et al. Floristic changes and environmental drivers of soil fungi and archaea in different salt-tolerant plant communities in the intertidal habitat of coastal wetlands [J]. Environ Geochem Health, 2024, 46(5): 167.

8 Meinzer M, Ahmad N, Nielsen B L. Halophilic Plant-Associated Bacteria with Plant-Growth-Promoting Potential [J]. Microorganisms, 2023, 11(12).

9 Goswami S K, Kashyap A S, Kumar R, et al. Harnessing Rhizospheric Microbes for Eco-friendly and Sustainable Crop Production in Saline Environments [J]. Curr Microbiol, 2023, 81(1): 14.

10 Sharma A, Chakdar H, Vaishnav A, et al. Multifarious Plant Growth-Promoting Rhizobacterium Enterobacter sp. CM94-Mediated Systemic Tolerance and Growth Promotion of Chickpea (Cicer arietinum L.) under Salinity Stress [J]. Front Biosci (Landmark Ed), 2023, 28(10): 241.

11 Han D, Zhang D, Han D, et al. Effects of salt stress on soil enzyme activities and rhizosphere microbial structure in salt-tolerant and -sensitive soybean [J]. Sci Rep, 2023, 13(1): 17057.

12 Wang Y, Hu C, Wang X, et al. Selenium-induced rhizosphere microorganisms endow salt-sensitive soybeans with salt tolerance [J]. Environ Res, 2023, 236(Pt 2): 116827.

13 Colette M, Guentas L, Patrona L D, et al. Dynamic of active microbial diversity in rhizosphere sediments of halophytes used for

bioremediation of earthen shrimp ponds [J]. Environ Microbiome, 2023, 18(1): 58.

14 Hui Q, Li S, Chen Z, et al. Inoculation with Salt-Tolerant Rhizobia and AMF Regulates K ($^{+}$)/Na ($^{+}$) Homeostasis in Robinia pseudo acacia L. Alleviating Salinity [J]. Plant Cell Environ, 2025.

15 Guo L, Zhang X, Liu Y, et al. Salt-alkali-tolerant growth-promoting Streptomyces sp. Jrh8-9 enhances alfalfa growth and resilience under saline-alkali stress through integrated modulation of photosynthesis, antioxidant defense, and hormone signaling [J]. Microbiol Res, 2025, 296: 128158.

16 Flores Clavo R, Suclupe-Campos D O, Castillo Rivadeneira L, et al. Harnessing PGPRs from Asparagus officinalis to Increase the Growth and Yield of Zea mays L [J]. Microb Ecol, 2025, 87(1): 174.

17 PENG M, JIANG Z, ZHOU F, et al. From salty to thriving: plant growth promoting bacteria as nature's allies in overcoming salinity stress in plants [J]. Front Microbiol, 2023, 14: 1169809.

18 Shahid M, Singh U B, Khan M S, et al. Bacterial ACC deaminase: Insights into enzymology, biochemistry, genetics, and potential role in amelioration of environmental stress in crop plants [J]. Front Microbiol, 2023, 14: 113277

19 Shahid M, Zeyad M T, Syed A, et al. Stress-Tolerant Endophytic Isolate Prestia Aryabhata BPR-9 Modulates Physio-Biochemical Mechanisms in Wheat (Triticum aestivum L.) for Enhanced Salt Tolerance [J]. Int J Environ Res Public Health, 2022, 19(17).

20 Wei H, He W, Li Z, et al. Salt-tolerant endophytic bacterium Enterobacter ludwigii B30 enhance bermudagrass growth under salt stress by modulating plant physiology and changing rhizosphere and root bacterial community [J]. Front Plant Sci, 2022, 13: 959427.

21 Chauhan J, Gohel S. Exploring plant growth-promoting, biocatalytic, and antimicrobial potential of salt tolerant rhizospheric Georgenia soli strain TSm39 for sustainable agriculture [J]. Braz J Microbiol, 2022, 53(4): 1817-28. 22 Y ang S, Hao X, Xu Y , et al. Meta-Analysis of the Effect of Saline-Alkali Land Improvement and Utilization on Soil Organic Carbon [J]. Life (Basel), 2022, 12(11).

23 Niu Y, Li X, Xu C, et al. Analysis of drought and salt-alkali tolerance in tobacco by overexpressing WRKY 39 gene from Populus trichocarpa [J]. Plant Signal Behave, 2021, 16(7): 1918885.

24 Zhao H, Chang J, Miao Z, et al. Application of coal-based solid waste artificial soil in the restoration of saline alkali land-taking the saline alkali land restoration of the Emao River in China as an example [J]. Heliyon, 2024, 10(17): e37095.

25 Pan Y, She D, Shi z, et al. Salinity and high pH reduce denitrification rates by inhibiting denitrifying gene abundance in a saline-alkali soil [J]. Sci Rep,

2023, 13(1): 2155.

26 Dong F, Wang Y, Tao J, et al. Arbuscular mycorrhizal fungi affect the expression of PxNHX gene family, improve photosynthesis and promote Populus simonii×P. nigra growth under saline-alkali stress [J]. Front Plant Sci, 2023, 14: 1104095.

27 Feng G, Wu Y, Yang C, et al. Effects of coastal saline-alkali soil on rhizosphere microbial community and crop yield of cotton at different growth stages [J]. Front Microbiol, 2024, 15: 1359698.

28 Tang H, Du L, Xia C, et al. Bridging gaps and seeding futures: A synthesis of soil salinization and the role of plant-soil interactions under climate change [J]. Science, 2024, 27(9): 110804.

29 Cui Y, Ning Z, Li M, et al. Microbial network-driven remediation of saline-alkali soils by salt-tolerant plants [J]. Front Microbiol, 2025, 16: 1565399.

30 Jain A, Sarsaiya S, Singh R, et al. Omics approaches in understanding the benefits of plant-microbe interactions [J]. Front Microbiol, 2024, 15: 1391059.

31 Xie J, Zhu H, Zhang L, et al. The alleviating effect of salt-tolerant endophytic bacteria in plant species belts on the salt stress of Festuca palustris [J]. Grassland and Turf: 1-14.

32 Nguyen N K, Nguyen P M, Chau A T T, et al. Long-term changes in soil biological activity and other properties of raised beds in Longan orchards [J]. Peer J, 2024, 12: e18396.

33 Sun Y, Wang Z, Liu Y, et al. Identification of the extracellular polysaccharide biosynthesis gene cluster of plant lactobacillus WCFS1 and functional study on differentially expressed genes in its transcriptome [J]. Food Industry Science and Technology: 1-21.

34 Barrón-Sandoval A, Martiny J B H, Pérez-Carbajal T, et al. Functional significance of microbial diversity in arid soils: biological soil

crusts and nitrogen fixation as a model system [J]. FEMS Microbiol Ecol, 2023, 99(2).

35 Wu X, Rensing C, Han D, et al. Genome-Resolved Metagenomics Reveals Distinct Phosphorus Acquisition Strategies between Soil Microbiomes [J]. mSystems, 2022, 7(1): e0110721.

36 Zheng Y, Xu Z, Liu H, et al. Patterns in the Microbial Community of Salt-Tolerant Plants and the Functional Genes Associated with Salt Stress Alleviation [J]. Microbiol Spectra, 2021, 9(2): e0076721.

37 Abdelaziz M E, Abdelsattar M, Abdeldaym E A, et al. Piriformospora indica alters Na+/K+ homeostasis, antioxidant enzymes and LeNHX1 expression of greenhouse tomato grown under salt stress [J]. Scientia Horticulturae, 2019, 256: 108532.

38 Yasmin H, Naeem S, Bakhtawar M, et al. Halotolerant rhizobacteria Pseudomonas pseudo alcaligenes and Bacillus subtilis mediate systemic tolerance in hydroponically grown soybean (Glycine max L.) against salinity stress [J]. PLOS ONE, 2020, 15(4): e0231348.

39 Yuan Z, Labbé J, et al. Specialized Microbiome of a Halophyte and its Role in Helping Non-Host Plants to Withstand Salinity [J]. Scientific Reports, 2016, 6(1): 32467.

40 Qin Y, Pan X, et al. Microbially Mediated Plant Salt Tolerance and Microbiome-based Solutions for Saline Agriculture [J]. Biotechnology Advances, 2016, 34(7): 1245-59.

41 Li Y, Li X, Liu Y, et al. Genetic diversity and community structure of rhizobia modulating Sesbania cannabina in saline–alkaline soils [J]. Systematic and Applied Microbiology, 2016, 39(3): 195-202.

42 Ahsan T, Tian P C, Gao J, et al. Effects of microbial agent and microbial fertilizer input on soil microbial community structure and diversity in a peanut continuous cropping system [J]. J Adv Res, 2024, 64: 1-13.

43 Graham P H, Vance C P. Legumes: Importance and Constraints to Greater Use [J]. Plant Physiology, 2003, 131(3): 872-7.
44 Warembourg F R, Roumet C, Lafont F. Differences in rhizosphere carbon-partitioning among plant species of different families [J]. Plant and Soil, 2003, 256(2): 347-57.
45 Zheng Y, Liang J, Zhao D-L, et al. The Root Nodule Microbiome of Cultivated and Wild Halophytic Legumes Showed Similar Diversity but Distinct Community Structure in Yellow River Delta Saline Soils [J]. Microorganisms, 2020, 8(2): 207.
46 Chen D, Rui K, Zeng T, et al. Isolation, Identification and Function Evaluation of a Bacillus amyloliquefaciens with IAA Production Capacity [J]. Tropical Agriculture in China, 2021, (03): 50-8.
47 Cheng M, Cheng M, Wang Z, et al. Synergistic composite filler of different salt-tolerant plants for enhancing the purification of saline wastewater by artificial wetlands [J]. Environmental Engineering, 2021, 39(08): 7-14.
48 Kang Y, Shen M, Wang H, et al. Root-associated microbial communities and the diversity of symbiotic bacteria and their screening strategies [J]. Hubei Agricultural Sciences, 2012, 51(24): 5553-5558.
49 Samari E, Sharifi M, Karimi F, et al. Regulation of lignan biosynthesis through signaling pathways mediated by $H(_2)O(_2)$ in Linum album cell culture in response to exogenous putrescine [J]. Sci Rep, 2025, 15(1): 13553.
50 Zhang Y, Liu S, Liang X, et al. GmFER1, a soybean ferritin, enhances tolerance to salt stress and root rot disease and improves soybean yield [J]. Plant Biotechnol J, 2025.