Diversity Analysis of Rhizosphere Soil Microbial Population Structure and Screening and Identification of Dominant Salt-Tolerant Strains of Juncao"oasis no. 1"

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Abstract

Purpose: As a newly cultivated grass species widely used in saline-alkali soil treatment, Juncao"Oasis No.1" can effectively enhance the microbial population abundance in saline-alkali soil. It has a good effect on improving saline-alkali soil.

Methods: In order to further study the microbial community in the rhizosphere soil of Juncao"Oasis No.1", the dominant strains with important ecological value were screened out. In this paper, soil samples were collected from saline-alkali soil planted with Juncao"Oasis No. 1" and unplanted Juncao"Oasis No. 1", and the microbial populations were sequenced by 16SrDNA high-throughput sequencing, in order to identify the difference of microbial population, the dominant bacteria in rhizosphere soil of Juncao"Oasis No. 1" was selected, and the dominant bacteria with important ecological value were isolated and cultured and identified by Colony PCR.

Results: The results showed that Pseudomonas fluorescens and Bacillus thuringiensis. were the dominant strains with important ecological value in rhizosphere soil of Juncao"Oasis No.1". After isolation and culture, Colony PCR identification showed that the dominant strain was successfully isolated and cultured.

Conclusions: In this paper, the dominant strains with important ecological value in rhizosphere soil of Juncao”Oasis No. 1” were screened and cultured, which provided theoretical basis for the improvement of saline-alkali soil by using Juncao”Oasis No. 1”, it is of great guiding significance.

Keywords: Juncao”Oasis No.1”, 16srdna High-Throughput Sequencing, Rhizosphere Soil Microorganism, Pseudomonas Flou-rescens, Bacillus Thuringiensis, Isolation and Culture, Colony PCR.

Introduction

JunCao refers to a new type of herbaceous plant that can be used for cultivating edible and medicinal fungi after systematic breeding by the three-stage systematic screening method of cultivated edible and medicinal fungi [1]. JunCao technology was invented in 1987 by Lin Zhanxi, a researcher of mycelia research Institute of Fujian Agriculture and Forestry University. JunCao technology was originally used to replace trees to grow edible and medicinal fungi [2]. That is, by using fungus instead of trees as the substrate for cultivating edible and medicinal fungi, the prominent "fungus forest contradiction" caused by massive felling of trees for cultivation of edible and medicinal fungi was alleviated.

After the invention of the JunCao technology, it immediately attracted the attention of THE United Nations Development Program and FAO. In 1994, it was listed as a "Priority Cooperation Project between China and Other Developing Countries" by the United Nations Development Program, and in 2017, it was listed as a key project of the United Nations Peace Development Fund and promoted to the world. Contribute Chinese wisdom and Chinese plan to the international poverty reduction cause. With the development of The Times, the JunCao technology has been widely used in the field of ecological management, playing an important role in the process of soil salinization control.

JunCao"Oasis No.1", a new grass species selected and cultivated by the fungus technology in recent years, has been widely used in soil salinization control. From 2013 to 2017, Fujian Agriculture and Forestry University National JunCao Engineering Technology Center has carried out experimental studies on planting JunCao"Oasis No. 1" to treat saline-alkali land in several regions of China, and achieved ideal treatment results.

Relevant research data show that the JunCao"Oasis No.1" has developed root system, rapid growth and is rich in various crude
proteins, which has a quick and good effect in the treatment of saline-alkali land. Most importantly, planting JunCao"Oasis No. 1" can significantly increase the number of soil microorganisms, thus improving the population structure of soil microorganisms, fundamentally improving the soil physical and chemical properties of saline-alkali land, reducing the salinity of saline-alkali soil, and effectively restoring the soil fertility of saline-alkali soil [1]. This is also the core mechanism of JunCao "Oasis No.1" to improve saline-alkali soil.

With the development of modern molecular biology technology, high-throughput sequencing technology plays an important role in the detection and identification of microbial populations, among which 16SrDNA high-throughput sequencing technology is the most widely used in the identification of microbial populations due to its good specificity [3]. 16SrDNA is located on the small ribosomal subunit of prokaryotic cells, including 10 Conserved Regions and 9 Hypervariable Regions [4, 5]. There is little difference between bacteria in Conserved Regions, and the Hypervariable Regions are genus or species specific [6]. There are certain differences with the different genetic relationship. Therefore (Luan et al, 2020), 16SrDNA can be used to reveal the characteristic nucleic acid sequence of biological species, and is considered to be the most suitable indicator for bacterial phylogeny and taxonomic identification [7, 8]. 16SrDNA Amplicon Sequencing refers to the selection of one or several mutated regions, the design of universal primers for PCR amplification using the conserved regions, and then the Sequencing analysis and species identification of the highly mutated regions [9]. 16SrDNA amplicon sequencing has become an important means to study the composition and structure of microbial communities in environmental samples [10].

Meanwhile, colony PCR has been widely used in the identification of positive clones [11]. Colony PCR directly uses the DNA exposed after thermal hydrolysis of bacterial bodies as a template for PCR amplification, which saves a series of complex processes such as extraction of bacterial body DNA [12]. Compared with the traditional method of extraction of bacterial body DNA and then PCR amplification, it is more time-saving and labor-saving and suitable for rapid batch identification of bacterial strains [13].

Therefore, it is only necessary to design specific amplification primers for dominant strains and detect whether the isolated and cultured strains can generate corresponding specific target gene fragment bands through Colony PCR amplification reaction, so as to determine whether the isolated and cultured strains are the desired dominant strains [14].

**Materials and Methods**

**Soil Sample Collection**

The Five-point sampling method was used to extract deep soil with a depth of about 20cm in the Blank saline-alkali area, where no JunCao"Oasis No. 1" plants were planted and no plants were growing on the surface, as Blank soil samples. Plant soil was selected in the planting section of JunCao"Oasis No. 1" and the rhizosphere soil of JunCao"Oasis No. 1" with a rhizosphere depth of about 20cm was extracted as soil sample of Experimental group [Table 1].

**Table 1: Soil Sample Collection Information**

| The sample Group | Sampling Method       | Sampling Depth | Sample Location       | Sample Number |
|------------------|-----------------------|----------------|-----------------------|---------------|
| Blank soil sample| Five point sampling method | 20 cm         | Blank saline area     | K.B.1-K.B.8   |
| Experimental soil sample | Five point sampling method | 20 cm         | Rhizosphere of JunCao | S.Y.1-S.Y.8   |

**Medium**

Salt-tolerant Kim B solid medium: Using for isolation culture and purification of dominant Salt tolerance strains in rhizosphere soil of JunCao"Oasis No. 1" plant.

Its formula is: hydrolyzed peptone 20.0g, NaCl 20.0g, MgSO4 1.5g, K2HPO4 1.5g, Agar 15.0g, pH 7.3 ± 0.2.

Solid medium for salt-tolerant Bacillus: Used for isolation culture screening and purification of dominant Salt tolerance strains in rhizosphere soil of JunCao"Oasis No.1" plant.

Its formula is:Glucose 10.0g, NaCl 20.0g, CaPO4 5.0g, (NH4)2SO4 0.5g, KCl 0.2g, (MgSO4)7H2O 0.1g, MnSO4 0.0001g, FeSO4 0.0001g, Yeast extract 0.5g, Agar 20.0 g, pH 7.0 ± 0.2.

**Main Reagent Materials**

MP Biomedicals Fast DNA Soil Sample Extraction Kit, 2×Taq Master Mix reagent (containing Taq enzyme, dNTP, Mg2+), ddH2O, 6×DNA Loading Buffer reagent, DL2000 DNA Marker reagent, Gleggure nucleic acid gel dye, 1×TAE gel electrophoresis buffer.

**Difference Analysis of Soil Microbial Population Between Blank Group and Experimental Group**

According to the operating instructions of MP Biomedicals Fast DNA soil sample extraction Kit, total DNA of soil microorganisms in Blank group and Experimental group are extracted, and 8 total DNA samples of soil microorganisms in each group are extracted. The 8 total DNA samples of soil microorganisms in Blank group are numbered as follows: KB1, KB2, KB3, KB4, KB5, KB6, KB7, KB8; The total DNA samples of 8 soil microorganisms in the Experimental group were numbered as SY1, SY2, SY3, SY4, SY5, SY6, SY7 and SY8. After passing the test, the total DNA extracted from soil microorganisms was used as the template to amplify the 16SrDNA fragment of bacteria using PCR technology. After the amplification, the PCR amplification products were recovered and analyzed by computer.

**Isolation Screening and Purification of Dominant Strains from Rhizosphere Soil of Juncaoo"Oasis no.1."

The rhizosphere soil of the JunCao"Oasis No. 1" plant stored at -80°C of 9.0g was weighed and placed in a sterilized 250ml conical flask. 90ml physiological saline was added to form a soil
suspension with a concentration gradient of 10-1. After sealing, it was placed in a constant temperature oscillation incubator at 37°C and 150r/min for 120 min to make the microorganisms in the soil fully dissolved in physiological saline. And then successively diluted into soil suspensions with concentration gradients of 10-2, 10-3, 10-4, 10-5, 10-6, 10-7, 10-8 and 10-9 [15].

Soil suspensions with concentration gradients of 10-4, 10-5, 10-6 and 10-7 were selected, 100 microliters of soil suspensions were absorbed with a 200-microliter pipette gun and inoculated into bacterial culture dishes containing sterilized TSB medium, which were evenly smeared on the surface of TSB medium with a coating stick. Soil suspensions with each concentration gradient were inoculated into 4 culture dishes. After sealing, pour into a constant temperature incubator at 37°C for 3-6 days.

White and yellow single colonies growing on the surface of TSB medium were respectively picked out after the inoculating ring was burned red and cooled by alcohol lamp. After sterilization for 20 min at 1×105Pa, continuous lines were drawn on the TSB medium until all colonies growing on the surface of TSB medium were white and yellow.

**Colony PCR Identification of Dominant Strains in Rhizosphere Soil of Juncao oasis no. 1**

The white and yellow single colonies on the surface of TSB medium were picked with the tip of a sterilized pipetting gun. the colonies were placed into a 1.5ml PCR tube filled with 20μl ddH2O. After stirring, the selected acidobacteria were thoroughly dissolved in ddH2O. Then, the 1.5-ml PCR tube containing the liquid of the dominant strain was covered and placed in an induction cooker with boiling water at 100°C for 5min, so that the dominant strain was fully cracked under heating conditions and DNA was fully exposed [16].

Specific primers for the dominant strain were designed [Table 2] using the DNA of the dominant strain that was fully lysed as the template, corresponding PCR reaction system was configured [Table 3], and appropriate PCR reaction conditions were set [Table 4] for PCR amplification reaction.

**Table 2: Design of Specific Primers for Dominant Strains**

| Superiority strains         | Upstream primer | Downstream primers | Fragment length |
|-----------------------------|-----------------|--------------------|-----------------|
| Pseudomonas fluorescens    | Pse-121L: ACGCTAATACCGCATACG | Pse-678R: ACTGGTGTTCCTTCCTATATC | 557bp           |
| Bacillus thuringiensis     | Bac-819L: ACGATGAGTGTGCTAAGTC | Bac-1518R: ATACCGCTACCGTTGTACG | 699bp           |

**Table 3: Colony per Reaction System of Dominant Strains**

| PCR reaction system | 20 μL |
|---------------------|-------|
| The template        | 3 μL  |
| Upstream primer     | 1 μL  |
| Downstream primers  | 1 μL  |
| 2×Taq Master Mix    | 10 μL |
| ddH2O               | 5 μL (Fill the system to 20 μL) |

**Table 4: Conditions for Colony PCR Amplification of Dominant Strains**

| Reaction stage      | Reaction time |
|---------------------|---------------|
| 94°C Pre-degeneration| 1min 30sec    |
| 94°C Degeneration   | 30sec         |
| 50°C Annealing      | 30sec         |
| 72°C Extension      | 1min          |
| Cycle Number        | 34            |
| 72°C Extension      | 5min          |
| 4°C Save            | Forever       |

**Gram Staining Reaction of Dominant Strains**

The dominant strains were stained with The Gram kit, and a small number of bacteria were selected and evenly smeared on the surface of the slide. After drying under the alcohol lamp, the crystal violet reagent was smeared on the surface of the bacteria for 1min, and then washed with water. Then apply the iodine solution to the bacteria surface for staining for 1min, and rinse with water after the staining. Drop appropriate amount of anhydrous ethanol for decolorization for 20 seconds, then rinse with water. Finally, the saffron reagent was applied to the thall surface for dyeing for 1min, and then washed with water. After drying under an alcohol lamp, the staining results were observed under a microscope [17].

**Gel Electrophoresis Reaction of Colony PCR Reaction Products**

The electrophoresis voltage was 120V and the electrophoresis...
time was 30min. After the electrophoresis, gel electrophoresis imaging system was used to verify the effect of gel electrophoresis.

**Results and Analysis**

**Difference Analysis of OTUs Clustering Between Blank Group and Experimental Group**

The differential distribution of OTUs clustering of soil microbial populations in the Blank group and the Experimental group is shown in Fig1. As can be seen from the figure, except for K.B.7 samples in the blank group (which may be caused by the uneven distribution of microbial population in the soil), the OTUs clustering numbers of all samples in the Experimental group were significantly higher than those in the Blank group. This indicated that the abundance of microbial population in the soil samples of the Experimental group was significantly higher than that of the Blank group, and the cultivation of JunCao"Oasis No.1" significantly restored and improved the abundance of microbial population in saline soil.

**Figure 1:** OTUs cluster quantity diagram of soil microbial population in Blank group and Experimental group

**Table 5:** OTUs clustering numbers of microbial populations at different classification levels of soil samples in Blank group and Experimental group

| Sample_Name | Kingdom | Phylum     | Class     | Order  | Family  | Genus   | Species |
|------------|---------|------------|-----------|--------|---------|---------|---------|
| K.B.1      | 28947   | 24740      | 24313     | 17028  | 14105   | 10680   | 1430    |
| K.B.2      | 63888   | 58342      | 57837     | 55278  | 48743   | 46352   | 37711   |
| K.B.3      | 55521   | 46349      | 44564     | 33666  | 23107   | 12643   | 1940    |
| K.B.4      | 55837   | 48571      | 46858     | 33583  | 25159   | 17519   | 1475    |
| K.B.5      | 55480   | 46471      | 44680     | 31394  | 22897   | 13731   | 2847    |
| K.B.6      | 60962   | 51357      | 50217     | 34760  | 26991   | 18492   | 883     |
| K.B.7      | 58570   | 50246      | 48498     | 34045  | 26955   | 16071   | 2791    |
| K.B.8      | 57226   | 47128      | 45398     | 28047  | 19794   | 9443    | 1629    |
| S.Y.1      | 61627   | 53175      | 51108     | 45812  | 37932   | 14202   | 2042    |
| S.Y.2      | 60373   | 49326      | 46868     | 39540  | 30310   | 9482    | 1701    |
| S.Y.3      | 58767   | 50327      | 48938     | 43958  | 38046   | 12535   | 1471    |
| S.Y.4      | 57131   | 49098      | 48290     | 45285  | 41161   | 30101   | 4054    |
| S.Y.5      | 57557   | 49198      | 46649     | 40243  | 32258   | 12862   | 1681    |
| S.Y.6      | 55593   | 48979      | 46952     | 41203  | 34106   | 13295   | 1787    |
| S.Y.7      | 55685   | 47915      | 45874     | 39869  | 32574   | 12025   | 1610    |
| S.Y.8      | 53858   | 46709      | 44925     | 37963  | 30640   | 15860   | 3312    |
Analysis of Species Relative Abundance Differences at Phylum Level

According to the phylum level relative species abundance histogram [Figure 4] and Phylum level species clustering heat map [Figure 5] of soil microbial populations in the Blank group and the Experimental group, the changes in Phylum level relative species abundance between the two groups were analyzed.

It can be seen from Fig.4 that, at the Phylum level, Proteobacteria and Firmicutes with relatively high population abundance in the Blank group soil samples (K.B.1-K.B.8) decreased significantly in the Experimental group soil samples (R.S.1-R.S.8). While Crenarchaeota, Unidentified Bacteria, Actinobacteriota, Cyanobacteria, Bacteroidota, the relative abundance of phyla such as Chloroflexi did not change much.

The relative species abundance of Acidobacteriota and Desulfobacterota in experimental soil samples (R.S.1-R.S.8) has been effectively increased. The relative abundance of Acidobacteriota in the Blank group (K.B.1-K.B.8) was 1.0%, 0.74%, 2.7%, 2.0%, 2.8%, 1.6%, 2.3% and 2.2% respectively. The relative abundance of Acidobacteriota in the Experimental group (S.Y.1-S.Y.8) was 5.5%, 8.4%, 5.0%, 6.4%, 5.1%, 6.1%, 6.1% and 6.5% respectively. The relative abundance of Desulfobacterota in the Blank group (K.B.1-K.B.8) is 0.1%, 0.1%, 0.4%, 0.3%, 0.7%, 0.6%, 0.8% and 0.7% respectively. The relative abundance of Desulfobacterota in the Experimental group soil samples (S.Y.1-S.Y.8) is 3.9%, 5.1%, 2.2%, 1.2%, 5.0%, 9.5%, 3.7% and 2.6% respectively. The relative abundance of both was significantly increased [Table 6.1, Table 6.2].
Table 6.1: Relative abundance values of species at the taxonomic level of each sample Phylum

| Taxonomy     | Proteobacteria | Crenarchaeota | unidentifed_Bacteria | Firmicutes | Desulfovibrioacetotrophota |
|--------------|----------------|---------------|----------------------|------------|---------------------------|
| K.B.1        | 0.315268       | 0.14791       | 0.128256             | 0.073955   | 0.001105                  |
| K.B.2        | 0.480587       | 0.080311      | 0.050915             | 0.170535   | 0.001554                  |
| K.B.3        | 0.184594       | 0.078135      | 0.234162             | 0.056615   | 0.004905                  |
| K.B.4        | 0.244525       | 0.139585      | 0.16228              | 0.043558   | 0.003454                  |
| K.B.5        | 0.26487        | 0.074162      | 0.196408             | 0.043834   | 0.017893                  |
| K.B.6        | 0.258515       | 0.222556      | 0.151572             | 0.033022   | 0.006321                  |
| K.B.7        | 0.291434       | 0.054439      | 0.188636             | 0.048566   | 0.032884                  |
| K.B.8        | 0.30266        | 0.031054      | 0.217755             | 0.020587   | 0.023731                  |
| S.Y.1        | 0.219965       | 0.206321      | 0.159827             | 0.015509   | 0.039206                  |
| S.Y.2        | 0.155889       | 0.142453      | 0.155302             | 0.010881   | 0.051364                  |
| S.Y.3        | 0.212332       | 0.275509      | 0.13513              | 0.008325   | 0.022211                  |
| S.Y.4        | 0.332055       | 0.033679      | 0.093679             | 0.023903   | 0.012124                  |
| S.Y.5        | 0.235406       | 0.120587      | 0.193817             | 0.003454   | 0.050363                  |
| S.Y.6        | 0.206183       | 0.129706      | 0.171192             | 0.017651   | 0.095509                  |
| S.Y.7        | 0.217478       | 0.16038       | 0.182245             | 0.014197   | 0.037617                  |
| S.Y.8        | 0.253782       | 0.100104      | 0.161071             | 0.046908   | 0.026321                  |

Table 6.2: Relative abundance values of species at the taxonomic level of each sample Phylum

| Taxonomy     | Acidobacteriota | Actinobacteriota | Cyanobacteria | Bacteroidota | Chloroflexi | Others |
|--------------|-----------------|------------------|---------------|--------------|-------------|--------|
| K.B.1        | 0.010535        | 0.052919         | 0.038204      | 0.016718     | 0.015648    | 0.199482 |
| K.B.2        | 0.018269        | 0.044491         | 0.001382      | 0.015682     | 0.047358    | 0.307461 |
| K.B.3        | 0.020136        | 0.034059         | 0.038722      | 0.013995     | 0.048256    | 0.251434 |
| K.B.4        | 0.012243        | 0.045492         | 0.000383      | 0.009326     | 0.040389    | 0.278826 |
| K.B.5        | 0.011615        | 0.030984         | 0.000622      | 0.008946     | 0.031883    | 0.238964 |
| K.B.6        | 0.043903        | 0.045216         | 0.000898      | 0.007599     | 0.030294    | 0.256131 |
| K.B.7        | 0.033299        | 0.034922         | 0.000622      | 0.007945     | 0.04        | 0.287427 |
| S.Y.1        | 0.055959        | 0.016511         | 0.000864      | 0.00715      | 0.033955    | 0.244732 |
| S.Y.2        | 0.048411        | 0.020449         | 0.000553      | 0.005216     | 0.044491    | 0.329292 |
| S.Y.3        | 0.049085        | 0.013022         | 0.003212      | 0.012746     | 0.020725    | 0.247703 |
| S.Y.4        | 0.064525        | 0.07361          | 0.058826      | 0.051364     | 0.033437    | 0.222798 |
| S.Y.5        | 0.05133         | 0.022039         | 0.00494       | 0.007599     | 0.044525    | 0.265976 |
| S.Y.6        | 0.061278        | 0.031088         | 0.000898      | 0.010501     | 0.031952    | 0.244041 |
| S.Y.7        | 0.061727        | 0.019447         | 0.001934      | 0.009983     | 0.031917    | 0.263074 |
| S.Y.8        | 0.065112        | 0.037858         | 0.006218      | 0.018895     | 0.04        | 0.243731 |

As can be seen from [Figure 5], at the level of Phylum classification, Verrucomicrobiota, Entothelaeota, Thermoplasmata Myxococca, Desulfuromonas (desulfurization bacterium door), Myxococca, Acidobacteriota (acid bacillus door), The species clustering of Latiscibacterota was significantly higher than that of blank soil samples (K.B.1-K.B.8).

Desulfoacterbacterium and Actinobacteria are the dominant Phylum in the Experimental group soil samples (S.Y. 1-S.Y. 8) combined with the column chart of relative species abundance at Phylum level and the heat map of Phylum level clustering.

Analysis of Species Relative Abundance Differences at Order Level

According to the Order level relative species abundance histogram [Figure 6] and Order level species clustering heat map [Figure 7] of soil microbial populations in the Blank group and the Experimental group, the changes in Order level relative species abundance between the two groups were analyzed.
It can be seen from [Figure 6] that the relative abundance of species at Order level showed obvious differentiation between samples from Blank group (K.B.1-K.B.8) and Experimental group (S.Y. 1-S.Y. 8). The relative species abundance of experimental soil samples (S.Y. 1-S.Y. 8) at Order level was significantly higher than that of blank soil samples (K.B.1-K.B.8).

The relative species abundance of Rhodospirillales, Sphingomonadales and Burkholderiales in the Experimental group (S.Y. 1-S.Y. 8) was significantly higher than that in the Blank group (K.B.1-K.B.8). The relative abundance of Rhodospirillales in experimental soil samples (S.Y. 1-S.Y. 8) was 4.2%, 6.1%, 4.4%, 3.3%, 5.1%, 4.4%, 5.9%, 3.6% respectively. The relative species abundance of Sphingomonadales in Experimental group soil samples (S.Y. 1-S.Y. 8) was 0.6%, 0.6%, 0.7%, 6.6%, 1.4%, 0.7%, 0.6%, 0.9% respectively, and in Blank group soil samples K.B.3, K.B.4, K.B.5. The relative abundance of Sphingomonadales was 0, and the relative abundance of Sphingomonadales in the other Blank groups was extremely low. The relative species abundance of Burkholderiales in experimental soil samples (S.Y. 1-S.Y. 8) was 6.2%, 3.8%, 6.3%, 10.9%, 4.9%, 4.8%, 5.2%, 4.9% respectively. Compared with the Blank group (K.B.1-K.B.8), the relative abundance of Rhodospirillales, Sphingomonadales and Burkholderiales in the Experimental group (S.Y. 1-S.Y. 8) was increased [Table 7].

**Table 7 : Relative abundance of dominant flora species at soil sample Order level in Experimental group**

| Taxonomy           | Burkholderiales | Nitrosopumilales | Paenibacillales | Rhodospirillales | unidentified_Desulfuromonadia | Sphingomonadales |
|--------------------|-----------------|------------------|-----------------|-----------------|------------------------------|-----------------|
| K.B.1              | 0.001796        | 0.144525         | 0.000587        | 0.002453        | 0.000622                     | 0.001209        |
| K.B.2              | 0.411952        | 0.080138         | 0.130432        | 0.004111        | 0.000829                     | 0.033472        |
| K.B.3              | 0.002349        | 0.072021         | 0.001105        | 0.002383        | 0.001762                     | 0.000484        |
| K.B.4              | 0.002487        | 0.134853         | 0.001104        | 0.005838        | 0.016442                     | 0.000415        |
| K.B.5              | 0.004387        | 0.071641         | 0.00104         | 0.005838        | 0.001676                     | 0.000415        |
| K.B.6              | 0.002038        | 0.215648         | 0.0000104       | 0.002591        | 0.004767                     | 0.000311        |
| K.B.7              | 0.018549        | 0.050708         | 0.000207        | 0.009223        | 0.024905                     | 0.005389        |
| K.B.8              | 0.006287        | 0.028566         | 0.000138        | 0.006183        | 0.018618                     | 0.001174        |
| S.Y.1              | 0.062142        | 0.205803         | 0.000104        | 0.042798        | 0.025941                     | 0.005838        |
| S.Y.2              | 0.037686        | 0.141623         | 0.000138        | 0.061036        | 0.042832                     | 0.002453        |
| S.Y.3              | 0.063005        | 0.274577         | 0.000207        | 0.044283        | 0.0162                       | 0.005043        |
| S.Y.4              | 0.108601        | 0.03323          | 0.003351        | 0.021209        | 0.007634                     | 0.065596        |
| S.Y.5              | 0.04943         | 0.119931         | 0.000242        | 0.051054        | 0.035959                     | 0.013748        |
| S.Y.6              | 0.048394        | 0.127288         | 0.00076         | 0.044352        | 0.084421                     | 0.006701        |
| S.Y.7              | 0.052435        | 0.157686         | 0.000173        | 0.059413        | 0.026736                     | 0.005561        |
| S.Y.8              | 0.049257        | 0.09658          | 0.000415        | 0.036408        | 0.017375                     | 0.009119        |
It can be clearly seen from [Figure 7] that Rhodospirillales, Rhizobiales, Sphingomonadales, Pseudomonadales in soil samples of Experimental group (S.Y. 1-S.Y. 8). The species clustering of Micrococcales was significantly higher than that of blank soil samples (K.B.1-K.B.8). Pseudomonadales is one of the most important growth-promoting bacteria in plant rhizosphere.

Combined with the histogram of relative abundance of species at Order level and the heat map of cluster of species at Order level, Pseudomonadales and Sphingomonadales were dominant in the soil samples of Experimental group (S.Y. 1-S.Y. 8).

**Analysis of species relative abundance differences at Family level**

According to the Family level relative species abundance histogram [Figure 8] and Family level species clustering heat map [Figure 9] of soil microbial populations in the Blank group and the Experimental group, the changes in Family level relative species abundance between the two groups were analyzed.

As can be seen from [Figure 8], the differences in relative species abundance at Family level between samples from Blank group (K.B.1-K.B.8) and Experimental group (S.Y. 1-S.Y. 8) were more obvious. In experimental soil samples (S.Y.1-S.Y.8), Magnetospiraceae, Sphingomonadaceae, Oxalobacteraceae, the relative species abundance of Desulfuromonadaceae is significantly higher than that of blank soil samples (K.B.1-K.B.8). The relative species abundance of Magnetospiraceae in experimental soil samples (S.Y. 1-S.Y. 8) was 4.0%, 5.9%, 4.2%, 2.0%, 4.8%, 4.2%, 5.7%, 3.4% respectively. The relative species abundance of Sphingomonadaceae in experimental soil samples (S.Y. 1-S.Y. 8) was 0.6%, 0.5%, 0.5%, 6.5%, 1.4%, 0.7%, 0.6%, 0.9% respectively. The relative abundance of Sphingomonadaceae was 0 in K.B.3, K.B.4, K.B.5 and K.B.6 samples of Blank group, while the relative abundance of Sphingomonadaceae was extremely low in other samples. The relative species abundance of Oxalobacteraceae in Experimental group soil sample (S.Y. 1-S.Y. 8) was 0.1%, 0.1%, 0.3%, 7.8%, 0.1%, 0.3%, 0.3%, 0.4% respectively, and in Blank group soil sample (K.B.1-K.B.8), The relative abundance of Oxalobacteraceae was 0. The relative species abundance of Desulfuromonadaceae in Experimental group soil samples (S.Y. 1-S.Y. 8) was 2.0%, 4.1%, 1.2%, 0.6%, 3.2%, 8.1%, 2.3% and 1.4% respectively [Table 8].

![Figure 8: Bar chart of species relative abundance at level of each Family](image)

![Figure 9: Cluster thermograms of species at the level of each Family](image)
Table 8: Relative abundance of dominant flora species at soil sample Family level in Experimental group

| Taxonomy                  | Magnetospiraceae | Paenibacillaceae | Sphingomonadaceae | Desulfuromonadaceae | Oxalobacteraceae |
|---------------------------|------------------|------------------|-------------------|--------------------|-----------------|
| K.B.1                     | 0.002383         | 0.000587         | 0.001209          | 0.000415           | 0.0000415       |
| K.B.2                     | 0.130432         | 0.033472         | 0.001209          | 0.000415           | 0.0000415       |
| K.B.3                     | 0.003282         | 0.001105         | 0.001209          | 0.000415           | 0.0000415       |
| K.B.4                     | 0.002038         | 0.001105         | 0.001209          | 0.000415           | 0.0000415       |
| K.B.5                     | 0.005285         | 0.001105         | 0.001209          | 0.000415           | 0.0000415       |
| K.B.6                     | 0.002314         | 0.001105         | 0.001209          | 0.000415           | 0.0000415       |
| K.B.7                     | 0.00867          | 0.001105         | 0.001209          | 0.000415           | 0.0000415       |
| K.B.8                     | 0.005596         | 0.001105         | 0.001209          | 0.000415           | 0.0000415       |
| S.Y.1                     | 0.040207         | 0.001105         | 0.001209          | 0.000415           | 0.0000415       |
| S.Y.2                     | 0.058756         | 0.001105         | 0.001209          | 0.000415           | 0.0000415       |
| S.Y.3                     | 0.042073         | 0.001105         | 0.001209          | 0.000415           | 0.0000415       |
| S.Y.4                     | 0.019793         | 0.001105         | 0.001209          | 0.000415           | 0.0000415       |
| S.Y.5                     | 0.048221         | 0.001105         | 0.001209          | 0.000415           | 0.0000415       |
| S.Y.6                     | 0.042383         | 0.001105         | 0.001209          | 0.000415           | 0.0000415       |
| S.Y.7                     | 0.057582         | 0.001105         | 0.001209          | 0.000415           | 0.0000415       |
| S.Y.8                     | 0.034059         | 0.001105         | 0.001209          | 0.000415           | 0.0000415       |

Desulfuromonadaceae, Pseudomonadaceae, Sphingomonadaceae in the Experimental group soil sample (S.Y.1-S.Y.8) can be clearly seen from Fig.9. The species clustering of Acidithiobacillaceae was significantly higher than that of the Blank group (K.B.1-K.B.8). Pseudomonadaceae is one of the most important growth-promoting bacteria in plant rhizosphere.

Combined with the histogram of relative abundance of species at Family level and the cluster heat map of species at Family level, Sphingomonadaceae, Pseudomonadaceae and Desulfuromonadaceae are the dominant Family in the experimental group soil samples (R.S.1-R.S.8).

**Analysis of Species Relative Abundance Differences at Genus Level**

According to the Genus level relative species abundance histogram [Figure 10] and Genus level species clustering heat map [Figure 11] of soil microbial populations in the Blank group and the Experimental group, the changes in Genus level relative species abundance between the two groups were analyzed.

![Figure 10: Bar chart of species relative abundance at level of each Genus](image)

**Figure 11: Cluster thermograms of species at the level of each Genus**

The differences in species relative abundance between samples from Blank group (K.B.1-K.B.8) and Experimental group (S.Y.1-S.Y.8) were most obvious at Genus level. Fig.10 shows that species relative abundance of Sphingomonas, Paenarthrobacter, Paenibacillus and Pseudomonas in Experimental soil sample (S.Y.1-S.Y.8) is higher than that in blank soil sample (K.B.1-K.B.8). The relative abundance of Sphingomonas in experimental soil samples (S.Y.1-S.Y.8) was 0.3%, 0.3%, 0.3%, 0.3%, 0.3%, 0.3%, 0.3%, 0.3% respectively. While that in Blank sample K.B.3, K.B.4, K.B.5, K.B.6, K.B.8 was 0. The relative abundance of species in the other Blank groups was extremely low.

The relative abundance of Pseudomonas in experimental soil samples (S.Y.1-S.Y.8) was 1.7% in S.Y.1, followed by 1.0% in S.Y.5. The relative abundance of species in S.Y.4, S.Y.6, S.Y.7, and S.Y.8 were about 0.7%, 0.5%, 0.5%, and 0.8% respectively.
The relative abundance of species in K.B.1 and K.B.2 samples of Blank group was 0, and the relative abundance of species in other Blank group samples was extremely low.

The relative species abundance of Paenibacillus in Experimental sample (S.Y. 1-S.Y. 8) was 0.3% in S.Y.4, while that in Blank sample K.B.1, K.B.3, K.B.5, K.B.6, K.B.7, K.B.8 was 0. The relative abundance of Paenibacillus species in K.B.2 was due to the heterogeneity of soil microbial population distribution [Table 9].

Table 9: Relative abundance of dominant flora species at soil sample Genus level in Experimental group

| Taxonomy          | Paenibacillus | Sphingomonas     | Paenarthrobacter | Pseudomonas |
|-------------------|----------------|------------------|------------------|-------------|
| K.B.1             | 0              | 0.001105         | 0                | 0           |
| K.B.2             | 0.130432       | 0.033472         | 0                | 0           |
| K.B.3             | 0              | 0.000484         | 0                | 0.001865    |
| K.B.4             | 0.001105       | 0.000415         | 0                | 0.001762    |
| K.B.5             | 0              | 0.000104         | 0.000173         | 0.00345     |
| K.B.6             | 0              | 0.000242         | 0                | 0.00152     |
| K.B.7             | 0              | 0.00487          | 0.001934         | 0.001796    |
| K.B.8             | 0              | 0.00076          | 0.000484         | 0.000898    |
| S.Y.1             | 0.000104       | 0.002902         | 0.00076          | 0.017168    |
| S.Y.2             | 0.000138       | 0.000829         | 0.000518         | 0.00494     |
| S.Y.3             | 0.000173       | 0.000228         | 0.001071         | 0.00677     |
| S.Y.4             | 0.00304        | 0.056718         | 0.02829          | 0.006874    |
| S.Y.5             | 0.000242       | 0.00456          | 0.00114          | 0.010501    |
| S.Y.6             | 0.000725       | 0.004836         | 0.002487         | 0.005492    |
| S.Y.7             | 0.000173       | 0.003212         | 0.002453         | 0.007634    |
| S.Y.8             | 0.000276       | 0.00601          | 0.004076         | 0.007703    |

As can be seen from [Figure 11], the species clustering of Sphingomonas and Pseudomonas in the Experimental group (S.Y.1-S.Y.8) was significantly higher than that in the Blank group (K.B.1-K.B.8). Pseudomonas is an important growth-promoting bacterium in plant rhizosphere. At the same time, Staphylococcus was distributed in soil samples of both the Blank group and the Experimental group. Although Staphylococcus was a common pathogenic bacterium in soil, it was distributed in soil samples of both the Blank group and the Experimental group due to its good salt tolerance. Therefore, Staphylococcus is a common strain of soil samples of Blank group and Experimental group.

Combined with the histogram of relative abundance of species at the Genus level and the cluster heat map of species at the Genus level, Sphingomonas, Paenibacillus and Pseudomonas were the dominant species in the Experimental group (S.Y. 1-S.Y.8). Staphylococcus was a common strain of soil samples from Blank group and Experimental group.

Ternaryplot Analysis

In order to find the differences of dominant Species among the three groups of samples at each classification level (Phylum, Class, Order, Family, Genus), the top 10 Species with average abundance at each classification level were selected to generate a Ternaryplot. In order to intuitively check the differences of dominant species among the three groups of samples at different classification levels [18]. Ternaryplot analysis using the R software VCD Ternaryplot command [19]. The three vertices in the figure represent three sample groups, and the circle represents species. The size of the circle is proportional to the relative abundance. The closer the circle is to the vertex, the higher the content of this species in this group [20]. Here, the three classification levels with the largest difference in microbial population structure between the Experimental group and the Blank group were selected for comparative analysis: Phylum classification level, Order classification level and Genus classification level.

Ternaryplot Analysis at The Classification Level of Each Sample Phylum

Ternaryplot analysis of classification level of each sample Phylum is shown in [Figure 12]. Content of Desulfobacterota and Acidobacteriota in the samples of the Experimental group is significantly higher than that in the samples of the Blank group. Desulfobacterota and Acidobacteriota are the dominant genuses in each sample in the Experimental group.

Ternaryplot analysis at the classification level of each Sample Order

Ternaryplot analysis of classification level of each sample Order is shown in [Figure 13]. Desulfuromonadia and Rhodospirillales content in the samples of the Experimental group is significantly higher than that in the samples of the Blank group. In addition, the content of Sphingomonadales in S.Y.4 was significantly higher than that in other Experimental groups, indi-
cating that the content of Sphingomonadales reached the highest level in S.Y.4.

Desulfuromonadia, Rhodospirillales and Sphingomonadales were the dominant species in the Experimental group.

**Ternaryplot Analysis at The Classification Level of Each Sample Genus**

Ternaryplot analysis of classification level of each sample Genus is shown in [Figure 14]. The content of Sphingomonas in all samples in the Experimental group is significantly higher than that in the Blank group. The results showed that the dominant bacteria in the Experimental group was Sphingomonas.

**Determine the Target Strain for Isolation and Culture**

Desulfuromonas and Acidobacteria are the dominant genera at the Phylum classification level in the experimental group soil samples (R.S.1-R.S.8). The dominant bacteria at the Order classification level were Rhodospirillales, Pseudomonadales and Sphingomonadales. The dominant genera at the Family classification level are Sphingomonadaceae, Pseudomonadaceae and Desulfuromonadaceae. The dominant bacteria at the Genus classification level were Sphingomonas, Paenibacillus and Pseudomonas.

Pseudomonas and Bacillus are important growth-promoting bacteria in plant rhizosphere and have good characteristics of salt and alkali tolerance and pest elimination. Therefore, *Pseudomonas and Bacillus* were selected as the target strains for isolation and culture based on the ecological value of soil salinization control and the ecological function of the strains themselves.

**Gram Staining Reaction Analysis of Target Strains**

Gram staining results of *Pseudomonas* strain are shown in [Figure 15]. It can be seen from the figure that the bacteria are elongated rods and stained red, indicating that the strain is Gram-negative. At the same time, the strain gave out visible fluorescence under uv light irradiation of 365nm [Figure 16], which proved that the *Pseudomonas* strain was *Pseudomonas fluorescens*.

![Figure 15: Gram staining results of Pseudomonas strain](image)

![Figure 16: Fluorescence production characteristics of Pseudomonas strains](image)

Gram staining reaction results of bacillus strain are shown in [Figure 17]. It can be seen from the figure that the bacterium is elongated rod shaped and stained purple, indicating that the strain is Gram-positive bacterium. Under the microscope, it was found that the strain could produce a large number of rhomboid spore crystal [Figure 17], which proved that the bacillus strain was *Bacillus thuringiensis*.

![Figure 17: Gram staining results of Bacillus strain](image)

**Colony PCR Gel Electrophoresis Analysis**

The gel electrophoresis images of PCR reaction products of *Pseudomonas fluorescens* and *Bacillus thuringiensis* colonies are shown in [Figure 18]. It could be seen that the PCR target bands of *Pseudomonas fluorescens* colony (557bp) and *Bacillus thuringiensis* colony (699bp) were clear and bright, neatly arranged, and the length and distribution location of the target bands were accurate. Therefore, it can be determined that the target strains isolated from the rhizosphere soil of "Oasis 1" are *Pseudomonas fluorescens* and *Bacillus thuringiensis* strains.
Finally, Pseudomonas fluorescens and Bacillus thuringiensis strains were successfully isolated from the rhizosphere soil of JunCao"Oasis No. 1". The dominant genera at the Family classification level were Sphingomonadales. The dominant bacteria at the Order classification level were Rhodospirillales, Pseudomonadales and Sphingomonadales. The dominant genera at the Family classification level are Sphingomonadaceae, Pseudomonadaceae and Desulfuromonadales. The dominant bacteria at the Genus classification level were Sphingomonas, Paenibacillus and Pseudomonas. Finally, Pseudomonas fluorescens and Bacillus thuringiensis were isolated and cultured from the rhizosphere soil of JunCao"Oasis No. 1".

**Results**

In this paper, 16SrDNA high-throughput sequencing technology was used to sequence the microbial population structure of blank soil samples (K.B.1-K.B.8) and experimental soil samples (R.S.1-R.S.8). The relative abundance columns and species clustering of Phylum, Order, Family and Genus were determined. Desulfuromondia and Acidobacteria are the dominant genera at the Phylum classification level in the Experimental group soil samples (R.S.1-R.S.8). The dominant bacteria at the Order classification level were Rhodospirillales, Pseudomonadales and Sphingomonadales. The dominant genera at the Family classification level are Sphingomonadaceae, Pseudomonadaceae and Desulfuromonadales. The dominant bacteria at the Genus classification level were Sphingomonas, Paenibacillus and Pseudomonas. Finally, Pseudomonas fluorescens and Bacillus thuringiensis were isolated and cultured from the rhizosphere soil of JunCao"Oasis No. 1".

**Discussion**

As a new grass species, JunCao"Oasis No. 1" can effectively control soil salinization. Pseudomonas fluorescens and Bacillus thuringiensis have a high abundance in rhizosphere soil. Pseudomonas fluorescens and Bacillus thuringiensis are important growth-promoting bacteria in plant rhizosphere [21]. Both of which can synthesize and secrete a large number of insecticidal toxins [22]. It plays an important role in plant resistance to pests [23]. Therefore, current studies on Pseudomonas fluorescens and Bacillus thuringiensis are focused on the field of resistance to disease and insect pests, and there are very few studies on their salt and alkali resistance [24]. Studies on the salt and alkali resistance of Pseudomonas fluorescens and Bacillus thuringiensis are conducive to fully exploring the great application potential of Pseudomonas fluorescens and Bacillus thuringiensis in environmental protection and industrial production [25].

In this paper, purified Pseudomonas fluorescens and Bacillus thuringiensis strains were successfully isolated from the rhizosphere soil of JunCao"Oasis No. 1" plant with abundant Pseudomonas fluorescens and Bacillus thuringiensis species by using Colony PCR identification method. It laid a solid theoretical foundation for the subsequent saline-alkali resistance of Pseudomonas fluorescens and Bacillus thuringiensis.

At the same time, it is also confirmed from the side that the mycelium JunCao"Oasis No. 1" plant has a good saline-alkali soil control function, which provides a new method and idea for the treatment of saline-alkali soil by biological methods, and has a good practical guiding significance and application value.

**Declarations**

**Statement of Funding and Informed Consent**

The fund of this paper is provided by the National Engineering Technology Research Center of JunCao, Fujian Agriculture and Forestry University. The project is supported by the Interdisciplinary integration to promote the high-quality development of JunCao science and industry (KJJC-712021030) and the Key technology Research and application of Mycelia Germplasm Innovation and Industrial Utilization of Fujian Province (2021NZ0101).

As the corresponding author of this article, I hereby declare: XiaoZhiqi, the first author of this paper, is my student, who is responsible for the data analysis and article writing of this paper. I have planned and communicated with the first author for many times about the fund operation and various writing work of this paper, and I am fully aware of it.

This paper was written and submitted with my full knowledge and communication with the first author. Accurate data analysis, capital operation in place. Meet the journal submission standards.

**Ethical Declarations**

I declare that this paper is the research result of data analysis and corresponding writing completed by my student XiaoZhiqi under my full knowledge and guidance.

The data in this paper are all from experiments, and all experimental data are clear and accurate. It does not include any research results published or written by others, and there is no plagiarism. If found to have infringed upon the intellectual property rights of others, he/she shall bear due responsibilities.

**The Author Contributions**

I declare that the first author of this thesis is XiaoZhiqi, who as my student is mainly responsible for the development of the thesis experiment, the analysis of the experimental data of the thesis and the compilation of the thesis.

As the corresponding author, I am mainly responsible for the final revision and submission guidance of this thesis.

**Data Availability Description**

I declare that all the experimental data in this paper are obtained from the experiment of XiaoZhiqi, the first author of this paper, the data is true and accurate, all available.
The data related to this paper has not been uploaded to a fixed data repository and will be submitted as a separate file. All data in this paper are true and reliable, and do not include any research results published or written by others. There is no plagiarism, and I am fully aware of it. If found to have infringed upon the intellectual property rights of others, he/she shall bear due responsibilities.

Consent to Publication
I declare that I am the corresponding author of this paper. Xiao Zhiqi, the first author of this paper and I agree that this paper will be published and published in the Annual Journal of Microbiology if it meets the publication requirements.

Statement of Competitive Interest
As the corresponding author of this paper, I hereby declare that I have not had any interest dispute or interest competition with anyone or any institution in the process of experiment and compilation of this paper. The funds for the experiment are from the special research funds of the university, and no individual donation is required.

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Conflicts of Interest and Acknowledgements
I hereby declare that there is no conflict of interest with any individual or institution in the experiment and writing process of this paper, and there is no risk of any conflict of interest. In the process of experiment, my student Xiao Zhiqi paid hard work, he is the actual author and the first author of this paper, and his colleague is also the direct analysis of experimental data. For this, I would like to extend my solemn thanks to my student Xiao Zhiqi!

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