Accumulation of beneficial bacteria in the rhizosphere of maize (Zea mays L.) grown in a saline soil in responding to a consortium of plant growth promoting rhizobacteria

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Abstract

Purpose: Salt stress reduces plant growth and is now becoming one of the most important factors restricting the agricultural productivity. Inoculation of plant growth-promoting rhizobacteria (PGPR) has been shown to confer plant tolerance against abiotic stress, but the detailed mechanisms of how this occurs remain unclear and the application effects in different reports are unstable. In order to obtain a favorite effect of PGPR inoculation and improve our knowledge about the related mechanism, we performed this study to analyze the mechanism of a PGPR consortium on improving the salt resistance of crops.

Methods: A region-specific (Saline land around Bohai Sea in China) PGPR consortium was selected that contains three strains (Pseudomonas sp. P8, Peribacillus sp. P10, and Streptomyces sp. X52) isolated from rhizosphere of Sonchus brachyotus DC. grown in a saline soil. By inoculation tests, their plant growth-promoting (PGP) traits and ability to improve the salt resistance of maize were investigated and shifting in rhizosphere bacterial community of the inoculated plants was analyzed using the high-throughput sequencing technology.

Results: The three selected strains were salt tolerant, presented several growth promoting properties, and inhibited several phytopathogenic fungi. The inoculation of this consortium promoted the growth of maize plant and enriched the beneficial bacteria in rhizosphere of maize in a saline soil, including the nitrogen fixing bacteria Azotobacter, Sinorhizobium, and Devasia, and the nitrification bacteria Candidatus Nitrosospheara, and Nitrosavibrio.

Conclusions: The bacterial consortium P8/P10/X52 could improve plant growth in a saline soil by both their PGP traits and regulating the rhizosphere bacterial community. The findings provided novel information about how the PGPR helped the plants in the view of microbiome.

Keywords: Consortium inoculant, Saline soil, Rhizobacteria, Plant promoting trait, Maize

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Introduction

Soil salinity is a common problem and one of the main abiotic stress factors that inhibit plant growth and development (Egamberdieva et al. 2019). Salinization refers to the concentration increase or accumulation of water-soluble salts in soil. Salt-affected soils are classified by electrical conductivity (EC > 4 dS m⁻¹, ~ 0.3%), the exchangeable sodium percent (ESP > 6%), or pH usually over 8.5. The salts mainly caused by the flooding and seepage of seawater, rising of brackish groundwater in areas with low rainfall and high evaporation, or saline irrigation water and poor water management in agricultural areas (Ondrasek and Rengel 2021). The most important type of soil degradation, soil salinization seriously affects crop production mainly by decreasing the osmotic potential of the soil that makes the plants difficult to absorb water, but also by the direct toxic of salts. With the human activity in arable land, such as excessive and long term supply of chemical fertilizers, the degree of secondary salinization of soil becomes more and more serious. Therefore, soil salinization forms a limitation to fit the increased food demand companied with the development of human society and the increase of population.

In general, around 953 × 10⁶ ha or 20% of the irrigated areas of the world have been affected by salinization, and the salinity problem is more common in semi-arid regions, as reported in Brazil, India, and China, due to the low rainfall against high evaporative rate (Fagundes et al. 2020; Singh 2021). In China, there are about 36 × 10⁶ ha of land with saline soil, accounting for about 5% of the total available land base of the country. Although these regions presented low agricultural productivity, the saline soils forms an important potential resource for arable land.

Attempts to improve the crop production in the saline lands have been made in China since 1930s (Li et al. 2014). The related studies have been focused on two aspects: land management or technologies to promote the soil desalination; and breeding crop cultivars resistant or adapted to the saline soils. Some of the methods are also applied in other countries. Rice production in the Mekong River Delta of Vietnam (MRD) is endangered by sea-level rise and an associated increase in the incidence of salinity intrusion. Diffusion of salt tolerant rice varieties in the MRD has been performing to solve this problem (Paik et al. 2020). Some well-proven, widely used and cost-effective traditional ameliorative strategies (e.g., conservation agriculture, application of natural conditioners) helped the crops against salinity and other constraints, especially in developing countries (Ondrasek and Rengel 2021).

Some desalinization technologies have been developed in previous studies, such as the drainage-based cropping system, physical adsorption improvement, brine discharge pipe, slag adsorption etc., but all of them are fresh water consuming procedures that is not suitable for arid and semi-arid areas (Ilangumaran and Smith 2017). On the other hand, in order to improve the salt tolerance of plants, mechanisms (including genes) of the salt resistance in plants have been studied (Li et al. 2014; Ondrasek and Rengel 2021), including the use of plant growth-promoting rhizobacteria (PGPR), traditional breeding and genetic engineering (Fita et al. 2015; Yang et al. 2009). Traditional breeding and genetic engineering usually need a long period for obtaining progress or success, and PGPR can greatly improve the salt tolerance (Chen et al. 2016), drought resistance and growth of crops with low cost and short time. PGPR have been used in promoting plant growth and disease prevention that in turn could improve the productivity in saline land (Palaniyandi et al. 2014). Therefore, the application of PGPR is one of the most promising alternatives for improving plant growth in saline soil (Numan et al. 2018; Yang et al. 2009). At present, the use of PGPR to improve salt stress in crop production is becoming more and more common. Various salt-tolerant PGPR, including Azospirillum, Burkholderia, Rhizobium, Pseudomonas, Acetobacter and Bacillus associated with different plants have been successfully applied or tested for improving plant growth under salt stress (Chatterjee et al. 2017; Egamberdieva et al. 2015). Maize is one of the most important cereal crops that is mainly planted in irrigated agricultural areas in both arid and semi-arid regions (Nuss and Tanumihardjo 2010). As a moderately salinity-sensitive plant species, few salinity-tolerant maize cultivars have been commercialized, but PGPR can improve maize salt tolerance (Chen et al. 2016). However, these bacteria have not been fully investigated, especially in arid and semi-arid regions, and their effects in application were not suitable and unstable in some cases, in addition to their limited application range (Wu et al. 2012). Furthermore, few of the microbial strains presented wide and strong adaptability in the soil.

Considering that the biogeographic patterns of the soil bacteria (Chu et al. 2020; Li et al. 2020) and plant associated bacteria (Román-Ponce et al. 2016; Zhang et al. 2011) are determined by the soil physiological and biological traits, we propose that region-specific PGPR might have a stable effect on improve the salt resistance of the local crops. In order to verify this hypothesis, we performed the present study by using a set of efficient bacteria screening methods for searching salt-tolerance PGPR against Sonchus brachyotus DC. (common name Qumacai or Kujucai in China) from rhizosphere of plants grown in a saline land in Haixing County, Hebei Province of China. A consortium composed of three strains was selected based upon their salt resistance and...
PGP traits, and its effects on maize growth and rhizosphere microbiota were investigated. The results provided theoretical support for application of the PGPR consortium in improving the productivity in saline land and in sustainable agriculture.

**Materials and methods**

**Bacterial isolation**

Rhizobacteria were isolated from *Sonchus brachyotus* rhizosphere soil collected in saline field in Haixing County, Cangzhou City, Hebei Province of China. A rhizosphere soil suspension was obtained by shaking off and discarded the loosely soil then rinsing the roots with adhered soil in phosphate-buffered for 10 min at 150 rpm. The suspension was serially diluted to 10⁻⁶, and aliquots of 0.1 mL of the last three dilutions were spread separately onto 1/3 KingB medium (KMB, 6.5 g Peptone, 0.5 g K₂HPO₄, 0.5 g MgSO₄.7H₂O, 3 mL Glycerol, 18 g agar, add dH₂O to 1 L) and incubated at 30 °C for 48 h. Single colonies with different morphology traits (shape, color, size, luster, texture, light transparency, edge integrity) were picked up and re-streaked several times on the same medium to obtain the pure culture. The purified bacteria were maintained at slants of 1/3 KMB medium at 4 °C for short time storage (3–4 weeks) or in broth of the same medium supplied with 30% (w/v) glycerol at –80 °C for long-term storage.

**Selection of the PGPR and estimation of their PGP traits**

The salt resistance of the isolates was tested by grown them on 1/3 KingB medium supplied with 0%, 5%, and 10% (w/v) of NaCl. The growth promoting properties of the isolates were tested according the reported methods. Briefly, the production of IAA (indole-3-acetic acid) was determined by the method of Khalid et al. 2004 and Bric et al. 1991; the production of ACCD (1-aminocyclopropane-1-carboxylic acid deaminase) was determined by the method of Khalid et al. 2004 and Bric et al. 1991; the production of siderophores was estimated based on the work of Schwyn and Neilands 1987; phosphate solubilization potential was determined by the method of Vyas et al. 2007; and nitrogen fixation capacity was investigated using the method of Dobereiner et al. 1976. All the tests were performed in triplicate by incubation at 30 °C for 48–96 h. IAA production and ACCD activities were also quantitatively determined with the methods described previously (Khalid et al. 2004; Penrose and Glick 2003). Based upon their salt tolerance and PGP traits, isolates with strong ACCD activity, high nitrogen fixation ability, high phosphate solubilization, and high IAA and siderophore production were selected to compose a consortium as PGPR inoculant. In addition, these isolates with different colony morphologies without nutritional competition were also considered as the selection criteria.

All the selected isolates were tested for their in vitro inhibition against the phytopathogenic fungi *Pythium aphanidermatum* MJ-1, *Fusarium oxysporium* Lsp. *curvum* *merinum* HG-11, *Curvularia lunata* sp. WM-1, *Cochliobolus heterostrophus* sp. WM-2, and *Phytophthora capsici* LJ-3 on 1/3 KingB medium by plate confrontation method and plate diffusion method according to the references (Fernandez-Garayzabal et al. 1992). Diameter in mm of the fungal colonies in both the control (without bacterial inoculant) and the test (co-incubated with bacteria) was measured after 7 days incubation at 28 °C. The inhibition (%) was presented as 100 × (radius of control colony – radius of colony in test)/radius of control colony. All the tests were performed in triplicate.

**Bacterial identification**

For identification of the selected isolates, bacterial genomic DNA was extracted by the CTAB/NaCl method (Andreou 2013) from 5 mL of culture in broth of 1/3 KingB medium incubated at 30 °C for 24 h with agitation, and the almost complete 16S rDNA was amplified from the DNA extract by using PCR with the universal bacterial primers 27F and 1492R (Frank et al. 2008). The PCR product was sequenced commercially in Invitrogen (Shanghai) with the same primers and the acquired sequences were analyzed with BLASTn to identify the isolates at genus/species level.

**Plant growth conditions and treatments**

The saline soil used for the pot experiment was obtained from 0 to 30 cm in depth at the Haixing Farm (38.04° N 117.24° E) that located in the same region for the bacterial isolation. The soil was air-dried and sieved to 2.5 mm before filling in the pot. The basic physicochemical properties of the soil were pH 8.39, total salt 3.09%, organic matter 4.45 g kg⁻¹, total N (TN) 0.27 g kg⁻¹, available P (AP) 10.39 mg kg⁻¹, total potassium (K) 19.46 g kg⁻¹, total phosphorus (P) 27.19 mg kg⁻¹, available P (AP) 10.39 mg kg⁻¹, total potassium (K) 18.59 g kg⁻¹, total phosphorus (P) 0.69 g kg⁻¹, available K (AK) 101.75 mg kg⁻¹, and available K (AK) 150.00 mg kg⁻¹. For the subsequent analysis, a
mixture of the two soils (1 g of the saline soil + 9 g of the non-saline soil) was prepared to get a soil with 0.31% of total salt, which is the limit concentration for surviving of maize in a preliminary test.

To define whether the bacteria had an effect on the growth of maize in saline condition, surface-sterilized and synchronized seeds of cultivar Zhengdan 958 were incubated with bacterial suspension (10⁶ CFU/mL) prepared by mixing the three selected isolates P8, P10, and X52 in the same ratio (1:1:1 in volume) or sterilized water solution as a control for 12 h and then pregerminated on sand under 0.0% and 0.3% NaCl concentrations and on the mixed soil containing 0.3% total salt. The germinated seeds were sown in pots filled with the natural soil samples (200 g soil in each 1 litter pot, one seed per pot). Potted plants were placed in a growth chamber at 25 °C with a 16/8 h light/dark cycle. Deionized water was supplied from the bottom of the pot. Finally, 25 days after sowing, plant agronomic traits were determined, including shoot length, root length, shoot dry weight, and root dry weight. Each treatment has three replicates (n = 3).

**Rhizosphere bacterial community analysis by 16S rDNA gene sequencing**

To investigate the effect of P8/P10/X52 consortium on maize rhizosphere microbiota in saline soil, sterilized and synchronized seeds were inoculated by immersing the seeds with the consortium suspension (1:1:1, 10⁶ CFU/mL/strain) or with sterilized water solution as a control for 12 h and then pregerminated on the mixed soil containing 0.31% of total salt. Preamplified seeds were sown in a stone pot with dimensions of 305 mm (height) × 225 mm (open top) × 205 mm (flat bottom) which were filled with 7 kg of the mixed soil samples supplied with inorganic fertilizers (kg ha⁻¹) in the ratio of 150 N, 75 P₂O₅ and 75 K₂O. Each treatment had 48 replicate pots. The growth conditions were the same as described above and supplied each pots in equal watering every 15 days. The rhizosphere soil was sampled at the three-leaf stage (10 days after sowing), jointing stage (27 days after sowing), tillering stage (52 days after sowing), and maturity stage (100 days after sowing) in three replicates for analyzing the microbiota by the high throughput DNA sequencing described subsequently.

The rhizosphere soil samples were obtained according to previous research (Hu et al. 2020). In brief, they were sampled by brushing off the soils attached to the root surface with a soft toothbrush. Samples from the three plants (repeats) were compiled to form a composite sample and were stored at – 80 °C until it was used for DNA extraction.

The metagenomic DNA was extracted from 0.5 g rhizosphere soil and purified by using PowerSoil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer’s instructions. The quality and quantity of the extracts were measured by the Nano Drop™2000 spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA). Primer set of F515 (5’-GTGCCAGCMGCGG-3’) and R907 (5’-CCGTCAATTCMTTTRAGTTT-3’) was used to amplify the V4-V5 hypervariable regions of bacterial 16S rRNA gene by PCR (Zhou et al. 2011). PCR products were purified and sequenced on a single lane of Illumina MiSeq platform at the Shanghai Personal biotechnology Co., Ltd (Personalbio, Shanghai, China).

Sequencing libraries were generated using the Illumina Nano DNA LT Library Prep KitTruSeq DNA PCR-Free Library Preparation Kit (Illumina, San Diego, USA) by following the manufacturer’s recommendations. Library quality was assessed on a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, USA) and an Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, USA). Finally, the library was sequenced on an Illumina MiSeq platform and 300 bp paired-end reads were generated (Personalbio, Shanghai, China). Raw reads were filtered by QIIME (Quantitative Insights Into Microbial Ecology) quality filters (Bokulich et al. 2013). The remaining reads from the original DNA fragments were merged using the FLASH tool (Magoc and Salzberg 2011). Paired-end reads were assigned to each sample according to unique barcodes with QIIME (Edgar 2010). OTUs (operational taxonomic units) with identities of 97% were determined for the quality filtered reads using the Mothur software (http:// www.mothur.org), from which the Shannon diversity index and Chao1 richness were estimated (White et al. 2009). The sequence with the highest relative abundance from each OTU was selected as the representative sequence to search the similar sequences in the National Center for Biotechnology Information (NCBI) nucleotide non-redundant database. PLS-DA (Partial least squares discriminant analysis) was also introduced as a supervised model to reveal the microbiota variation among groups, using the “plsda” function in R package “mixOmics” (Chen et al. 2011). Each group represents by one color and has three dots, which represent three repetitions and are marked with an ellipse. It would be better if the same color dots are closer, and the different color dots are farther.

Abundances of taxa at the genus levels were statistically compared among samples or groups by Metastats (http://metastats.cbcb.umd.edu/) (White et al. 2009). The difference in quantity (relatively abundance) of the OTUs was tested by pairwise comparison. Each time Metastats compares generated the corresponding P and Q values. The corrected P value is called Q value, and when P value < 0.05, the closer the Q value is to 0, the lower the probability of false positive.
Results

Selection of PGPR consortium

Based on the PGP traits (Table 1), three isolates P8, P10, and X52 were able to grow in the presence of 5% and 10% (w/v) NaCl were selected for constructing the consortium. Isolate P8 showed the best N fixation ability and the highest ACCD activity; P10 presented the best phosphate solubilization and N fixation ability, while X52 produced the highest IAA production and considerable siderophore production (Table 1). The quantitative analyses of IAA and ACCD revealed that X52 produced IAA as high as 70.58 μg mL⁻¹, and P8 reached the ACCD activity of 8.01 mM mg⁻¹ h⁻¹ (Table 1).

Inhibition to phytopathogenic fungi by P8, P10, and X52

The antimicrobial activities of the selected isolates were summarized in Table 1 (details available as Suppl. Table S1). All the three isolates presented a certain degree of inhibition on all the tested fungal strains, in which the isolate X52 had the strongest inhibitory effects on four of the five pathogens (except *F. oxysporium* f. sp. *cucumerinum* HG-11), with the inhibition rates from 43.00% for *F. oxysporium* f.sp. *cucumerinum* HG-11 to 82.98% for *B. maydis*. For *Fusarium*, the highest inhibition (69.50%) was found in culture with isolate P10. The broad antifungal spectrum presented in the three selected bacterial isolates, especially X52, evidenced them the potential for biological control application.

Molecular identification of the selected bacteria

The acquired 16S rDNA sequences of isolates P8, P10, and X52 were approximately 1.5 kb in length, which have been deposited in GenBank database under the accession numbers MT879460, MT878549, and MT878548, respectively. The BLAST search of these sequences against the GenBank database demonstrated that P8, P10 and X52 shared more than 99.7% of sequence identity with reference strains for *Pseudomonas silesiensis*, *Peribacillus simplex*, and *Streptomyces microflavus*, respectively (Table 1). Therefore, the three isolates were identified as *Pseudomonas* sp. P8, *Peribacillus* sp. P10, and *Streptomyces* sp. X52.

Enhancing maize salt stress tolerance by inoculation of P8/P10/X52 consortium

As shown in Fig. 1, the maize seedlings grew similarly in the sand substrate with 0 and 0.3% NaCl concentrations, and no significant difference was observed among different treatments in all the four growth traits: shoot length, root length, shoot dry weight, and root dry weight. So, maize growth was not affected by the salinity of 0.3% of NaCl. However, the growth of maize seedlings was significantly improved by inoculation of the mixture of P8, P10, and X52 in sand despite the tested level of NaCl, on the 25th day after sowing, comparing with the control group (*P* < 0.05) (Fig. 1). These results implied that the consortium could improve the maize growth under...
low level of saline stress (0.3% NaCl). The effects of P8/P10/X52 consortium on maize growth were also verified in saline soil (0.3% NaCl) (Fig. 1), by analysis of the same growth traits on the 25th day after sowing (P < 0.01) (Fig. 1). These results suggest that P8/P10/X52 consortium could promote the maize growth with or without slight salt stress.

Promotion of the beneficial bacteria in maize rhizosphere by consortium inoculation

Compared with the treatment of P8/P10/X52 consortium, germination time of control seeds is 1 day later and the plants grew with more pests and diseases in the mixed 0.3% saline soil. The plant growth and root development in control were also the worst, and the lower leaves became yellowish at the tasseling period. Finally, the maize yield of the P8/P10/X52 inoculation treatment increased by 16.52% (P < 0.05) compared to that of the control.

In the metagenomic analysis, a total of 626,785 high-quality reads for the V4–V5 region of the 16S rRNA genes were obtained from the samples, with an average of 286,683 and 281,592 reads per sample of treatments and controls, respectively. These reads were rarefied to 6909 bacterial genes of each sample, corresponding to a total of 233,637 OTUs. The raw data have been deposited in SRA database under the accession numbers PRJNA562815. The OTU Shannon rarefaction curve (see Supplementary Fig. S1) tended to be flat and the Simpson index (see Supplementary Table S2) for most of the samples were greater than 0.90 (ranging from 0.76 to 0.99), suggesting that the sequencing depth of the samples can reflect the species diversity in the samples. The analysis of OTUs revealed differences in microbial community structure in rhizosphere of different growth stages and between control and P8/P10/X52 consortium inoculation (Fig. 2). The number of OTUs in the soil at the time of planting was the least; as the maize grew, the number of OTUs in the rhizosphere was increased; meanwhile, the bacterial abundance of treatment inoculated with P8/P10/X52 consortium was lower than that of control.
According to the PLS-DA (Fig. 3), the largest species diversity varied along the growth of maize plant. As maize grew, the difference in diversity between the P8/P10/X52 inoculated treatments and the controls increased, and at the final maturity stage, the difference between the P8/P10/X52 treatments and the controls was the largest, suggesting that the microbial community composition in rhizosphere of maize was always changed related to the P8/P10/X52 inoculation.

The rhizosphere microbiota of maize varied among the four sampling stages, and the bacterial genera with significantly abundance were different according to the treatments and the growth stages of maize (Table 2). Some genera were more abundant in controls, but others were more abundant in P8/P10/X52 treatments, while several genera only existed in controls or in P8/P10/X52 treatments. For instance, Exiguobacterium was only found in control at the three-leaf stage, and Cupriavidus only found in controls and Saccharopolyspora only recorded in P8/P10/X52 treatments at the jointing stage. At the maturity stage, the significantly different genera were the highest, and all of them presented higher abundances in P8/P10/X52 treatment. It’s worth noting that most of the genera with significant difference in abundance at the maturity stage were related to the biological nitrogen fixation (such as Azotobacter, Sinorhizobium, and Devasia) and to nitrification (such as Candidatus Nitrosospaera, Nitrosovibrio), which are beneficial for restoring the soil ecology. These results suggest that inoculation of P8/P10/X52 consortium can enrich the rhizosphere microbiota and may promote the beneficial bacteria in the rhizosphere of maize grown in saline soil.

**Discussion**

The use of PGPR in agriculture as a sustainable and eco-friendly approach is a recommended strategy and an emerging trend in agriculture, including in saline soil (Numan et al. 2018; Yang et al. 2009). In the previous studies, salt-tolerant PGPR with various PGP traits have been tested (Chatterjee et al. 2017; Egamberdieva et al. 2015; Numan et al. 2018; Yang et al. 2009). However, most of the related studies used single strain as inoculant (Chatterjee et al. 2017; Egamberdieva et al. 2015), and the inoculation effects were unstable in some cases (Wu et al. 2012). For getting PGPR inoculant efficiently improving the growth and yield of staple crops in saline land, we tried in the present study to use consortium of PGPR as inoculant by combining strains with complementary PGP traits and without nutrient competition, and the results were encouraging.

In the present study, a set of efficient bacteria screening methods was employed for searching salt-tolerance PGPR from the rhizosphere of herbal plant Sonchus brachyotus grown in saline soil. Three strains of Pseudomonas sp. P8, Peribacillus sp. P10, and Streptomyces sp. X52 were selected based upon their salt tolerance and several growth promoting properties, as well as inhibiting several common pathogenic fungi (Table 1). The
inoculation of consortium composed of these three strains could promote the seed germination and growth of maize plant in saline soil (Fig. 1). Previously, *Pseudomonas* and *Bacillus* strains, as well as *Azospirillum*, *Burkholderia*, *Rhizobium*, *Acetobacter*, and *Raoultella planticola*, have been used individually as PGPR to improve growth of various plants grown in saline soils, like red pepper and cotton plants, (Chatterjee et al. 2017; Egamberdieva et al. 2015; Wu et al. 2012). Among the three strains we selected, P10 belonged to the genus *Peribacillus* that was proposed recently as a new genus separated from *Bacillus* (Patel and Gupta 2020), while *Streptomyces* strains were also reported as PGP bacteria for different crops, like wheat grown in saline soil (Akbari et al. 2020; Olanrewaju and Babalola 2019). So, the isolation and identification of the three strains in the present study further revealed that *Pseudomonas*, *Peribacillus*, and *Streptomyces* are common PGPR in saline soils. Although the sequence identity (99.73% to 99.87%) of 16S rRNA gene between these three strains and the closely related reference strain for defined species were apparently greater than the suggested species threshold (97%) (Gevers et al. 2005), we did not affiliate them into species, since the phylogeny of 16S rRNA gene is not sensitive enough for species definition in many cases.

The mechanisms for the PGP effects of PGPR have been reported and reviewed (Numan et al. 2018) previously, which can be sorted into two aspects: (1) PGPR stimulate the metabolic pathways of plants, such as stimulating plant synthesis of growth hormones, triggering the antioxidant system and starting siderophore production of plants, as well as augmenting nutritional capacity of the plants. (2) The PGPR themselves produce various phytohormones like auxins and cytokinins to improve the growth of both roots and shoots, or improve nutrient supplement by phytopathogenic antagonism, mineral solubilization, and nitrogen-fixation. In the present study, the three selected strains presented some of the PGP traits (Table 1), which might be a part of their mechanism to help the maize plant in alleviating stresses of salinity as reported previously (Etesami and Maheshwari 2018). Furthermore, these three strains were originately isolated from *Sonchus brachyotus*, but the consortium formed by them showed significant PGP effects on maize grown in the same soil; therefore, their host specificity is not strong, and they may also be used for other crops.

Except directly stimulating the plant growth, inoculation of the consortium in our study also regulated the microorganisms in the rhizosphere of maize: decreased
the amount of phytopathogenic bacteria and increase the relative abundance of beneficial bacteria, especially the nitrogen-fixing bacteria and nitrifying bacteria (Table 2). These results added the third possible mechanism of PGPR: regulating the microbiota in rhizosphere and in turn promoting the growth of plants, in addition to the two aspects mentioned above (Numan et al. 2018). The variations in the rhizosphere microbiota of maize in respect to the consortium inoculation and the four growth stages (Table 2, Fig. 2) demonstrated that the rhizosphere microbiota was affected by the interactions between the inoculated consortium and the plant biophysical status. This estimation was also supported by the results of PLS-DA (Fig. 3), in which a clear separation in the rhizosphere microbiota of maize in different growth stages and treatments was observed. For instance, some of the microorganisms from samples of different treatments (with/without inoculation) and different growth stages showed close relationships, while those from the original soils and from the inoculation treatment in the maturity stage formed two groups with great difference. These findings were consistent with the previous observations on the plant associated bacteria that their community composition was determined by the interactions among soil properties, the plant species and the microbes (Román-Ponce et al. 2016; Zhang et al. 2011).

In the present study, genus *Exiguobacterium* was only detected in control at the three-leaf stage. As salt-tolerance bacteria, *Exiguobacterium* usually presented in saline environments (Patel et al. 2018; Remonsellez et al. 2018; Zhang et al. 2019). *Cupriavidus* was only found in control and *Saccharopolyspora* was only detected in the consortium inoculated samples in the jointing stage. *Cupriavidus* usually exists in environments loaded with metal ion, and the bacteria in this genus have been screened from metal ion environments for bioremediation of metal contamination (Huang et al. 2019; von Rozycki and Nies 2009). Therefore, both *Exiguobacterium* and *Cupriavidus* might be selected by maize for salt resistance in different growth stages, when the consortium inoculant was absent. The genus *Saccharopolyspora* contains potential producers of diverse natural products, including antibiotics (Sayed et al. 2020) and insecticide with an excellent environmental and mammalian profile (Tao et al. 2019). So, its augmentation by the consortium inoculation might be beneficial to maize for improving insect resistance, which may be the reason why the inoculation treatment showed fewer pests and diseases (data not shown). At the maturity stage, the presence of more significantly enriched genera in P8/P10/X52 inoculated treatment (Table 2) might be related its greater biomass accumulation (Fig. 1), which made the plant produce more root exudates to support greater abundant

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**Table 2** The difference in quantity (relatively abundance) of the rhizosphere microbiota of maize at each stage

| Period            | Genus            | Quantity (Control) | Quantity (PPG x PG) | q-value |
|-------------------|------------------|--------------------|---------------------|--------|
| The three-leaf stage | *Lactobacillus* | 0.001922          | 0.000305            | 0.00191 |
|                   | *Rubrivivax*     | 0.00351            | 0.00186             | 0.00206 |
|                   | *Actinobacteria* | 0.001119           | 0.00075             | 0.00185 |
|                   | *Pseudomonas*    | 0.00726            | 0.00311             | 0.00206 |
|                   | *Erigeron*       | 0.00189            | 0.00020             | 0.00185 |
|                   | *Provasi*        | 0.001168           | 0.00020             | 0.00206 |
|                   | *Azotobacter*    | 0.00217            | 0.00793             | 0.00206 |
|                   | *Lysobacter*     | 0.00128            | 0.00124             | 0.00206 |
|                   | *Gemminaltus*    | 0.000000           | 0.000000            | 0.00206 |
|                   | *Cupriavidus*    | 0.001168           | 0.001168            | 0.00206 |
|                   | *Saccharopolyspora* | 0.000000         | 0.000000            | 0.00206 |
|                   | *Pseudomonas*    | 0.001168           | 0.001168            | 0.00206 |
|                   | *Streptomyces*   | 0.004351           | 0.004351            | 0.00206 |
| The jointing stage | *Daucus*         | 0.005292           | 0.007980            | 0.00206 |
|                   | *Cathelicidae*   | 0.001168           | 0.001168            | 0.00206 |
|                   | *Cupriavidus*    | 0.001168           | 0.001168            | 0.00206 |
|                   | *Xylemonosporum* | 0.000981           | 0.000981            | 0.00206 |
|                   | *Cupriavidus*    | 0.001168           | 0.001168            | 0.00206 |
|                   | *Cupriavidus*    | 0.001168           | 0.001168            | 0.00206 |
|                   | *Cupriavidus*    | 0.001168           | 0.001168            | 0.00206 |
|                   | *Cupriavidus*    | 0.001168           | 0.001168            | 0.00206 |
|                   | *Cupriavidus*    | 0.001168           | 0.001168            | 0.00206 |
|                   | *Cupriavidus*    | 0.001168           | 0.001168            | 0.00206 |
|                   | *Cupriavidus*    | 0.001168           | 0.001168            | 0.00206 |
|                   | *Cupriavidus*    | 0.001168           | 0.001168            | 0.00206 |
| The flowering stage | *Myxococcus*     | 0.000000           | 0.000000            | 0.00206 |
|                   | *Arthrobacter*   | 0.001270           | 0.000430            | 0.00206 |
|                   | *Provasi*        | 0.001270           | 0.000430            | 0.00206 |
|                   | *Arthrobacter*   | 0.001270           | 0.000430            | 0.00206 |
|                   | *Provasi*        | 0.001270           | 0.000430            | 0.00206 |
|                   | *Provasi*        | 0.001270           | 0.000430            | 0.00206 |
|                   | *Provasi*        | 0.001270           | 0.000430            | 0.00206 |
| The maturity stage | *Pedobacter*     | 0.000981           | 0.000981            | 0.00206 |
|                   | *Azotobacter*    | 0.001168           | 0.001168            | 0.00206 |
|                   | *Provasi*        | 0.001270           | 0.000430            | 0.00206 |
|                   | *Provasi*        | 0.001270           | 0.000430            | 0.00206 |
|                   | *Provasi*        | 0.001270           | 0.000430            | 0.00206 |
|                   | *Provasi*        | 0.001270           | 0.000430            | 0.00206 |
|                   | *Provasi*        | 0.001270           | 0.000430            | 0.00206 |

The quantity is relatively abundance, red indicates that the genus quantity is higher in that group.
and diverse rhizosphere microbes. For example, more root exudates (sugars, organic acids, amino acids etc.) can offer greater carbon source to the biological N-fixation and more ammonia to stimulate nitrification, which could be explained why the abundances of *Azotobacter*, *Sinorhizobium*, *Devosia* (N-fixers) and *Candidatus Nitrosophaera*, *Nitrosovibrio* (nitrification bacteria) were increased in the inoculation treatment. *Azotobacter* is usually applied for nitrogen fixation (Kennedy and Toukdarian 1987), but also tolerant to abiotic stresses such as temperature, pH, and insecticides (Chennappa et al. 2016). Root inoculation with *Azotobacter chroococcum* 76A could promote tomato plant growth, stress tolerance, and nutrient assimilation efficiency under moderate and severe salinity (Van Oosten et al. 2018). So, the change in rhizosphere microbiota by the consortium promoted the accumulation of beneficial bacteria in the rhizosphere of maize in saline soil. Therefore, the consortium composed of *Pseudomonas* sp. P8, *Peribacillus* sp. P10, and *Streptomyces* sp. X52 is a promising inoculant, which has the potential to improve crop growth in saline soil. Meanwhile, such consortium inoculant may have a more stable and effective impact than inoculants contained only one strain/species of microorganism, and it could be the future development direction of microbial inoculants.

Although *Pseudomonas* sp. P8, *Peribacillus* sp. P10, and *Streptomyces* sp. X52 have beneficial effects in maize growth and altered composition of rhizosphere bacterial community, the results of high-throughput sequencing show that their number or abundance in the rhizosphere of inoculated maize was not high. Therefore, they are likely to affect the growth of maize by stimulating and regulating certain genes of plants, as reported in other studies (Pieterse et al. 2012; Yang et al. 2009), and mediating the rhizosphere microbiota (Table 2, Fig. 2) to affect the growth of maize. PGPRs initially recognized as agents to enhance defense capacity of above ground parts, which was described as induced systemic resistance (ISR) with different pathways (Kawaharada et al. 2015; Pieterse et al. 2012; Zamioudis and Pieterse 2012). In the future, we will study such regulation mechanism, so as to find more efficient PGPR inoculants.

**Conclusion**

P8/P10/X52 consortium can promote the growth of maize and promote the accumulation of beneficial bacteria in the rhizosphere of maize in saline soil, which revealed the possibility to use bacterial consortia as inoculants to enhance the crop production in saline soils.

**Abbreviations**

PGPR: Plant growth-promoting rhizobacteria; PGP: Plant growth-promoting; EC: Electrical conductivity; ESP: Exchangable sodium percent; pH: Potential of hydrogen; mm: Millimeter; ha: Hectare; min: Minute; rpm: Revolutions per minute; mL: Milliliter; KMB: King’s B medium; IAA: Indole-3-acetic acid; ACCD: 1-aminocyclopropane-1-carboxylic acid deaminase; CFU: Colony forming unit; rDNA: Ribosomal deoxyribonucleic acid; rRNA: Ribosomal ribonucleic acid; QIME: Quantitative insights into microbial ecology; NCBI: National Center for Biotechnology Information; SRA: Sequence read archive; OTUs: Operational taxonomic units; PLS-DA: Partial least squares discriminant analysis; BLAST: Basic local alignment search tool; IR: Inhibition rates
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