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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Research Article

Keywords: Juncao"Oasis No.1", 16SrDNA high-throughput sequencing, Rhizosphere soil microorganism, Pseudomonas fluorescens, Bacillus thuringiensis, Isolation and culture, Colony PCR

Posted Date: February 1st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1240877/v1

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.

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(Zhou et al, 2021).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(Liu et al, 2015).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, thus 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(Zhou et al, 2021).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(Reuter et al, 2015), among which 16SrDNA high-throughput sequencing technology is the most widely used in the identification of microbial populations due to its good specificity. 16SrDNA is located on the small ribosomal subunit of prokaryotic cells(Young et al, 2017), including 10 Conserved Regions and 9 Hypervariable Regions(Goldmann et al, 2021). There is little difference between bacteria in Conserved Regions, and the Hypervariable Regions are genus or species specific(Vezzulli et al, 2018). 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(Elberri et al, 2020). 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(Kosacki et al, 2020). 16SrDNA amplicon sequencing has become an important means to study the composition and structure of microbial communities in environmental samples(Laranjo et al, 2004).

Meanwhile, colony PCR has been widely used in the identification of positive clones(Woodman et al, 2016).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(Asano et al, 2011). 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(Kong et al, 2005).

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(Zeng et al, 2020).

1. Materials And Methods

1.1 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

1.2 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,$MgSO_4$ 1.5g, K_2HPO_4 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,$CaPO_4$ 5.0g, $(NH_4)_2SO_4$ 0.5g, KCl 0.2g, $(MgSO_4)7H_2O$ 0.1g, $MnSO_4$ 0.0001g, $FeSO_4$ 0.0001g, Yeast extract 0.5g, Agar 20.0 g, pH 7.0 ± 0.2.

1.3 Main reagent materials

MP Biomedicals Fast DNA Soil Sample Extraction Kit, 2×Taq Master Mix reagent (containing Taq enzyme, dNTP, Mg^{2+}), ddH_2O, 6×DNA Loading Buffer reagent, DL2000 DNA Marker reagent, Glegreen nucleic acid gel dye, 1×TAE gel electrophoresis buffer.

1.4 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.

1.5 Isolation screening and purification of dominant strains from rhizosphere soil of JunCao"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 120min 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}(Li et al, 2020).

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 1×10^5Pa, continuous lines were drawn on the TSB medium until all colonies growing on the surface of TSB medium were white and yellow.

1.6 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 1.5mlPCR tube filled with 20μL ddH_2O. After stirring, the selected acidobacteria were thoroughly dissolved in ddH_2O. Then, the 1.5-mlpcr 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(Pesce et al, 2019).

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: ACGATGAGTGCTAAGTGT	Bac-1518R: ATACGGCTACCTTGTTACG	699bp

Table 3
Colony PCR 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
ddH_2O	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

1.7 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(Moyes et al, 2009).

1.8 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

2. Results And Analysis

2.1 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 Fig. 1. 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 OUTs 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.

2.2 Difference analysis of OTUs clustering numbers of microbial populations at different classification levels between blank group and experimental group soil samples

The OTUs clustering numbers of microbial populations at different classification levels of soil samples from the blank group and the experimental group were shown in Fig. 2, Fig. 3 and Table 5. It can be concluded that the OTUs clustering number of microbial population of each soil sample in the experimental group is significantly improved at the level of phylum classification and genus classification compared with that of each soil sample in the blank group. This indicated that the microbial population abundance of the soil samples in the experimental group was significantly improved at the level of Phylum classification, Order classification, Family classification and genus classification, which was also an important reason for the obvious difference in the microbial population structure of the soil samples in the experimental group compared with the soil samples in the blank 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

2.2.1 Analysis of species relative abundance differences at Phylum level

According to the phylum level relative species abundance histogram (Fig. 4) and Phylum level species clustering heat map (Fig. 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 species 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	unidentified_Bacteria	Firmicutes	Desulfobacterota
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	0.007427	0.044491	0	0.002556	0.029361	0.132263
K.B.3	0.027496	0.042211	0.001382	0.015682	0.047358	0.307461
K.B.4	0.020138	0.034059	0.038722	0.01399	0.048256	0.251434
K.B.5	0.028428	0.045492	0.00038	0.009326	0.04038	0.278826
K.B.6	0.016615	0.030984	0.000622	0.008946	0.031883	0.238964
K.B.7	0.043903	0.045216	0.000898	0.007599	0.030294	0.256131
K.B.8	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	0.084111	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	0.022003	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 Fig. 5, at the level of Phylum classification, Verrucomicrobiota, Entotheonellaeota, Thermoplasmata Myxococcota, Desulfuromondia (desulfurization bacterium door), Myxococcota, Acidobacteriota (acid bacillus door), The species clustering of Latescibacterota was significantly higher than that of blank soil samples (K.B.1-K.B.8).

Desulfobracterota and Acidobacteriae 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.

2.2.2 Analysis of species relative abundance differences at Order level

According to the Order level relative species abundance histogram (Fig. 6) and Order level species clustering heat map (Fig. 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 Fig. 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 (R.S.1-R.S.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 (R.S.1-R.S.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	0.000829	0.033472
K.B.3	0.002349	0.072021	0	0.004111	0.001934	0.000794
K.B.4	0.002487	0.134853	0.001105	0.002383	0.001762	0.000484
K.B.5	0.004387	0.071641	0.000104	0.005838	0.016442	0.000415
K.B.6	0.002038	0.215648	0.000104	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 Fig. 7 that Rhodospirillales, Rhizobiales, Sphingomonadales, Pseudomondales 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). Pseudomondales 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, Pseudomondales and Sphingomonadales were dominant in the soil samples of Experimental group (S.Y. 1-S.Y. 8).

2.2.3 Analysis of species relative abundance differences at Family level

According to the Family level relative species abundance histogram (Fig. 8) and Family level species clustering heat map (Fig. 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 Fig. 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).

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	0.000415
K.B.2	0	0.130432	0.033472	0	0
K.B.3	0.003282	0	0	0	0
K.B.4	0.002038	0.001105	0	0	0.000484
K.B.5	0.005285	0.000104	0	0.001002	0
K.B.6	0.002314	0.000104	0	0	0.000449
K.B.7	0.00867	0.000207	0.005389	0.004525	0.002867
K.B.8	0.005596	0.000138	0.001174	0.001727	0.000794
S.Y.1	0.040207	0.000104	0.005838	0.019206	0.001105
S.Y.2	0.058756	0.000138	0.002453	0.040794	0.001451
S.Y.3	0.042073	0.000207	0.005043	0.012055	0.002556
S.Y.4	0.019793	0.003351	0.065596	0.005907	0.078342
S.Y.5	0.048221	0.000242	0.013748	0.032332	0.000967
S.Y.6	0.042383	0.00076	0.006701	0.080587	0.002694
S.Y.7	0.057582	0.000173	0.005561	0.023247	0.002832
S.Y.8	0.034059	0.000415	0.009119	0.014128	0.003972

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).

2.2.4 Analysis of species relative abundance differences at Genus level

According to the Genus level relative species abundance histogram (Fig. 10) and Genus level species clustering heat map (Fig. 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.

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%, 5.7%, 0.5%, 0.5%, 0.3%, 0.6% 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%, 0.8%, 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	0.000345
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	0.00228	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 Fig. 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 at the Genus level of soil samples from the Gxperimental group (S.Y. 1-S.Y.8). Staphylococcus was a common strain of soil samples from Blank group and Experimental group.

2.3 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(Hong et al, 2021). Ternaryplot analysis using the R software VCD Ternaryplot command(Brzosko et al, 2021). 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(Marć et al, 2020). 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.

2.3.1 Ternaryplot analysis at the classification level of each sample Phylum

Ternaryplot analysis of classification level of each sample Phylum is shown in Fig. 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.

2.3.2 Ternaryplot analysis at the classification level of each sample Order

Ternaryplot analysis of classification level of each sample Order is shown in Fig. 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, indicating 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.

2.3.3 Ternaryplot analysis at the classification level of each sample Genus

Ternaryplot analysis of classification level of each sample Genus is shown in Fig. 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.*

2.4 Determine the target strain for isolation and culture

Desulfuromondia and *Acidobacteriae* are the dominant genuses 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, Pseudomondales* and *Sphingomonadales*. The dominant genuses 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.

2.5 Gram staining reaction analysis of target strains

Gram staining results of *Pseudomonas* strain are shown in Fig. 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 (Fig. 16), which proved that the *Pseudomonas* strain was *Pseudomonas fluorescens*.

Gram staining reaction results of bacillus strain are shown in Fig. 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 (Fig. 17), which proved that the bacillus strain was Bacillus thuringiensis.

2.6 Colony PCR gel electrophoresis analysis

The gel electrophoresis images of PCR reaction products of Pseudomonas fluorescens and Bacillus thuringiensis colonies are shown in Fig. 18. It could be seen that the PCR target bands of Pseudomonas fluorescens colony (557bp) and Bacillus thingiensis 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.

3. 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 *Acidobacteriae* are the dominant genuses 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, Pseudomondales* and *Sphingomonadales*. The dominant genuses at the Family classification level are *Sphingomonadaceae, Pseudomonadaceae* and *Desulfuromonadaceae*. 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".

4. Discuss

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(Rojas-Ruiz et al, 2015). Both of which can synthesize and secrete a large number of insecticidal toxins(Durán et al., 2021).It plays an important role in plant resistance to pests(Shahid et al., 2021).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(Huang et al, 2007).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(Hernández-Pacheco et al, 2021).

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 (XKJC-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.

Acknowledgements

As the corresponding author of this paper, I would like to thank the first author of this paper, my student Xiao Zhiqi. He is the data analyst and author of this paper. For this paper's overall conception and saved to write made a great contribution!

At the same time, I also want to thank my colleagues, who have provided many valuable references for the analysis of the data and the compilation of the paper. Finally, I would like to thank the editors of Annals of Microbiology for reviewing my paper in their busy schedule. I hope the paper can be published smoothly!

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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Figures

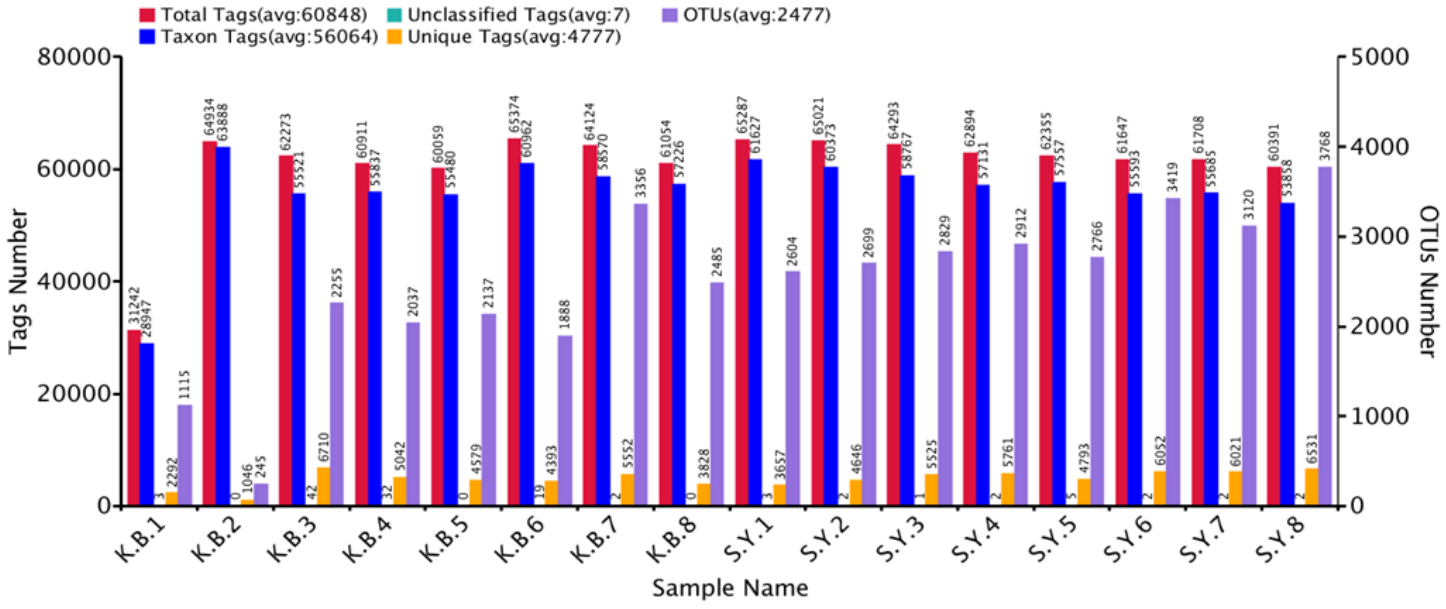

Figure 1

OTUs cluster quantity diagram of soil microbial population in Blank group and Experimental group

Figure 2

OTUs clustering numbers of microbial populations at different classification levels in blank group and experimental group soil samples

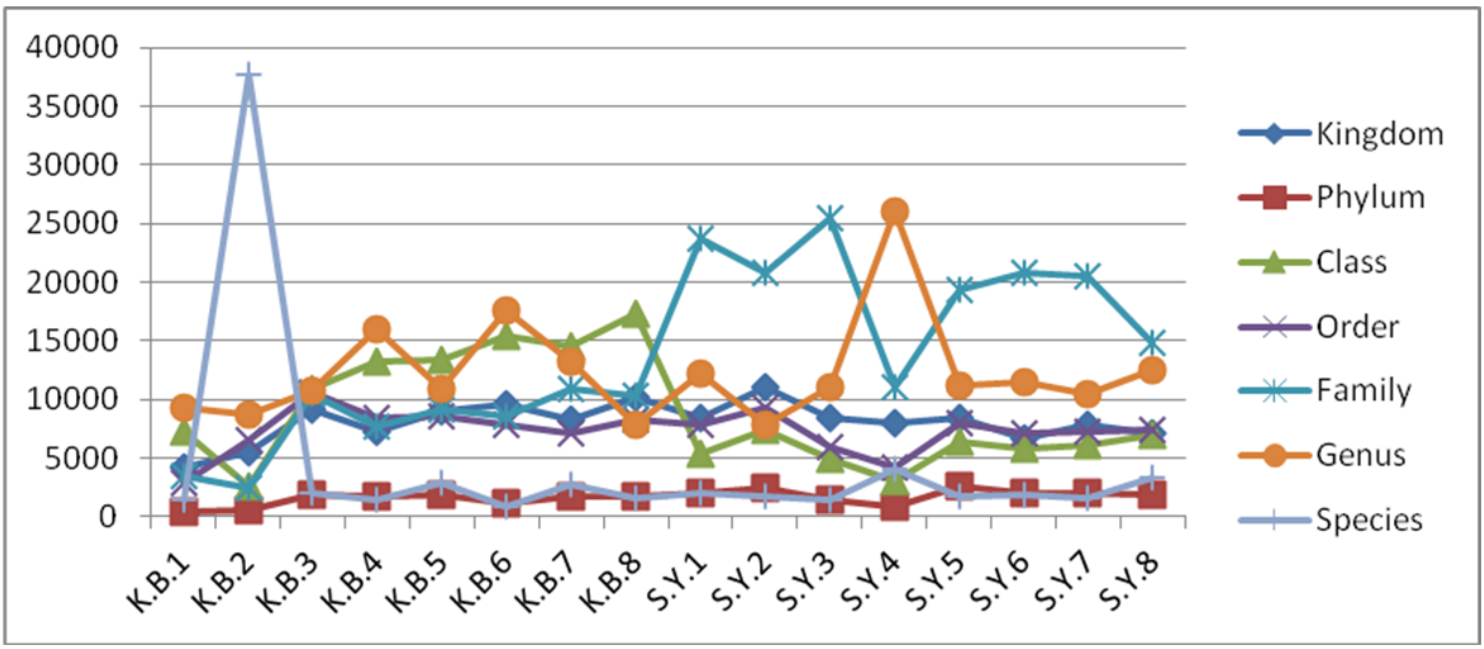

Figure 3

OTUs cluster number curve diagram of microbial populations at different classification levels in blank group and experimental group soil samples

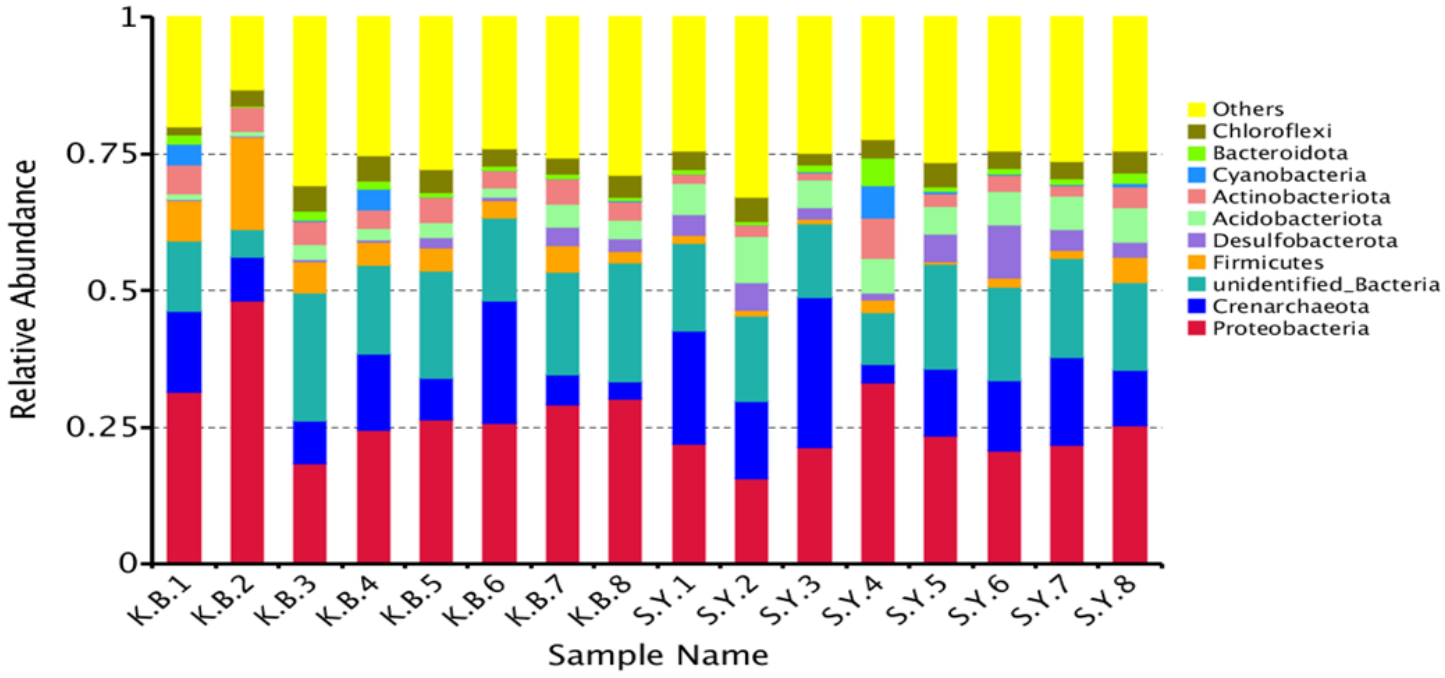

Figure 4

Bar chart of species relative abundance at the level of each Phylum

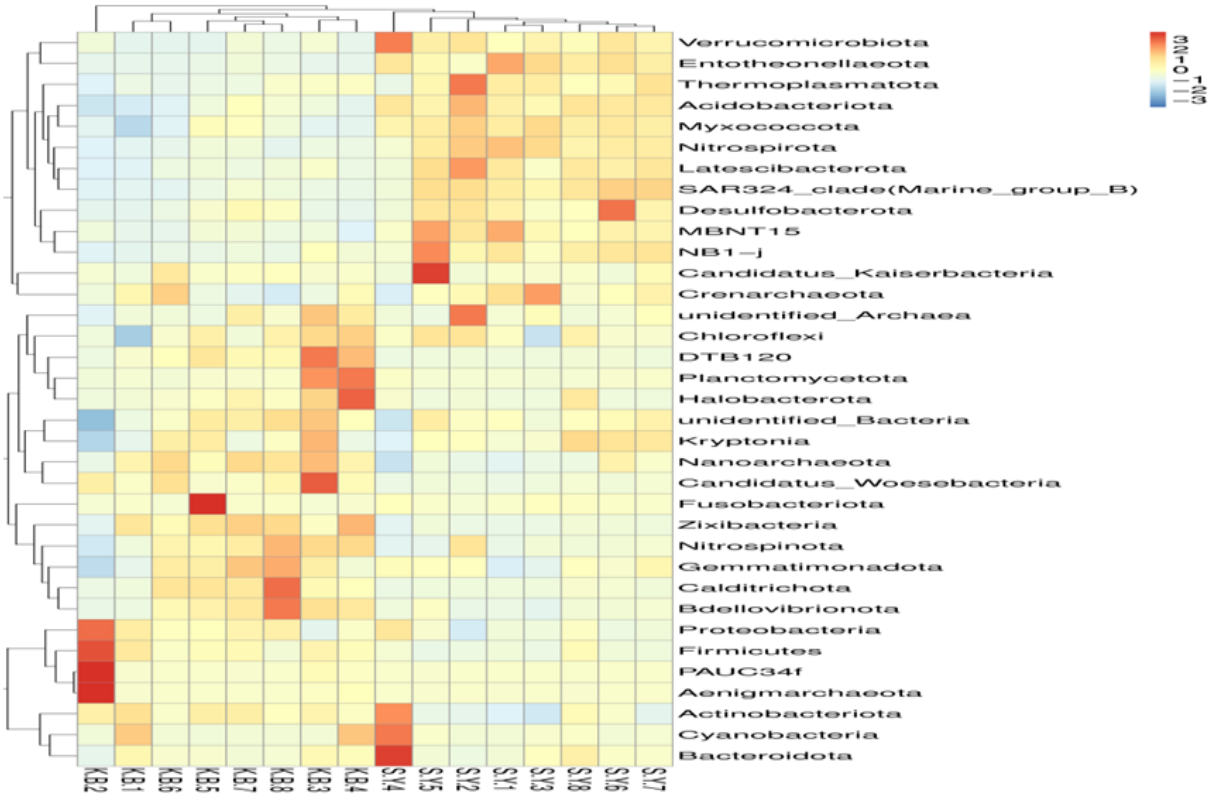

Figure 5

Cluster thermograms of species at the level of each Phylum

Figure 6

Bar chart of species relative abundance at level of each Order

Figure 7

Cluster thermograms of species at the level of each Order

Figure 8

Bar chart of species relative abundance at level of each Family

Figure 9

Cluster thermograms of species at the level of each Family

Figure 10

Bar chart of species relative abundance at level of each Genus

Figure 11

Cluster thermograms of species at the level of each Genus

Figure 12

Ternaryplot analysis at the classification level of each sample Phylum

Figure 13

Ternaryplot analysis at the classification level of each sample Order

Figure 14

Ternaryplot analysis at the classification level of each sample Genus

Figure 15

Gram staining results of Pseudomonas strain

Figure 16

Fluorescence production characteristics of Pseudomonas strains

Figure 17

Gram staining results of Bacillus strain

Figure 18

Figure of Colony PCR amplification of Pseudomonas fluorescens and Bacillus thuringiensis

Note: M is the DNA Marker indicator band of DL2000, 1-5 were pseudomonas fluorescens PCR results, and 7-11 were Bacillus thuringiensis PCR results.