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Microbiome-metabolome analysis directed isolation of rhizobacteria capable of enhancing salt tolerance of Sea Rice 86

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Runing Title: PGPR induced salt tolerance of plants

Microbiome-metabolome analysis directed isolation of rhizobacteria capable of enhancing the salt tolerance of Sea Rice 86 (SR86)

Abstract:

Introduction: Sea rice 86 (SR86) is a new rice cultivar domesticated from a wild strain which could grow in saline-alkaline soil. The application of SR86 is significant for saline soil restoration, crop production and food security. However, there are knowledge gaps on the salt tolerance mechanisms in SR86, especially the roles of rhizobacteria.

Objective: To illustrate the effects of salt stress on the rhizosphere ecology, the rhizobacterial community diversity and rhizosphere metabolites composition of SR86 seeding plants under salt stress were analyzed, and the candidates of plant growth promoting rhizobacteria were isolated to explore their application in inducing plant salt tolerance.

Methods: Stable salt stress conditions were established using a pot-scale method with diluted seawater. Rhizosphere soil and plants of SR86 cultivated under different salinities were collected. The rhizobacterial diversity was analyzed via high throughput sequencing and bioinformatics, the rhizosphere metabolites composition was determined using LC/MS analysis, and the correlation between rhizobacteria and rhizosphere metabolites was further determined. Cultivation-dependent methods was applied to isolate salt tolerance PGPR.

Results: SR86 could grow under salinity 0.4% and salt stress significantly changed the rhizobacterial diversity and rhizosphere metabolites composition of SR86. The constructed co-occurrence network of rhizobacteria under salt stress revealed the keystone taxa potentially involved in the salt tolerance of SR86, and the correlation analysis of keystone taxa and rhizosphere metabolites demonstrated that some specific compounds might play an important role in plant-microbe interaction mediated plant salt tolerance. Further, four rhizobacterial strains, capable of inducing plant salt tolerance, were isolated and characterized.

Conclusion: Salt stress significantly changed the rhizosphere bacterial and metabolites composition of SR86. PGPRs induced the seedling plant salt tolerance of SR86.

Keywords: Sea rice 86, Rhizobacteria, Microbiome, Metabolome, PGPR, *Bacillus*

Introduction

Food security is a major global sissue because of the increasing population, global warming and decling arable land. Soil salinization has been recognized as one of the main factors causing the the decrease in cultivated land area. Approximately 20% of global agriculture land is affected by soil salinization [1-3]. Salinity has become the major cause of reductions in plant productivity and the degradation of land [4, 5]. Globally, approximate 5.2 billion hectare of fertile land is affected by salinity and soil salinity is claiming about three hectares of arable land from conventional crop farming every minute [6, 7]. Therefore, research on the restoration and utilization of salinized land is of great importance and timely.

As one of the major routes of saline soil restoration and utilization, promoting plant growth under saline conditions has great significance. Specifically, the investigation of salt-tolerant rice (*Oryza sativa*) is attracting wide attentions since rice is a worldwide important cereal crops for human consumption [8]. In the past decades, a variety of measures has been explored to improve the salt tolerance of rice by breeding salt tolerant plant varieties, through genetic engineering technology, and by application of beneficial microbes [9, 10]. Although huge attempts have been made on salt tolerant plant breeding, the progress for increasing salinity tolerance or crop yield by such approaches was slow and the potential reasons have been summarized by Qin et al. [4]. Meanwhile, the application of genetically modified crops has faced more difficulties such as security and legislative barriers. Thus, the understanding and developing of plant salt tolerance from the ecological perspective is becoming more promising. Now, it is well-acknowledged that plant fitness and adaptation is not only related to the plant itself but might also be significantly related to multiple biotic factors of the environment [7, 11, 12].

Plants depend upon beneficial interactions between roots and microbes for nutrient uptake, growth promotion, and defence against adversity stress [13]. Roots provide three separate rhizo-compartments for microbe colonization: the endosphere (root interior), rhizoplane (root surface), and rhizosphere (soil close to the root surface), each of them has been found to harbor a distinct microbiome [14]. The plant rhizosphere is a complex ecosystem inhabited by numerous microbes (primarily bacteria and fungi) which play an important role in the process of plant adaptation to osmotic stress [15, 16]. The rhizosphere microbes have tailored their community assembly and structure to specific environmental pressures and have coevolved with the hosts during plant fitness and adaptation [14, 17, 18]. The beneficial rhizobacteria are dominated by plant growth promoting rhizobacteria (PGPR) and endophytic microbes

such as abuscular mycorrhizal fungi (AMF), dark septate endophytes (DSEs), *Trichoderma* spp. and *Serendipita indica* [4]. Since PGPR showed remarkable positive effects on crop yield and fitness, they have been extensively developed as biofertilizers for several years [19-21].

Recently, researchers have attempted to illustrate the potential contribution of rhizosphere microbes to plant salt tolerance by rhizosphere microbiome and metabolome (mainly focus on root excludes and microbial metabolites) [22-26]. Great efforts have been made to illustrate the direct and indirect mechanisms underlying the rhizosphere bacteria and fungi that confer plant salt tolerance. Numerous PGPR strains have been isolated and characterized, including genus of *Bacillus*, *Pseudomonas*, *Azospirillum*, *Enterobacter*, *Ochrobactrum*, and *Agrobacterium* (references). Some PGPR are directly or indirectly involved in the regulation of plant endogenous hormone levels by producing aminocyclopropane-1-carboxylate deaminase (ACC deaminase) or phytohormones, and ultimately reprogram the hormone signaling in plants [27] [28] [29] [30] [31]. Most of the isolated PGPR are capable of increasing the antioxidative systems in plants through reactive oxygen species (ROS) scavenging with some antioxidant enzymes such as superoxide dismutase (SOD)[32], peroxidase (POD) [33], catalase (CAT) [34]. Further, PGPR might produce some important osmolytes (proline and polyamines) to protect plants from osmotic stress[35, 36]. PGPR generated exopolysaccharides (EPSs) might ameliorate salinity stress in plants, since EPSs can bind with the toxic Na^+ which will restrict Na^+ influx into roots[2] and benefit the PGPR colonization on roots[37]. To reduce the toxic effects of Na^+ in plants under saline conditions, PGPR can regulate the expression or status of some specific membrane proteins in roots such as high-affinity K^+ transporter (HKT)[38] and plasma membrane integral proteins (PIPs)[39] which could influence the influx and efflux of Na^+, K^+, Ca^{2+}, and water. Meanwhile, PGPR could alleviate plant osmotic stress via activating the signaling pathways[40], promoting metabolic efficiency[41], and producing some bioactive compounds[42].

Sea rice 86 (SR86) is a new rice cultivar domesticated from a wild strain of rice which was first found in 1986 in sea water submerged, saline-alkaline soil near the coastal region of Zhanjiang City, Southeast China[43, 44]. After more than 30 years of breeding and selection, SR86 retains many unique features such as the ability to grow in saline-alkaline and infertile soil, submergence and water logging tolerance, disease and pest resistance, and can grow in marginal lands while producing meaningful yields. To have an insight into the salt tolerance mechanisms of SR86, whole genome sequencing and transcriptome analysis of SR86 have been conducted and several candidate genes related to salt

adaptation have been identified which might be valuable for further functional investigation [43]. However, the potential contribution of rhizobacteria to the salt tolerance of SR86 remains underexplored.

In this study, we examined the rhizosphere bacterial diversity and soil metabolome of SR86 under different salinity. The purposes of this study were to: i) determine the influences of salt stress on SR86 rhizobacterial community and metabolome; ii) construct the rhizobacterial co-occurrence network of SR86 under salt stress and further examine the relationship between keystone taxa and rhizosphere metabolites; iii) isolate and characterize PGPR capable of inducing seedling plants salt tolerance of SR86. These findings might provide novel insights into the mechanisms of PGPR induced plant salt tolerance as well as novel strategies for alleviating plant salt stress and augmenting yield of plants.

2. Materials and methods

2.1. Rice seeds, soil and seawater

The seeds of SR86 were kindly provided by Professor Risheng Chen. The seeds of SR86 were germinated at 30 °C and then grown in the farmland around Guangdong Ocean University and normal seedling management was performed. When the seedling plants grew to the trifoliate stage (approximately 16 days after planting), the rice seedlings showing similar status were selected for the following assays. To make the experimental conditions close to the real situation, the diluted seawater was selected as the irrigation water in this study. Seawater was collected from Zhanjiang Bay (Zhanjiang, China) and the characteristics of seawater are presented in Table S1 (Suppementary Materials). The experimental soil was collected from rice-planting farmland and the detailed information of soil is presented in Table S1.

2.2 Establishment of culture conditions and determination the salt tolerance of SR-86

The pot experiments were conducted in plastic pots (110 cm length by 60 cm width by 50 cm depth) under controlled conditions at Guangdong Ocean University, and the schematic diagram of pot experiment is shown in Figure S1. Prior to the pot experiments, the collected seawater was filtered with gauze to remove the particles and the collected soil was sieved with a 4-mm-pore size mesh. Then, the soil was mixed and covered with diluted seawater (diluted with ddH_2O). The salinity of water phase was adjusted to the targeted range (0-?). The prepared soil and seawater mixture was kept at room temperature overnight, and then the salinity of water phase was recorded. The salinity of water phase was maintained at the initial salinity approximately, by adding sterilized pure water or diluted seawater. Finally, the stable

salt stress conditions with different salinity was constructed.

To determine the salt tolerance range of SR86, the survival rates of SR86 seedling plants under different salinities was measured through serial salinity cultivation assays. Six salinity gradients (0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.6%, *w*/*v*) and one control treatment (ddH2O served as the irrigation water) were employed to determine the salt tolerance of SR86 and the pots were prepared as described above, all in triplicate. When the seedling plants of SR86 grew to the trifoliate stage, the rice seedlings were removed to the pots with target salinity to obtain 20 plants per pot (Figure S1, 5×4, the distance of plants was set as ~12 cm). After that, all pots were cultivated under controlled conditions and the salinities of all pots were measured, recorded and adjusted every day. After 15 days cultivation, the salinities with survival rates above 80.0% were selected for the following assays.

2.3 Experimental design and sample collection

Based on the detected the survival rates of SR86 under different salinities, two groups of rhizosphere soils, non-salt stressing group (NSS, irrigated with ddH_2O, S0) and salt stressing group (SS, S2 (salinity of 0.2%, *w*/*v*) and S4 (salinity of 0.4%, *w*/*v*)), were selected for metabolome and microbiome analysis. Briefly, the pot experiments with S0, S2 and S4 were conducted as described above. Seeds of SR86 were germinated and subsequently grown to trifoliate stage. Then, twenty rice seedlings with the same status in trifoliate stage were planted in each pot and all treatments were performed in triplicate. The rhizosphere soils of survived plants in each treatment were sampled after 15 days cultivation. Meanwhile, the original bulk soil (BS) was also collected for the microbiome analysis. For the rhizosphere soil sample collection, the attached soil was removed by gentle shaking and then the soil attached to the root was collected. All samples were packed into polyethylene bags and transported on ice packs (4 °C) to the laboratory. In total, 108 rhizosphere samples (3 salinities × 3 pots per salinity×12 plants per pot) and 3 bulk soil samples were collected. Randomly, 6 rhizosphere samples in each pot were merged as one sample. Each sample was then divided into three parts: one was applied for soil characteristics analysis, one was used for DNA extraction, and the rest was applied for metabolome analysis. After thorough homogenization, all soil samples were stored at -80 °C for further anaslysis.

2.4 Rhizosphere soil metabolites analysis

For each sample, 0.1 g of freeze-dried soil was added to an EP tube, and 500 μL extract solution (acetonitrile: methanol: water = 2: 2: 1) containing isotopically-labelled internal standard mixture (???) was added. After 30 s vortex, the mixture was extracted by ultrasound with a working frequency of 45

kHz (5 min) on an ice-water bath. The mixture was incubated at -40 °C for 1 h and centrifuged at 12,000 rpm for 15 min at 4 °C. Subsequently, supernatants were transferred to a fresh tube dried in a vacuum concentrator at 37 °C. Then, the resulting extracts were redissolved in 200 μL of 50% acetonitrile by sonication for 10 min in ice-water bath. The solution was then centrifuged at 13,000 rpm for 15 min at 4 °C, and 75 μL of supernatant was transferred to a fresh glass vial for ultra-high pressure liquid chromatography-mass spectrometry (UHPLC/MS) analysis. The quality control sample was prepared by mixing an equal aliquot of the supernatants from all of the samples.

The UHPLC separation was performed with a Exion LC Infinity series UHPLC System (AB Sciex), equipped with a UPLC BEH Amide column (2.1 mm × 100 mm ×1.7 μm, Waters). The mobile phase consisted of 25 mmol/L ammonium acetate and 25 mmol/L ammonia hydroxide in water（pH = 9.75）(A) and acetonitrile (B). The analysis was carried out with elution gradient as follows: 0-0.5 min, 95% B; 0.5-7.0 min, 95%-65% B; 7.0-8.0 min, 65%-40% B; 8.0-9.0 min, 40% B; 9.0-9.1 min, 40%-95% B; 9.1-12.0 min, 95% B. The column temperature was maintained at 25°C. The auto-sampler temperature was 6 °C, and the injection volume was 2 μL.

The Triple-TOF 5600 mass spectrometry (AB Sciex) was used for its ability to acquire MS/MS spectra on an information-dependent basis (IDA) during an LC/MS experiment. In this mode, the acquisition software (Analyst TF 1.7, AB Sciex) continuously evaluates the full scan survey MS data as it collects and triggers the acquisition of MS/MS spectra depending on preselected criteria. In each cycle, the most intensive 12 precursor ions with intensity above 100 were chosen for MS/MS at collision energy (CE) of 30 eV. The cycle time was 0.56 s. ESI source conditions were set as following: Gas 1 as 60 psi, Gas 2 as 60 psi, Curtain Gas as 35 psi, Source Temperature as 650 °C, Declustering potential as 60 V, Ion Spray Voltage Floating (ISVF) as 5000 V or -4000 V in positive or negative modes, respectively. MS raw data (.wiff) files were converted to the mzXML format by ProteoWizard, and processed by R package XCMS. The process includes peak deconvolution, alignment and integration. Minfrac and cut off are set as 0.5 and 0.3 respectively. In-house MS2 database was applied for metabolites identification.

2.5 High throughput sequencing and bioinformatics analysis

Approximately 0.5 g soil sample was used for DNA extraction with the Power Soil DNA kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's instruction. For one sample, DNA extracts in triplicate were merged, quantified and qualified using a NanoDrop® TM ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, United States). The full length of 16S rRNA

genes were amplified using the primers 27F/1492R. PCR amplicons were purified, quantified, and pooled in equimolar concentrations to form a composite DNA sample. Sequencing was performed on an PacBio RS II platform at Biomarker Technologies CO. Ltd. (Beijing, China).

Raw reads were filtered using the Trimmomatic (v0.33)[45], paired-end reads were assembled using FlASH (v1.2.11)[46], and chimera sequences were removed through UCHIME (v 8.1)[47]. Reads were de-replicated and singletons were excluded to yield quality reads. The quality sequences were clustered into operational taxonomic units (OTUs) with a 97% similarity cutoff using UPARSE (v10.0)[48]. Representative sequences in each OTU were taxonomically clustered using the Ribosomal Database Project (RDP) classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (release128, http://www.arb-silva.de) 16S rRNA database using a confidence threshold of 70%. Normalized reads were used in all the subsequent analysis. The OTUs with a relative abundance ≥1% of each sample were defined as abundant taxa[49].

2.6 Bioinformatics analysis

The alignment of the rarefied OTU table was accomplished via pyNAST (v1.2.2)[50]. Bacterial α-diversity indexes, such as Chao1 richness estimator (Chaol), Ace richness estimator (Ace), Shannon-Wiener diversity index (Shannon), and Simpson diversity index (Simpson), were calculated from the representative OTU table via QIIME2[51]. Principal co-ordinates analysis (PCoA), based on unweighted Unifrac metrics of abundant communities, were performed to determine the bacterial community structures (β-diversity) among the samples. Redundancy analysis (RDA) was exploited to analyze the relationship between significant factors and the bacterial community compositions. Nonparametric multivariate analysis of variance (Adonis) and analysis of molecular variance (AMOVA) were conducted to determine the differences of the bacterial communities among different treatments.

The co-occurrence network was constructed with the ‘WGCNA’ R package by using the Spearman correlation. The correlations between pairs of OTUs and the corresponding p-value were calculated, and the p-values were adjusted with the method of false discovery rate (FDR). The adjusted p-values with a cutoff value ≥ 0.001 were applied for the construction of co-occurrence network. The topological parameters of the constructed networks were calculated and visualized via the interactive platform Gephi (v.9.2)[52]. Keystone species were selected as the OTUs with the highest ‘between centrality’ and with a ‘closeness centrality’ higher than 0.41[53-55]. Further, the Spearman correlation matrix of identified metabolites with the keystone taxa were obtained by using the R package psych (v.1.8.12). The

correlations between metabolites and OTUs were calculated as well as their *p*-values. The relationship between metabolites and keystone taxa with FDR-adjusted *p*-values had a 0.05 cutoff. Subsequently, we selected metabolites, which were tightly correlated with keystone taxa for generating a heatmap plot using the R package 'pheatmap'. All the bioinformatics analyses were performed in R software version 3.4.0 (R Core Team, 2013).

2.6 Isolation, identification and characterization of potential PGPR in plant salt tolerance

The schematic diagram for the isolation and confirmation of potential PGPR contributing to plant salt tolerance is shown in Figure 1. The modified media for the isolation were prepared according to the predicted keystone taxa information with Known Media Database (KOMODO, http://komodo.modelseed.org/)[56]. The detailed information of rhizobacteria isolation media (RIM) was presented in Table S2. The rhizosphere soil of alive plants with salinity 0.4% and 0.5% was used for the isolation. Approximately 0.5 g rhizosphere soil was added into 5 mL sterilized water. After fully mixing by vortex, the water phase was were serially diluted and spread onto the solid RIM. Single colonies on the plates were selected and inoculated into liquid RIM. After 48 h incubation (160 rpm, 30 °C), cells were harvested by centrifugation (6,000 rpm, 5 min) and resuspended in phosphate buffer solution (PBS, 0.2 M, pH 7.2). The centrifugation and washing were repeated for three times. The cell pellets were finally resuspended in PBS (0.2 M, pH 7.2) to obtain a concentration of OD_{600} = 1.0 and served as inoculums. The rice seedlings of SR86 were prepared as described above and planted in soil with a salinity of 0.7%. Subsequently, 1 mL of the prepared inoculum was inoculated into the rhizosphere of SR86 while the inoculation of same volume of PBS was served as control treatment. All the plants were cultivated and managed as described above. The status of each plant was recorded daily and the withered plants were abandoned. After 10-day cultivation, inoculants ensuring the rice seedlings alive were selected as candidates, and the candidates were confirmed by repeating the steps above. After 5 times of functional confirmation, the isolates potentially induced the plant salt tolerance were applied for bacterial identification. Meanwhile, re-isolation of target strain was conducted with the inoculated rhizosphere soil to verify whether the target strain colonized and survived in rhizosphere. Finally, the bacterial strains, capable of promoting the salt tolerance of SR86 and colonizing in its rhizosphere, were selected for further evaluation and characterization.

2.8 Analytic methods

The soil characteristics, including pH, concentration of Na^+, soil total potassium (TK), total

phosphorus (TP) and total nitrogen (TN), were measured to quantify the impact of salt stress on the rhizosphere soil SR86 seedling plants. Soil pH was measured with a fresh soil to water ratio of 1:5 using a pH monitor (FE28, Mettler Toledo, Shanghai, China). Prior to soil characteristics analysis, soil was air dried and sieved (1 mm mesh). The concentration of Na^+ in soil was determined by atomic absorption spectrometry (iCE 3300, Thermo Scientific, Waltham, USA). Soil total potassium (TK) was measured by an inductively coupled plasma (ICP, Prodigy XP, Leeman Labs, USA). Soil total phosphorus (TP) and total nitrogen were measured by an Elemental Analyzer (LECO CNS-2000, St. Joseph, MI, USA).

For the collected plants, the dry weight of the plants was measured. The content of Na^+, K^+ and Ca^{2+} in dried leaves and roots were measured by an ICP (Prodigy XP, Leeman Labs, USA) while the concentration of Cl^- in dried leaves and roots was determined by an ion chromatograph (ICS-4000, Dionex, USA). Total free proline content in fresh root was measured according to the method of Bates[57]. The chlorophyll content in the fresh leaves was determined according to the method of Moran and Porath[58]. Malondialdehyde (MDA) in fresh roots was extracted and quantified following the method of Alexander[59].

2.8 Accession numbers

Strain RL-WG26, RL-WG62, RL-WG133, and RL-WG347 have been deposited in Guangdong Microbial Culture Collection Center (GDMCC) under accession numbers of GDMCC 61956, GDMCC61835, GDMCC 61955, and GDMCC 61957, respectively. The 16S rRNA gene of strain RL-WG26, RL-WG62, RL-WG133, and RL-WG347 are accessible in GenBank with accession number of MZ318256, MZ407651, OK275042, and OK275101, respectively. Raw sequence data for the rhizosphere and bulk soil bacterial community is accessible in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (RSA) with accession number PRJNA772703. The raw metabolite data for the soil metabolites is available from supplemental data1.

3. Results and Discussion

3.1 Metabolite characteristics of rhizosphere soils

A total of 3,358 metabolites from 18 rhizosphere soil samples were detected by LC-MS/MS (Table SX). The orthogonal partial least squares discrimination analysis (OPLS-DA) demonstrated a clear separation between SS group and NSS group (Figure 2A). According to OPLS-DA results(variable importance in projection [VIP] > 1.0, p < 0.01), we obtained 1,189 differentially expressed rhizosphere

soil metabolites between different groups and the pattens of differentially expressed metabolites were dominant by up-regulated metabolites under salt stressing (Table SX). A Venn diagram was used to demonstrate the distribution and relationship of differential metabolites between groups (Figure 2B). Principal component analysis (PCA) also revealed the clearly different patterns of rhizosphere soil metabolites between groups of SS and NSS (Adonis, $p < 0.05$, Figure 2C). Among these detected metabolites, 1,002 known metabolites were identified using known MS/MS database (Table SX). And 164 of these identified metabolites were further annotated to 32 specific categories (Table S4) via search against human metabolome database (HMDB), including glycerophospholipids (68), carboxylic acids and derivatives (10), fatty acyls (8), et al. By annotating with Kyoto Encyclopedia of Genes and Genomes database (KEGG), 181 of the identified metabolites were assigned to 71 metabolic pathways (Table S5), including glycerophospholipid metabolism (70), linoleic acid metabolism (40), alpha-Linolenic acid metabolism (39), arachidonic acid metabolism (39), et al. It is noteworthy that, compared with NSS group, the abundance of lipids and lipid related metabolites in SS groups were upregulated. HMDB and KEGG annotation suggested that the differentially expressed rhizosphere soil metabolites were correlated with lipid metabolism.

The rhizosphere is densely populated with a variety of organisms, including nematodes, fungi, bacteria, and arthropod herbivores[60]. These organisms could interact with plants via various routes and further confer specific functions to the plants, such as modulating plant growth, phytopathogen defense, against the environmental stress, et al[22]. Interactions between plants and rhizosphere organisms are mainly achieved via chemical communication[25]. Metabolic profiles in rhizosphere soil contain an array of primary and secondary metabolites from plants and rhizosphere community members. The rhizosphere metabolome changed dynamically according to plant developmental stages, genotypes, and environment conditions, which might play an important role in plant-soil feedback, shaping the composition of the rhizosphere microbiota via recruiting or expelling specific microbial species[61]. Thus, metabolites diversity in rhizosphere soil may determine microbial diversity in soils and govern the plants' survival under abiotic and biotic stresses. The correlation analysis of rhizosphere metabolome and specific microbiomes will provide novel insights into the contribution of microbial communities to plant growth and stress resistance. As metabolic profile and microbial diversity of rhizosphere could be influenced by abiotic and biotic stresses, we thus examined the rhizosphere soil metabolome and microbiome of SR86 from different salinities. Our results may illustrate the influences of salinity stress on rhizosphere

metabolome and microbiome and also shed light on the pivotal importance of small molecular metabolites and specific species in enhancing the salt tolerance of SR86.

The metabolome analysis suggested that metabolites in rhizosphere soils from SR86 plants with different salinities were highly complex mixtures, and the metabolites composition in the rhizosphere soil showed significant differences between SS group and NSS group. Meanwhile, the statistical analysis revealed that the differentially expressed rhizosphere metabolites were dominated by lipids and their derivatives, demonstrating the vital role of lipids in the response to salt stress. Numerous studies on belowground plant-microbe interactions have already been conducted and lots of works have already demonstrated that the rhizosphere of plants plays an important role in the process of plant-microbe communication. Since other forms of communication are not feasible belowground, the plant-microbe communication was mainly achieved via chemical communication[25]. Thus, metabolomics analysis of rhizosphere can potentially help us to better understand the dialogue between plants and rhizosphere organisms during abiotic stress. Lipids are vital components of plasma membrane, as well as one predominant category of rhizosphere metabolites, which could facilitate abiotic stress adaptation of plants. Certain lipids have been found to function in response to salt stress[62, 63]. It has been reported that up-regulation of lipid metabolism and glycine betaine synthesis are associated with choline-induced salt tolerance in halophytic seashore paspalum[64]. Meanwhile, elevated concentration of choline in rhizosphere soil was induced by salt stress which indicated that choline and lipid were potentially contributed to the salt tolerance of SR86. The investigation of *Pseudomonas putida* KT2400 suggested that lipopolysaccharide and/or exopolysaccharide modification can be relevant for KT2440 to cope with salinity stress in vitro and in association with plants[65]. Phosphoinositides have been demonstrated to be involved in the complex networks of plant growth and adaptation, including salt and osmotic stress[63, 66]. Although studies about the roles of lipids in plant root-microbe interactions have come a long way, the contribution of lipids in rhizosphere to plant salt tolerance is still underexplored and further investigation is needed to elucidate the underlying mechanisms of lipids-contributed salt tolerance of plants.

3.2 Bacterial community compositions in rhizosphere soils

A total of 137,143 effective circular consensus sequences (CCSs) were obtained from 12 rhizosphere soil samples. The numbers of CCS per sample ranged from 10,069 to 14,559 (average = 11,429 CCSs). The length of CCSs ranged from 1,456 bp to 1,463 bp (average length = 1,459 bp). The

detailed information of the obtained CCS was presented in Table S6. The effective CCSs were clustered into 2,272 OTUs, with a mean of 1,617 OTUs per sample (min=1,384, and max=1,789). A Venn diagram was used to demonstrate the distribution of OTUSs in differential groups (Figure 3A). The top six phylum with the highest relative abundance were *Proteobacteria* (26.3%), *Firmicutes* (10.4%), *Desulfobacterota* (9.2%), *Verrucomicrobiota* (5.6%), *Acidobacteriota* (5.2%), and *Nitrospirota* (4.6%) (Figure 3B). Specifically, the relative abundance of *Firmicutes* was significant higher in bulk soil and NSS group, while the relative abundances of *Proteobacteria* and *Desulfobacterota* were significant higher in SS groups. The α-diversity indexes indicated that rhizosphere soil bacterial community highly differed from bulk soil bacterial community, and salt stress increased the α-diversity of rhizobacterial community (Figure 3C and Table S7). Principal coordinates analysis (PCoA) indicated that the bacterial community compositions of different treatment exhibited significant differences (Figure 3D). RDA score plot was used to illustrate the relationships between the rhizosphere soil physiochemical properties and bacterial communities (Figures 3E and 3F). The typical eigenvalues of Axis 1 and Axis 2 were 47.66% and 15.26%, respectively. The impacts of each physiochemical property on rhizosphere bacterial communities were represented by the length of arrows, and the cosine angle between arrows illustrated their relationship (smaller angle indicated more significant correlation). The concentration of Na^+ was negatively correlated with K^+ concentration. The rhizosphere soil bacterial communities of S4 group were significantly correlated with Na^+ concentration while the rhizosphere soil bacterial communities of S0 group were significantly correlated with K^+ concentration. Further, the rhizosphere K^+ concentration was positively correlated with genus of *Vicinamibacter*, Candidatus_*Magnetoovum*, Candidatus_*Udaeobacter*, and *Bacillus*, while the rhizosphere Na^+ concentration was positively correlated with genus of *Thiobacillus*, *Sideroxydans* and *Geobacter* ($p < 0.05$).

Table 1 A summary of representative salt tolerance PGPR

Phylum taxa	Genus	PGPR isolates	Plant species	References
Firmicutes	*Bacillus*	*B. subtilis* GOT9	Arabidopsis	[67]
	Bacillus	*B. amyloliquefaciens* SQR9	Rice	[68]
	Bacillus	*B. amyloliquefaciens* NBRISN13	Maize	[69]
	Bacillus	*B. pumilus* SB1-ACC3	Rice	[27]
	Bacillus	*B. aryabhattai* RS341	Canola	[1]
Proteobacteria	*Pseudomonas*	*P. frederiksbergensis* OS261	Red pepper	[70]
	Pseudomonas	*P. fluorescens* 002	Maize	[71]
	Pseudomonas	*P. putida* R4	Cotton	[72]
	Enterobacter	*Enterobacter* sp. SA187	Arabidopsis	[73]
	Enterobacter	*E. cloacae* W6	Wheat	[74]
	Enterobacter	*E. aerogenes* S14	Maize	[75]
	Ochrobactrum	*O. pseudogregnonense* IP8	Wheat	[76]
	Ochrobactrum	*Ochrobactrum* sp. TH－N-29	Rice	[27]
	Azospirillum	*A. lipoferum* GQ 255949	Wheat	[77]
	Azospirillum	*A. brasilense* NO40	Barley	[78]
	Agrobacterium	Agrobacterium	Rice	[79]

Although the communities of rhizobacteria are mainly developed from the bulk soil communities, research has demonstrated that the communities of rhizobacteria showed significant differences with the bulk soil communities[80]. Further, abiotic stress could alter the rhizobacterial composition and their relative abundance[81, 82], and these changes might be beneficial for counteracting salinity stress in plants[83-86]. In the present study, the statistics analysis revealed that the α-diversity indexes of bulk soil bacteria showed significant differences with rhizobacteria (Figure 3C) and the α-diversity indexes of rhizobacteria in SS group is higher than the α-diversity indexes of NSS rhizobacteria, which is in line with Yang and Santos who proposed that salinity stress might increase the diversity of rhizobacterial communities[26, 85]. Further, the microbiome analysis demonstrated that *Proteobacteria*, *Firmicutes*, *Desulfobacterota*, *Verrucomicrobiota*, *Acidobacteriota*, and *Nitrospirota* are dominant phyla in bulk soil and rhizosphere soil (Figure 3B). Among these dominant phyla, *Proteobacteria*, *Firmicutes* and *Actinobacteria* taxa are ubiquitously distributed in the rhizosphere microbiome, which suggested that they could well inhabit in rice root niches[87-90]. In accord with the reports by Canfora[91], Morrissey and Franklin[92], and Rath[93], rhizosphere *Proteobacteria* was found to be associated with high salinity, which indicated that *Proteobacteria* might harbor a high adaptability to salt stress, or *Proteobacteria* could proliferate since the salt sensitivity of its antagonists[94]. Meanwhile, in line with report by

Ding[90], we found *Acidobacteria* and *Chloroflexi* in higher relative abundance. Further, we observed that *Firmicutes* was associated with low salinity, which is in line with Santos[85]. Lots of studies demonstrated that several specific taxonomic groups (Table 1), including endophytes and rhizobacteria, are associated with the tolerance of increased salinity and could strengthen the salt tolerance of plants[90, 93, 95, 96]. In addition, the concentration of rhizosphere soil Na^+ and K^+ was found to be correlated with rhizosphere soil bacterial communities, as shown by RDA analysis (Figure 3E and Figure 3F). Our results agree with previous studies[97], suggesting that salt stress could change Na^+/K^+ ration of rhizosphere soil and therefore affected the rhizosphere bacterial community composition and diversity. The responses of rhizosphere bacterial taxa to salt stress varied from species, and some of these species were negatively correlated with rhizosphere Na^+ concentration and positively correlated with rhizosphere K+ concentration, which might be involved in inducing the salt tolerance of plants. To identify the rhizobacteria potentially induced the salt tolerance of plants, further analysis and characterization were needed.

3.3 Co-occurrence network and metabolites correlated with keystone species

To have a further insight into the effect of salt stress on rhizosphere soil bacterial communities, we constructed rhizobacterial co-occurrence networks based on the correlation analysis of OTUs in NSS and SS groups (bulk soil was excluded). The bacterial co-occurrence network of all rhizobacteria is composed of 74 nodes (OTUs) and 218 edges (links), including 191 (87.61%) positive and 27 (12.39%) negative interactions (Figure 4). The network has a diameter of 7, an average clustering coefficient of 0.717, an average path length of 2.528, and a modularity index of 0.785. The modularity index is larger than 0.4, suggesting that the real-world network has a modular structure potential keystone taxon[52]. A total of 8 modules were obtained from the network. Module 3 interacted with module 5 via OTU112 (*Bacillus acidiceler*), OTU242 (*Geobacter* sp.) and OTU1329 (*Sphingoaurantiacus* sp.), while module 1 interacted with module 6 via OTU1526 (*Paludibacter propionicigenes*), OTU1859 (*Spirochaeta aurantia*), OTU1503 (Candidatus_*Saccharimonas aalborgensis*), and OTU183 (*Geobacter* sp.). Fifteen species, which were distributed in four modules (module 1, 3, 5, and 6), were proposed as keystone taxa with a ‘closeness centrality’ higher than 0.41 and a ‘between centrality’ higher than 25 (Table S8).

The correlations between keystone taxa and metabolites with significant differences between SS and NSS groups were calculated via Spearman’s correlation analysis ($p<0.01$, Figure 5). Specifically, the metabolites were dominated by lipids and lipid related metabolites (79.3%). Meanwhile, several

exopolysaccharides and ion channel regulators were highly correlated with keystone taxa, including meta844 (indanyloxyacetic acid 94, IAA-94), meta97 (choline), meta1472 (CGS_7181), meta76 (3-methylellagic acid 8-(2-acetylrhamnoside), meta1631 (CDP-abequose), meta2902 (cholesteryl-β-D-glucoside), and meta2676 (celloheptaose). OTU112 (*Bacillus*) and OTU242 (*Geobacter*) were positively correlated with IAA-94 and CGS 7181, respectively. OTU183 (*Geobacter*) and OTU662 (*Novosphingobium*) was positively correlated with Choline. As to lipids and exopolysaccharides, they were mainly positively correlated with OTU1503 (Candidatus_*Saccharimonas*), OTU1853 (*Rhizobium*), OTU3412 (*Cronbergia*), OTU242 (*Geobacter*), OTU618 (*Enterobacter*), OTU183 (*Geobacter*), OTU662 (*Novosphingobium*), OTU670 (*Phaeospirillum*), and OTU1526 (*Paludibacter*), which were mainly distributed in module 1 and module 6.

As shown in previous studies, salt stress could modulate rhizobacterial diversity and community composition via influencing the ecological factors of rhizosphere soil[23, 98]. On the other hand, plants could recruit specific beneficial PGPR by exuding metabolites and therefore reconstruct the rhizobacterial co-occurring networks to enhance the salt tolerance of themselves. Thus, illustrating the interaction mechanisms between rhizosphere metabolites and keystone taxa could facilitate the isolation and application of specific PGPR and metabolites to improve the plant salt tolerance. However, the role of rhizosphere metabolites in connection with keystone taxa in microbial networks remained poorly understood. Here, 46 lipids related metabolites and 3 saccharides related metabolites had distinct correlations with 15 keystone taxa in the rhizobacterial network (Figure 5), which were highly central and connected and mainly included in module 1, 5 and 6 (Figure 4). Interestingly, most of the lipids and saccharides related metabolites were positively correlated with keystone taxa in module 1 and 6 whereas the keystone taxa in module 5 were positively correlated with IAA-94. These results indicated that the interaction between keystone taxa and rhizosphere metabolites showed distinct modularity. These results indicated that specific metabolites might recruit specific keystone taxa or specific keystone taxa might generate specific products to promote the salt tolerance of plants. It has been reported that certain lipids could function as secondary messengers during abiotic stress responses[62, 63, 99]. Lipids and saccharides related metabolites might be involved in the biosynthesis of lipopolysaccharide (LPS) and exopolysaccharide (EPS) which are known to play an important role in inducing the plant salt tolerance[65, 100-103]. Jasmonates, lipid-derived compounds, were also known as key signaling compounds in plant stress responses[104, 105]. Therefore, it could be deduced that lipids and saccharides

related metabolites show versatility for inducing the plant salt tolerance. In addition, the results show that choline was positively correlated with *Geobacter*_OTU183 and *Novosphingobium*_OTU662 in module 1. Choline may affect salt tolerance by regulating lipid and glycine betaine metabolism[106]. Previous study has demonstrated that up-regulation of lipid metabolism and glycine betaine synthesis were associated with choline-induced salt tolerance in halophytic seashore paspalum[64]. Meanwhile, *Bacillus*_OTU112 (module 5) was found to be highly positively correlated with IAA-94 (p<0.01), a known chloride channel blocker[107]. IAA-94 might play an important role in osmoregulation in higher plants[108]. Saleh and Plieth has proved that IAA-94 was capable to inhibit the salt-induced chloride influx in *Arabidopsis*.[109] To provide further insight into the interaction between rhizobacteria and rhizosphere metabolites, and the related mechanisms contributed to the enhanced plant salt tolerance, isolation and characterization of PGPR capable of inducing the plant salt tolerance is needed.

3.4 Isolation and identification of potential PGPR in plant salt tolerance

After several isolation and screening cycles, four bacterial candidates capable of increasing the survival rates of SR86 under salinity 0.7% to be higher than 80% were isolated from more than 700 colonies, and named as RL-WG26, RL-WG62, RL-WG133, and RL-WG347. With the help of these isolated salt tolerance promoting rhizobacteria, the survival rates of SR86 at different salinities are shown in Figure 6A and the morphological differences of SR86 are shown in Figure 6B (under salinity 0.6%). Specifically, highest plant survival rates were observed in the group inoculated with strain RL-WG62, which was therefore selected for further investigation. The colony morphology of these salt tolerance promoting rhizobacteria is shown in Figure 6D. The amplified 16S rRNA gene sequences were applied to BLAST search and the 16S rRNA gene sequences of related type strains were retrieved from List of Prokaryotic names with Standing in Nomenclature (LPSN, http://www.bacterio.net/). The phylogenetic analysis (Figure 6C) indicated that strain RL-WG26, RL-WG62, RL-WG133, and RL-WG347 belong to *Pseudomonas putida*, *Rossellomorea vietnamensis*, *Bacillus* sp., and *Bacillus velezensis*, respectively.

The isolation of salt-tolerant PGPRs has received scientific attention since these isolates showed great application potential in the reclamation of saline land, thereby increasing global food production. Further, the elucidation of salinity tolerance mechanisms of plants attributed by PGPR might provide novel strategies to combat salinity in agricultural fields. In the present study, four rhizobacterial strains, capable of inducing salt tolerance of SR86 seedling plants, were isolated from the rhizosphere soil. Compared with SR86 seeding plants without inoculation, the seeding plants inoculated with these

isolated strains showed higher survival rates and better plant growth under salt stress conditions (Figures 6A, 6B). Numerous PGPRs capable of improving plant salt tolerance have been isolated and characterized, including genus of *Bacillus*, *Pseudomonas*, *Enterobacter*, *Azotobacter*, etc., and some representative PGPRs are listed in Table 1. *Bacillus* spp. are a group of well-known PGPRs and widely used as inoculants to enhance the salt tolerance of plants. As to *Rossellomorea vietnamensis*, it is previously known as *Bacillus vietnamensis*. *Bacillus subtilis* strain GOT9 was isolated from soil collected from Gotjawal in Jeju island and was able to enhance the salt tolerance of *Arabidopsis thaliana* and *Brassica campestris*[67]. Hydroponic experiments with *Bacillus amyloliquefaciens* SQR9, a beneficial bacterium isolated from the rhizosphere soil of a healthy cucumber, indicated that strain SQR9 could help maize plants tolerate salt stress, promote the growth of maize seedlings and enhance the chlorophyll content[68]. Genus *Pseudomonas* is another widely reported taxonomic group of PGPR that has been isolated from the rhizosphere of various plants. It was reported by Chatterjee that the application of *Pseudomonas frederiksbergensis* OS261 could augment salt tolerance and promote red pepper plant growth[70]. One *Pseudomonas* strain isolated from date-palm rhizospheres could improve root growth and promote root formation in maize when exposed to salt and aluminum stress[71]. In addition to inducing salinity tolerance in cotton, *Pseudomonas* was found to be able to enhance the cotton resistance to Fusarium root rot via the modulation of indole-3-acetic acid[72]. The isolation of PGPR, capable of improving the salt tolerance of plants, might provide vital biotic resources for alleviating damages in plants from salt stress. Further, the investigation of mechanisms by which PGPR inoculation alleviates salt stress in plants would facilitate the application and modification of these PGPR.

3.5 Effects of strain RL-WG62 on the ion concentration in SR86

In order to explore the underlying mechanism by which strain RL-WG62 enhanced SR86 seedling salt tolerance, the effects of *Rossellomorea vietnamensis* strain RL-WG62 on the ion concentration (Ca^{2+}, K^+, Na^+ and Cl^-) in plants under salinities of 0.6% and 0.7% were evaluated. For the concentration of Ca^{2+} in root and leave, no significant differences were observed between inoculated and uninoculated plants, whereas the concentration of Ca^{2+} was increased along with the increasing salinity (Figure 6E(1) and 6E(5)). Although no significant differences of K^+ concentration in root were observed between treated and untreated plants, the concentration of K^+ in leaves of plants inoculated with strain RL-WG62 was significantly higher than the leaves of plants without inoculation of strain RL-WG62 under salt stress

(Figure 6E(2) and 6E(6)). Notably, the concentrations of Na^+ and Cl^- in both root and leave were significantly decreased in plants inoculated with strain RL-WG62 when treated with osmotic stress, compared with the plants without inoculation. Under salinity 0.6% and 0.7%, the concentration of Na^+ in leaves without inoculation of strain RL-WG62 was 13.53 g/kg and 20.68 g/kg respectively, while the concentration of Na^+ in leaves inoculated with strain RL-WG62 was 11.84 g/kg and 11.73 g/kg respectively. Although similar effects strain of RL-WG62 on the concentration of Na^+ in root were observed under salt stress conditions, higher concentration of Na^+ was observed in leave than in root within the same plant. The same situations occurred in the effects of strain RL-WG62 on the concentration of Cl^-. These results indicated that (i) strain RL-WG62 has no significant impact on the concentration of Ca^{2+} in SR86, (ii) strain RL-WG62 could increase the concentration of K+ in the leaves of SR86 and might alleviate the salinity stress in SR86 via adjusting Na^+/K^+ ratio in leave, and (iii) strain RL-WG62 could decrease the concentration of Na^+ and Cl^- in both root and leave of SR86 and could ameliorate of salinity stress to SR86 via decreasing the concentration of Na^+ and Cl^- in plants. Meanwhile, it is noteworthy that all analyzed ions might be accumulated in the leaves of SR86.

Salt stress adversely affects plant growth and development since salinity could alter water relations of plants' tissues, and cause nutrient imbalance and toxicity due to excess concentration of Na^+ and $Cl^−$ in cell. Thus, the general approaches to alleviate salt stress include (i) restricting Na^+ and Cl^- influx into roots, (ii) accelerating Na^+ and Cl^- efflux out from plant cells, and (ii) increasing the K^+/Na^+ ratio in plant cells. Soil microbes, and particularly PGPR, could help plants to limit Na+ entry into roots under salt stress conditions via various approaches. Some of PGPR could generate bacterial EPSs and LPSs which could bind the rhizosphere Na^+ and therefore restrict Na^+ influx into roots[65, 100, 103]. In this study, the increased concentration of lipid and saccharide related metabolites indicated that LPSs and EPSs might be involved in the binding of rhizosphere Na^+ under saline conditions. Although some PGPR could enhance the plant salt tolerance by increasing the K^+/Na^+ ratio by selectively enhancing K^+ uptake and avoiding uptake of Na^+ [74, 110], strain RL-WG62 did not enhance the salt tolerance of SR86 by increasing the K^+/Na^+ ratio since the K^+ concentration decreased with salt stress. Apart from the high concentration of Na^+, Cl^- is the predominant concomitant anion for high salinity. Higher plants are always sensitive to high concentration of Cl^- [111, 112] and the term 'chloride toxicity' has been well established[113]. In the present study, the concentration of Cl^- in SR86 seedling plants was significantly decreased with the inoculation of strain RL-WG62 and the metabolome analysis suggested that IAA-94

might act as the anion channel blocker during strain RL-WG62 mediated plant salt tolerance enhancing. Menwhile, even the concentrations of Ca^{2+} in inoculated and non-inoculated groups showed no significant differences, the results also indicated that the concentration of Ca^{2+} increased with salt stress. In summary, strain RL-WG62 could enhance the salt tolerance of SR86 seedling plants via inhibiting the uptake of Na^{+} and Cl^{-}.

3.6 Effects of strain RL-WG62 on the physiological-biochemical characteristics of SR86

To determine the potential influences of strain RL-WG62 on SR86 under salt stress conditions, we further investigated the response of SR86 to salt stress with inoculated and uninoculated treatments by analyzing the physiological-biochemical characteristics of plants. The plant grown with strain RL-WG62 under salt stress showed lower proline content in root in comparison to the uninoculated plants of SR86. The proline content of SR86 grown under salinity 0.6% and 0.7% without inoculation of strain RL-WG62 was 23.02 mg/kg and 24.05 mg/kg, respectively, while the proline content of SR86 inoculated with strain RL-WG62 was 12.32 mg/kg and 13.99 mg/kg, respectively. For the proline content in root of SR86 under control conditions, no significant differences was observed between inoculated and uninoculated plants. Salinity stress conditions resulted higher MDA accumulation in the root of uninoculated SR86 plants while the MDA content in the root of plant grown with strain RL-WG62 was significantly decreased. Approximately 9.40 μmol/g and 10.73 μmol/g of MDA were accumulated in the roots of uninoculated SR86 with salinities of 0.6% and 0.7%, respectively, whereas 5.23 μmol/g and 6.22 μmol/g of MDA were measured in the roots of SR86 inoculated with strain RL-WG62 under the same salinity conditions, respectively. Interestingly, the plant of SR86 inoculated with strain RL-WG62 under control conditions showed higher total chlorophyl (chlorophyl a and b) content and better plant growth in comparison to the uninoculated plants, and these suggested that strain RL-WG62 was a plant growth promoting rhizobacteria (PGPR). Under control conditions, the uninoculated SR86 plants had an average total chlorophyl content of 3.18 g/kg and the average total chlorophyl content of SR86 plants with inoculation of strain RL-WG62 was about 4.22 g/kg. Meanwhile, under salinity stress conditions, the plants of SR86 inoculated with strain RL-WG62 had a higher concentration of total chlorophyl in comparison with the plants without inoculation. Under salinities 0.6% and 0.7%, the concentrations of total chlorophyl in the leaves of SR86 without inoculation were 2.18 g/kg and 2.02 g/kg, respectively, whereas the concentrations of total chlorophyl in the leaves of SR86 inoculated with strain RL-WG62 were increased to 3.56 g/kg and 3.78 g/kg, respectively. Compared with the plants without inoculation, strain RL-WG62

also improved the average dry weight of SR86 plants from 0.71 g/plant to 0.87 g/plant under control conditions. Under salinity stress conditions, strain RL-WG62 could improve the average dry weight of SR86 from 0.51 g/plant (salinity 0.6%) and 0.43 g/plant (salinity 0.7%) to 0.78 g/plant and 0.69 g/plant, respectively.

It is well understood that high salinity would lead to an increased production of reactive oxygen species (ROS) which could damage plant cells by altering fatty acids, amino acids, pigments and other biomolecules[114]. Malondialdehyde (MDA), a product of lipids peroxidation, has been widely used as an indicator for the degree of salt stress[115]. When the seedling plants of SR86 were treated with high salinity, the root MDA content increased significantly within the group of non-inoculation while it was reduced significantly when inoculated with strain RL-WG62 ($p<0.01$) (Figure 6E(9)). These results indicated that the process of lipid peroxidation was attenuated by inoculating with strain RL-WG62. Proline is a well-known osmolyte and the biosynthesis of proline was up-regulated in plants to counteract salt stress[115-117]. The inoculation of strain RL-WG62 significantly reduces the content of proline also suggested that the inoculation of PGPR could effectively alleviate salt stress in seedling plants of SR86. Further, the results of the concentration of chlorophyll a & b and dry weight of plant (Figure 6E(11) and 6E(12)) showed that the inoculation of strain RL-WG62 could stimulate the growth of seedling plants SR86 under salt stress.

Conclusions

Overall, our results suggest that salt stress could significantly change the rhizobacterial diversity and rhizosphere metabolites composition. The constructed rhizobacterial network demonstrated that several rhizobacterial taxa might serve as keystone taxa and be involved in the interaction of SR86 with salt stress. The correlation analysis of keystone taxa and rhizosphere metabolites further indicated the potential contribution of keystone taxa and some specific metabolites in the process of SR86 salt tolerance. Protective layer consists of lipids and their derivatives (LPS and EPS) might protect SR86 from the negative effects of salt stress through binding Na^+, and Cl^- was blocked out of root cells with the assistant of anion channel blocker (IAA-94). Four rhizobacterial strains, capable of enhancing the salt tolerance of SR86, were isolated and characterized. The inoculation of isolated PGPR could alleviate the plant salt stress and promote plant growth. The findings provide novel insights into the mechanisms of plant-microbe interaction mediated plant salt tolerance, and promote the isolation of salt tolerance

PGPR and their application in the restoration and utilization of saline-alkali land.

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