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Plant growth-promoting rhizobacteria *Halomonas alkaliantarcticae M23* promotes the salt tolerance of maize by increasing the K^+/Na^+ ratio, antioxidant levels, and ABA levels and changing the rhizosphere bacterial community

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Abstract

Background Soil salinity is a global issue threatening crop growth and yield. Salt-tolerant plant growth-promoting rhizobacteria (PGPB) can survive in high-salinity environments and help plants adapt to stress, thus serving as an effective measure to mitigate salt stress.

Results In this study, a salt-tolerant plant growth-promoting bacterium, *Halomonas alkaliantarcticae M23* (M23), was isolated from the rhizosphere soil of the salt-tolerant plant *Suaeda salsa*. This study characterized the effects of M23 on maize growth, salt stress response, and the composition and structure of rhizosphere soil microorganisms, and preliminary explained the mechanism by which M23 enhances maize salt tolerance. M23 can tolerate up to 14% NaCl, produce auxin, and exhibit the ability to absorb Na^+ and accumulate K^+ under salt stress. This study also measured amino acid production by M23 under different salinity conditions and found that M23 could mainly produce glutamic acid (Glu), glutamine, proline, and lysine, with their contents significantly increasing as salinity rises. Inoculating maize with M23 enhances the salt tolerance by increasing the K^+/Na^+ ratio, improving the antioxidant levels, and regulating its ABA levels in maize. Additionally, inoculating with strain M23 not only increases soil diversity but also alters the composition of bacterial communities in the maize rhizosphere soil. Most species were significantly enriched in saline soil treated with M23 at the phylum level. At the genus level, some salt-tolerant plant growth-promoting bacteria such as *Bryobacter*, *Nocardioides*, and *Micromonosporaceae* were also significantly enriched.

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Conclusions *Halomonas alkaliantarcticae M23* could promotes the salt tolerance of maize by increasing the K^+/Na^+ ratio, antioxidant levels, and ABA levels and changing the rhizosphere bacterial community. This study demonstrates that M23 has great potential in promoting plant growth in saline-alkali soils.

Keywords Plant growth promoting bacteria, *Halomonas alkaliantarcticae M23*, Maize, Rhizosphere bacterial community

Background

According to the survey data of the Food and Agriculture Organization of the United Nations, at present, more than 1 billion hectares of salinized soil in the world cannot be effectively utilized due to the high salinity level. Moreover, under the joint action of climate change, frequent extreme climate, irrational farming system and other factors, the salinization of soil has become a worldwide problem [1]. Due to the increasingly serious impact of salt stress on land and crops, it is urgent to develop an efficient and feasible coping strategy to solve the global food security problem. The traditional method is to cultivate new salt-tolerant varieties or develop transgenic salt-tolerant plants [2], but due to its poor effect and certain limitations in the application process, it is urgent to find an effective and feasible alternative strategy. In recent years, it has become an effective means to improve saline-alkali soil and plant stress resistance through plant rhizosphere growth-promoting bacteria, and it is also an efficient and sustainable development strategy.

Soils with high salinity (i.e., conductivity EC > 4dS/M) are classified as saline soils [3, 4]. Due to various reasons such as low precipitation, high surface evaporation rate, weathering of native rocks, saline irrigation and poor cultural practices, salinization areas have increased dramatically. The damage caused by salt stress to plants is mainly ion toxicity, osmotic disorder and accumulation of toxic substances (reactive oxygen species). When plants are subjected to salt stress, high salinity reduces the water potential around the plant roots, which inhibits the absorption of water and nutrients, leading to osmotic stress. Secondly, high salt will lead to the accumulation of Na^+ and Cl^- in plants, resulting in ionic toxicity, damage the homeostasis of Na^+ and K^+, and hinder the absorption of Ca^{2+} [5]. Osmotic stress and ion stress caused by high salt can also lead to excessive accumulation of ROS, resulting in oxidative stress, while excessive accumulation of ROS in chloroplasts and mitochondria can affect photosynthesis and respiration of plants [6, 7]. ROS accumulation can also damage plant tissues, alter DNA, damage cell membranes, and degrade lipids, proteins, and other biomolecules [8, 9].

Salt stress has a serious impact on the growth of plants and caused huge losses to agricultural production, so in order to sustainable development of agriculture, it is necessary to formulate a series of mitigation strategies to deal with soil salinization. In order to reduce the adverse effects of salt stress on plants, scientists have adopted a series of measures, among which salt-tolerant crop breeding (conventional breeding and genetic engineering, etc.) is one of the commonly used technical means [2]. For example, overexpression of ion transporters (NHX1, SOS1, HKT1, etc.) significantly improved the salt tolerance of transgenic plants [10–12]. Although salt-tolerant crop breeding shows great application potential, its wide application is limited by its long breeding time, high cost and low success rate. At the same time, finding effective new salt tolerance genes that can balance crop yield and salt tolerance is also a huge challenge. In recent years, plant growth promoting bacteria (PGPB) have also been used to protect plants from salt stress in soil [13]. PGPB improves plant growth and enhances its tolerance to salt stress through direct effects, including nitrogen fixation, phosphorus enrichment, NH3, indole acetic acid (IAA) and iron carrier production, as well as indirect effects, including antioxidant defense, production of volatile organic compounds (VOC), extracellular polysaccharide (EPS) and osmotic balance mechanism [13–16]. Microorganisms with plant growth promotion function are natural tools to combat soil salt stress [8]. Moreover, the application of plant promoting rhizogenic bacteria (PGPB) is an ecologically friendly method to solve the base osmotic stress in agroecology. It has been reported that many native PGPB inhabit plant rhizosphere and promote plant growth through biofilm formation, chemotactic mechanism, phosphate (P) solubilization, nitrogen fixation (N_2), and production of exopolysaccharides, growth hormones, and deaminase [17, 18]. So far, many bacteria, including *Pseudomonas*, *Bacillus*, *Burkholderia*, *Panthenia* and *rhizobia*, have been reported to improve crop tolerance under different abiotic stress conditions [19]. In addition, the improper application of some fertilizer to increase production will lead to a gradual increase in soil salinity, and in the long run, the soil will become unfertile, while PGPB can reduce fertilizer input, reduce production costs, and increase soil fertility.

In this study, a salt-tolerant auxin producing bacterium was isolated from the rhizosphere soil of halophyte *Suaeda salsa* growing in the soil of saline-alkali soil in Shandong Province, and its growth-promoting effect and influence on the salt tolerance of plants were studied. It was found that the application of the bacterium could significantly promote the growth of plants under normal soil conditions. Salt stress can also significantly improve

plant salt tolerance. In addition, the bacteria can tolerate up to 14% NaCl, so it can be colonized in soil with high salinity. Moreover, the bacteria is a natural microorganism isolated from saline-alkali soil, so it can be made into bacterial fertilizer and applied in saline-alkali soil in the future, so as to achieve the effect of improving saline-alkali soil. The results of this study can provide candidate strain resources for the development of salt-tolerant biologic fertilizer/agent, so it has important application value.

Result

Strain M23 is a salt-tolerant auxin-producing bacteria

We isolated a salt-tolerant strain from the rhizosphere soil of the halophyte *Suaeda salsa* (Fig.S1A). PCR amplification and sequencing of the 16 S rRNA gene obtained a 1400 bp 16 S rRNA sequence. NCBI BLAST analysis revealed its closest phylogenetic relationship with *Halomonas alkaliantarcticae* CRSS. The strain was designated as *Halomonas alkaliantarcticae* M23 (Supplemental document), and its 16 S rDNA sequence has been deposited in NCBI (accession number: PP864102.1). The bacterium exhibits a rod-shaped morphology, stains gram-positive (Fig.S1B), and can be capable of producing exopolysaccharides (1.82 mg/g) (Fig.S1C). To determine the salt tolerance of strain M23, 100 μL of strain M23 suspension was inoculated into LB medium containing 0%, 3%, 5%, 10%, 14%, and 15% NaCl (w/v), and cultured in a shaker at 37 °C for 48 h. The OD value of the culture medium was measured at 12 h, 24 h, 36 h, and 48 h, showing that strain M23 can tolerate up to 14% salt concentration (Fig. 1A, Fig.S2). And the strain M23 could also produce IAA (Fig. 1B). In summary, M23 is a salt-tolerant auxin-producing bacteria.

Effect of inoculated strain M23 on Na^+ and K^+ content in culture medium

To investigate the effect of strain M23 on the Na^+ and K^+ content in the culture medium, we measured the changes in Na^+ and K^+ concentrations in the LB medium with different NaCl concentrations after inoculating M23. The results showed that compared with 0 h, under 1.5% and 3% NaCl concentrations, the Na^+ concentration in the culture medium decreased significantly with the increase in culture time after inoculating M23, indicating that M23 can absorb Na^+ from the culture medium (Fig. 2A). Compared with 0 h, the K^+ concentration in the culture medium also increased under 1.5% and 3% salt concentrations (Fig. 2B). The above results indicate that under salt stress conditions, M23 regulates the ion balance of the medium by absorbing Na^+ and releasing K^+, thereby reducing the environmental Na^+ concentration.

High salt levels lead to the accumulation of some amino acids in strain M23 cells

Under normal conditions, M23 primarily produces glutamic acid (Glu), glutamine, proline, and lysine, with their contents significantly increasing with rising salinity. Specifically, compared to the control, proline content under 3%, 5%, and 10% NaCl stress increased by 276.38%, 1571.22%, and 8162.14%, respectively. Glutamic acid content also increased by 48.28%, 25.04%, and 264.30% under 3%, 5%, and 10% NaCl stress, respectively, when compared to the control. Lysine content was also elevated by 532.54% under 10% NaCl stress compared to the control. Additionally, amino acids such as serine, glycine, threonine, alanine, L-ornithine, tyrosine, methionine, valine, isoleucine, leucine, phenylalanine, and tryptophan increased with rising salinity. Notably, arginine (Arg) accumulated only under salt stress conditions (Table 1).

M23 could promote the growth of maize under both normal growth conditions and salt stress conditions

The pot experiment demonstrated that M23 can significantly promote maize growth under both normal growth conditions and salt stress (Fig. 3A). Under normal growth conditions, inoculation with M23 notably enhances maize growth, evidenced by an increase in the fresh weight of the maize leaves (increased 46.55% compared with the control maize). Under salt stress conditions, salt stress significantly inhibits maize growth, however, inoculation with M23 alleviates the inhibitory effect of salt stress on aboveground compared to the control maize, the fresh weight of the maize leaves increased by 108% (Table 2). The ABA content in the M23-treated maize was significantly higher than that in the control maize under salt stress (Table 2). Additionally, the activities of GST, CAT, POD and APX in maize treated with M23 were also increased by 88.25%, 24.26%, 74.57% and 44.35% respectively. We also detected the Na^+ and K^+ content in the leaves of maize under both normal and salt condition. The result showed that inoculation with M23 could inhibit the absorption of Na^+ and promotes the absorption of K^+ in maize leaves (Fig. 3B-J). Consequently, this leads to a significant increase in the K^+/Na^+ ratio, both under salt stress conditions, thereby enhancing the salt tolerance of maize. Even under normal conditions, the application of M23 was found to elevate the K^+/Na^+ ratio, primarily due to its ability to facilitate K^+ absorption and hinder Na^+ absorption (Fig. 3B-D).

Ion transport and ABA synthesis and signaling pathways related genes in leaves of maize inoculated with M23 were significantly up-regulated under salt stress conditions

Since M23 can affect the Na^+ and K^+ content in maize leaves after salt stress, we further examined the expression of the ion transport-related genes in maize leaves

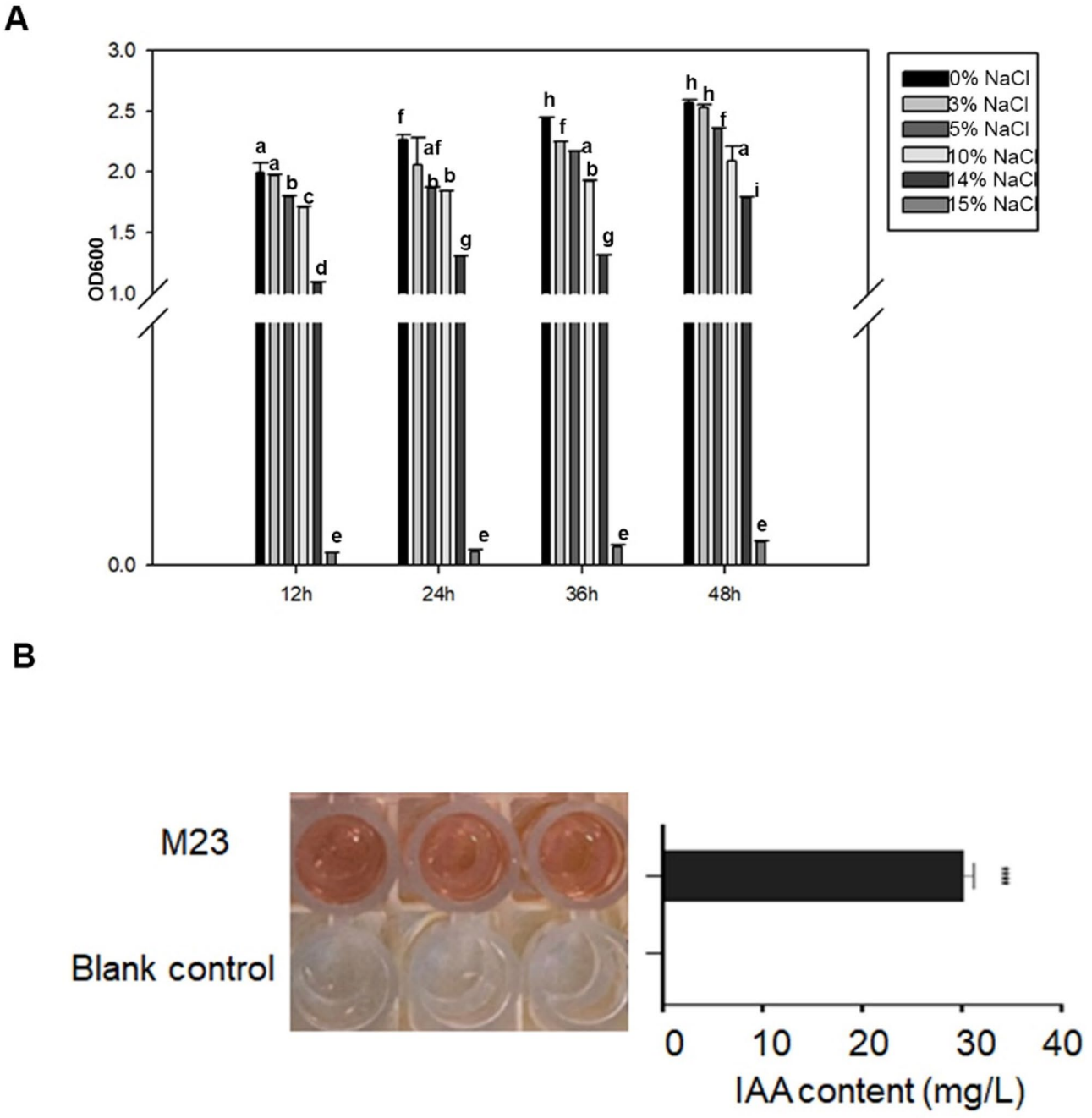

Fig. 1 Determination of the salt tolerance and auxin-producing ability of M23. A, The cell number (indicated by OD600) in the culture solution at different NaCl concentrations inoculated with M23. B, Determination of IAA-producing ability of M23. Each bar represents the mean ± SD ($n \geq 3$). Different lowercase letters above the bars represent significant differences based on one-way ANOVA (Duncan's multiple range, $p < 0.01$). Asterisks indicate significant differences between treatments according to Student's t-test (****$p < 0.0001$)

under both normal and salt-stress conditions. The result showed that M23 could induces the expression of Na^+/H^+ antiporter genes (*NHX5*, *NHX8*, *NHX9*, and *NHX14*), and potassium high-affinity transporter genes (*HAK2*, *HAK3*, *HAK4*, *HAK7*, *HAK8*, under the salt stress conditions (Fig. 4). And because of that the ABA content in the M23-treated maize was significantly higher than that in the control maize under salt stress, we also detected the expression level of the genes related the ABA synthesis and signaling pathways. It indicated that the genes related to ABA synthesis (*NCED2*, *NCED8*, *NCED* and *NCED9*) and the ABA signaling pathway (2 genes of PYL, 1 gene of SnRK, and 2 genes of ABF) were also up-regulated in maize inoculated with M23 under the salt stress. And we

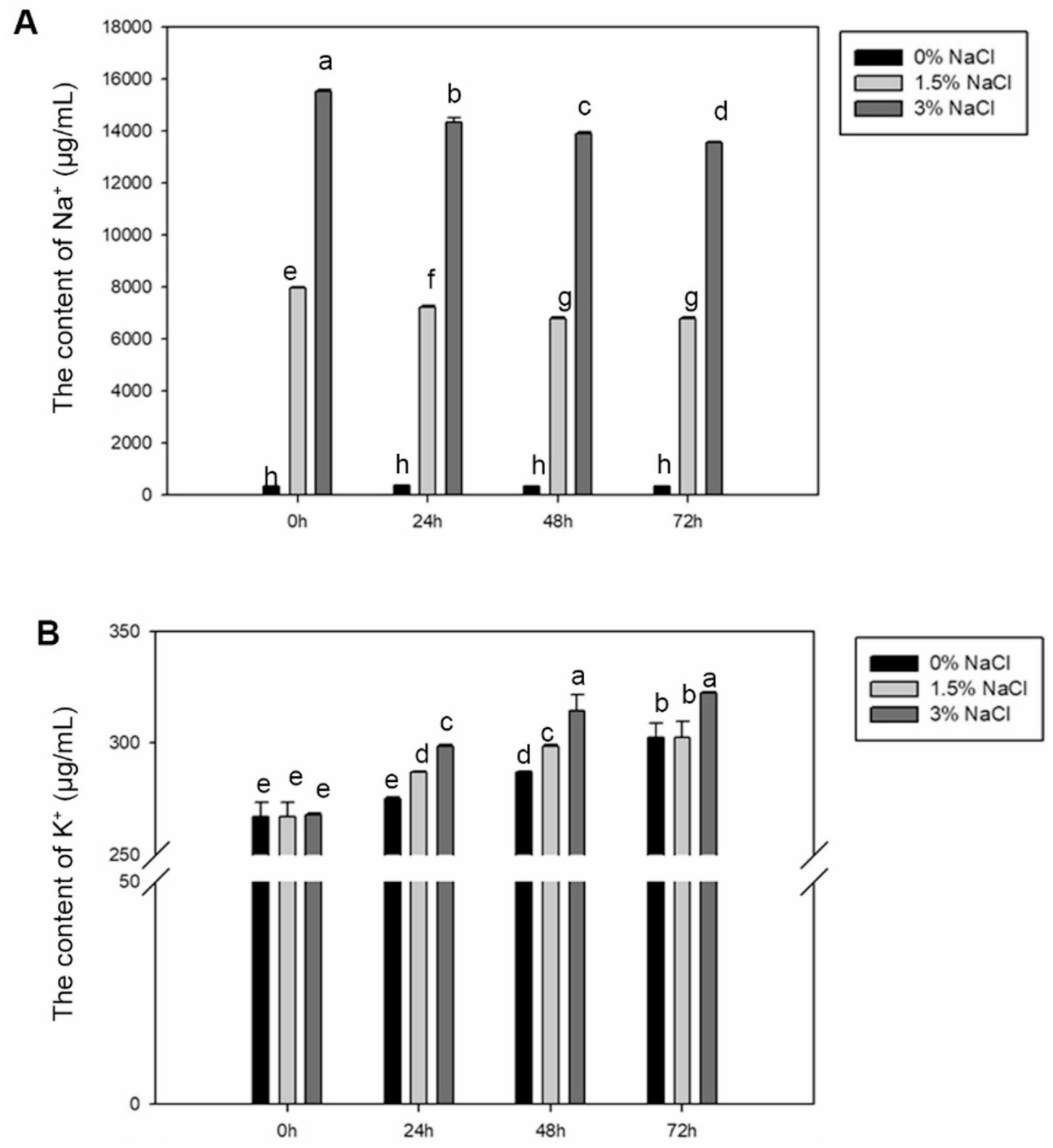

Fig. 2 The Na^+ and K^+ concentration in the culture solution at different concentrations of NaCl stress inoculated with strain M23. Values are means ± sd. Bars represent means ± sd ($n = 3$ repeats). Different letters indicate significant difference at $P < 0.01$

also found that the genes encoding of peroxidase 72 were also up-regulated in maize with M23 treatment (Fig. 4).

M23 could significantly improve the soil environment in both normal and salt-stressed soils

The soil enzyme activity is an important indicator for monitoring the dynamics and health of the soil ecosystem. Since the rhizosphere soil was in close contact with the roots, soil enzyme activity in the rhizosphere soil of the maize under the normal and salt stress condition were detected. And the result showed that M23 significantly increased the activity of soil alkaline phosphatase, urease and soil dehydrogenase (Table 3). Compared with uninoculated soil, the activities of urease,

Table 1 Analysis of the production of amino acids by *Halomonas alkaliantarcticae* M23 under different salt concentrations (0%, 3%, 5%, and 10% NaCl)

sampleCode	0% NaCl	3% NaCl	5% NaCl	10% NaCl
Histidine	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.10 ± 0.10
Arginine	0.00 ± 0.00	5.16 ± 0.18	5.01 ± 0.30	0.00 ± 0.00
Asparagine	0.16 ± 0.02	7.04 ± 0.16	0.95 ± 0.09	0.05 ± 0.01
Glutamine	32.10 ± 0.28	23.31 ± 0.53	23.16 ± 0.38	76.31 ± 0.88
Serine	2.24 ± 0.05	8.09 ± 0.16	1.53 ± 0.01	1.41 ± 0.02
Glycine	2.42 ± 0.03	9.44 ± 0.09	3.46 ± 0.06	3.15 ± 0.06
Aspartic acid	0.81 ± 0.02	3.80 ± 0.12	1.29 ± 0.01	1.81 ± 0.03
L-Citrulline	0.62 ± 0.02	3.17 ± 0.03	13.69 ± 0.12	344.79 ± 0.49
Glutamic acid	30.90 ± 0.69	45.82 ± 0.47	38.64 ± 0.21	112.57 ± 0.67
Threonine	1.03 ± 0.02	9.45 ± 0.11	5.33 ± 0.06	8.10 ± 0.07
Alanine	4.95 ± 0.03	15.70 ± 0.06	6.35 ± 0.07	2.51 ± 0.04
Gamma-Aminobutyric acid	0.17 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.33 ± 0.02
Proline	10.46 ± 0.07	39.37 ± 0.11	174.81 ± 1.36	864.22 ± 10.21
L-Ornithine	2.03 ± 0.02	10.45 ± 0.14	4.28 ± 0.21	37.69 ± 0.21
Lysine	22.06 ± 0.47	19.54 ± 0.28	16.41 ± 0.34	139.54 ± 0.38
Cystine	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Tyrosine	2.11 ± 0.05	12.85 ± 0.14	7.20 ± 0.14	13.00 ± 0.11
Methionine	1.51 ± 0.04	7.46 ± 0.10	5.07 ± 0.02	3.33 ± 0.06
Valine	3.67 ± 0.08	11.74 ± 0.03	5.28 ± 0.12	2.68 ± 0.04
Isoleucine	0.84 ± 0.02	10.46 ± 0.08	2.64 ± 0.01	0.89 ± 0.02
Leucine	3.05 ± 0.07	15.52 ± 0.17	5.29 ± 0.04	3.32 ± 0.02
Phenylalanine	3.92 ± 0.19	13.20 ± 0.13	5.63 ± 0.07	7.13 ± 0.09
Tryptophan	1.97 ± 0.04	3.55 ± 0.03	1.81 ± 0.02	5.52 ± 0.04

alkaline phosphatase, and soil dehydrogenase increased by 30.68%, 22.50%, and 35.47% respectively under salt stress. While under normal conditions, the activities of urease, alkaline phosphatase, and soil dehydrogenase were also increased by 31.46%, 54.54%, and 28.01%, respectively. These results indicated that the application of M23 could significantly improve the soil environment in both normal and salt-stressed soils.

Inoculation with M23 changed the bacterial community in maize rhizosphere under normal and salt stress

To analysis the effect of M23 on the rhizosphere bacterial community, high throughput sequencing of 16 S rDNA region was used to analyze the effects of M23 on rhizosphere bacterial community diversity and abundance under both normal and salt stress condition. The results of sparse curve analysis indicate that the curves of the 4 libraries generate were nearly smooth, indicating that the sequencing results adequately reflected the diversity contained in the current sample (Fig. 5A). The number of taxa showed that salt stress significantly reduced the number of bacterial species in soil, but inoculation of M23 alleviated the decline (Fig. 5B). The effect of bacterial inoculation on microbial community composition was assessed by PCoA analysis, and the bacterial community in the inoculated samples of strain M23 was significantly separated from that in the uninoculated samples (Fig. 5C). Analysis of the species composition showed that *Proteobacteria* was the main phyla, followed by were *Actinobacteriota*, *Gemmatimonadota*, *Chloroflexi*, *Bacteroidota*, *Acidobacteriota*, *Verrucomicrobiota*, *Firmicutes*, *Patescibacteia* and *Planctomycetota*. Although the bacterial community abundance of the soil treated with M23 compared with the soil without M23 treatment changed under normal and salt condition, the dominant species were similar (Fig. 5D). Analysis of the α diversity showed that in the absence of inoculation with strain M23, soil bacterial α diversity, including Shannon, Simpson, and Pielou, was significantly reduced under salt stress. Compared with uninoculated soil, the applied strain M23 significantly increased the Shannon and Simpson and Pielou indices under both normal and salt stress conditions (Fig. 5E). Random forest analysis in different level showed that the bacterial community abundances of the rhizosphere soil with M23 treatment were different with the soil without treatment both under normal and salt stress condition (Fig. 6). As shown in Fig. 6A, at the phylum level, most species were significantly enriched in the saline soil with M23 treatment. And at genus level, *Rubrobacter*, *Babeliales*, *Alcaligenaceae*, *Bryobacter*, *PLTA13*, *Nocardioides*, *Mycobacterium*, *Flindersiella*, *Pedobacter*, *Rhizocola*, *Rhodococcus*, *Candidatus_Alysiosphaera*, *Ensifer*, *alphaI_cluster* and *Pedomicrobium* were significantly enriched in M23 treatment soil (Fig. 6B).

Discussion

High salinity leads to osmotic stress, ionic toxicity, oxidative damage and nutrient imbalance in plants, thus reducing photosynthetic rate, inhibiting plant growth, and reducing plant yield and quality. Beneficial rhizosphere bacteria inhabit the roots of plants and can release a variety of metabolites to promote plant growth, development and resistance to various stresses. Therefore, PGPB from natural salt environments can be a real candidate to more effectively reduce the effects of salt stress on plants [20]. This study evaluated the effects of M23 inoculation on plant growth and development under normal growth conditions and salt stress. The results showed that inoculation of M23, a plant growth promoting bacterium, could not only promote the growth of maize under normal growth conditions, but also reduce the negative effects of salt stress on the plant and significantly promote the growth under salt stress conditions.

M23 can alleviate the ion stress caused by high salinity in maize

Na^+ homeostasis regulation is the key to maintain normal plant growth under salt stress [21]. Under normal

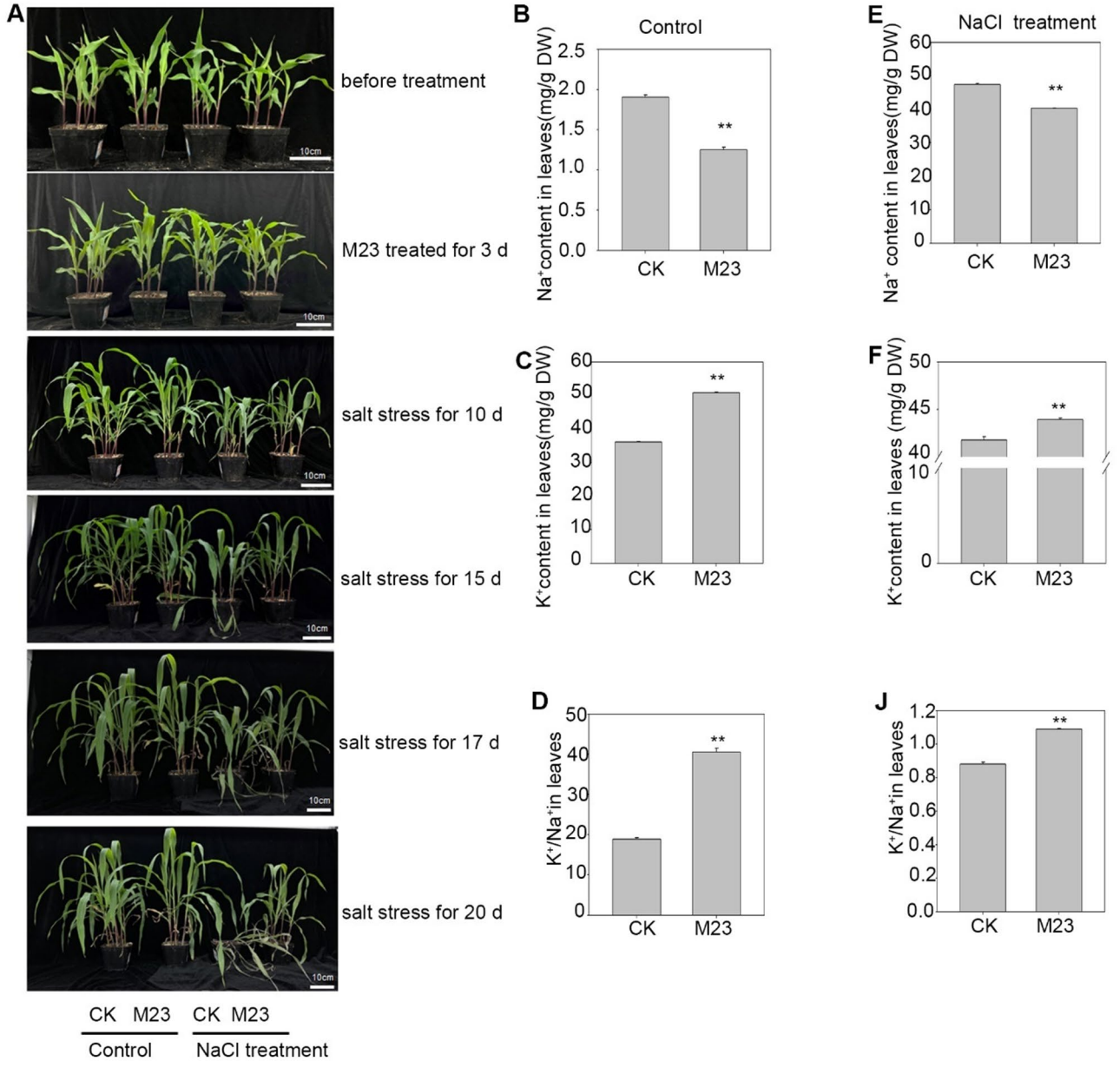

Fig. 3 Phenotypes of maize under normal and salt stress in soil culture condition with and without M23. A, Phenotypes of maize under normal (control) and salt stress (final soil salinity was 8.0 dS m^{-1}) in soil culture condition with and without *M23*. B-D, Na^+ content, K^+ content, and K^+/Na^+ ratio in the leaves of maize under normal condition. E-J, Na^+ content, K^+ content, and K^+/Na^+ ratio in the leaves of maize under salt stress. Values are means ± sd ($n = 3$ repeats). Significant differences are indicated by asterisks (**, $P \leq 0.01$)

Table 2 Effect of strain M23 on the FW, ABA content and the antioxidant enzymes (GST, CAT, POD and APX) of the maize

NaCl treatment (mM)	M23 treatment	FW(g/plant)	ABA(μg/g)	GST(U/g)	CAT(U/g)	POD(U/g)	APX(U/g)
0	No bacteria	2.234 ± 0.143	1.319 ± 0.021	1054.125 ± 56.363	26.940 ± 0.644	17.333 ± 1.955	1.003 ± 0.015
0	M23	3.274 ± 0.304**	2.433 ± 0.067**	2885.995 ± 157.906**	52.840 ± 0.794**	71.200 ± 0.600**	4.240 ± 0.168**
250	No bacteria	0.180 ± 0.083	2.231 ± 0.030	2104.085 ± 18.161	49.680 ± 1.191	38.017 ± 1.557	3.170 ± 0.097
250	M23	0.375 ± 0.107**	2.629 ± 0.018**	3960.955 ± 43.366**	61.733 ± 0.578**	66.367 ± 0.611**	4.576 ± 0.043**

and saline stress conditions, inoculating with M23 can restrict the accumulation of Na^+ in plants and increase K^+ content, thereby enhancing the K^+/Na^+ ratio. This indicates that M23 possesses certain mechanisms to limit the accumulation of Na^+ in plants. Additionally, we found that under salt stress, the concentration of Na^+ in the liquid medium inoculated with M23 significantly decreased, suggesting that this bacterium can consume Na^+ from

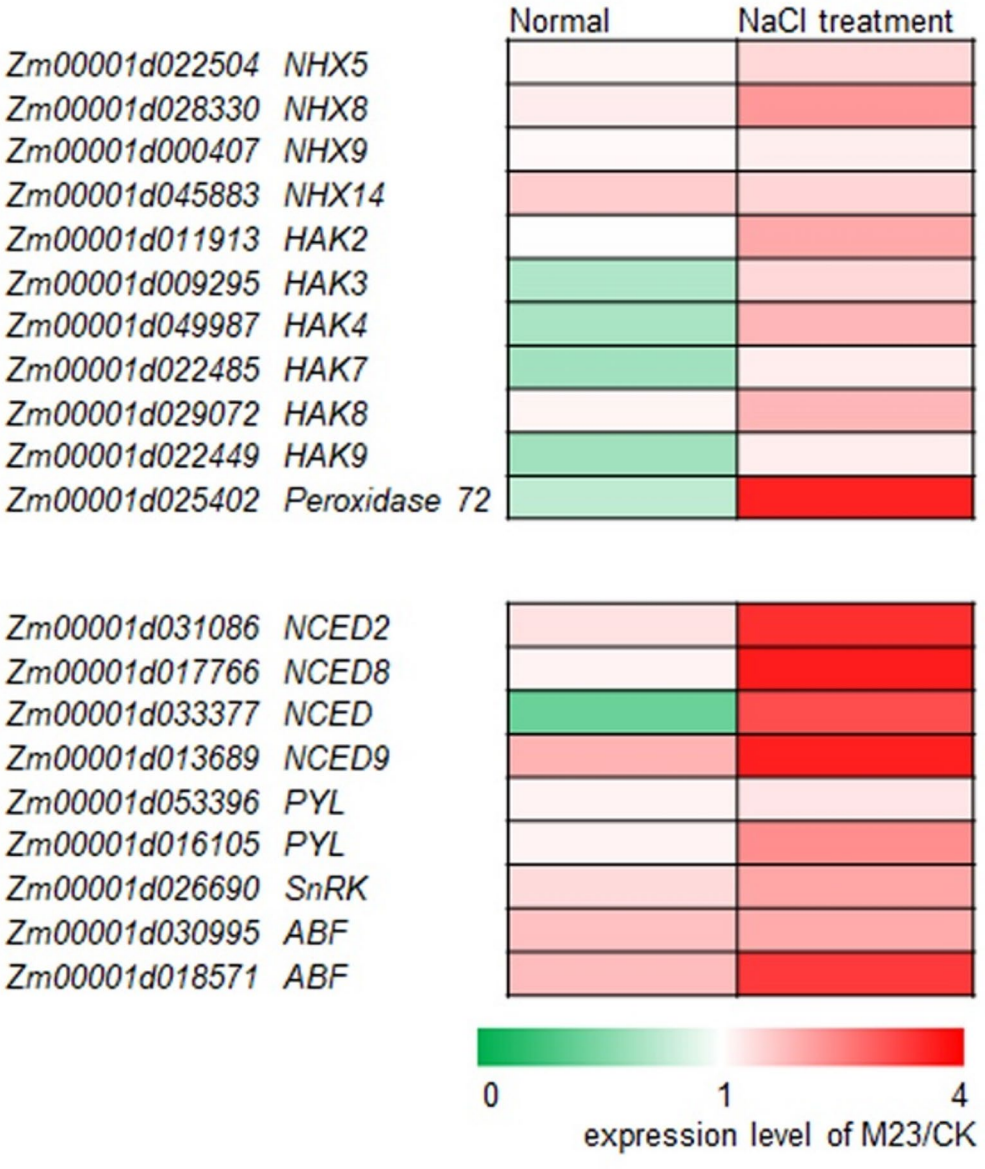

Fig. 4 Expression of genes related to ion transport and ABA synthesis and signaling pathways in leaves of maize inoculated with or without M23 under normal and salt stress conditions. Expression of genes related to ion transport and ABA synthesis and signaling pathways were analyzed by real-time RT-PCR, fold changes in transcripts were calculated by $2^{-\Delta\Delta Ct}$ method with *ZmTub* as an internal control. And the heatmaps of these genes were made using the value of M23 /CK of the relative expression level

Table 3 Effect of strain M23 on the enzymes activity of the urease, alkaline phosphatase, and dehydrogenase in rhizosphere soil of the maize under normal and salt condition

NaCl treatment (mM)	M23 treatment	Urease(U/g)	Alkaline phosphatase(U/g)	Soil dehydrogenase(U/g)
0	No bacteria	315.306 ± 3.535	854.139 ± 18.264	1607.964 ± 25.015
0	M23	414.516 ± 7.559**	1320.022 ± 27.599**	2058.318 ± 32.579**
250	No bacteria	328.031 ± 2.121	1136.577 ± 22.013	2235.132 ± 38.556
250	M23	428.656 ± 4.261**	1392.282 ± 10.106**	3027.834 ± 26.226**

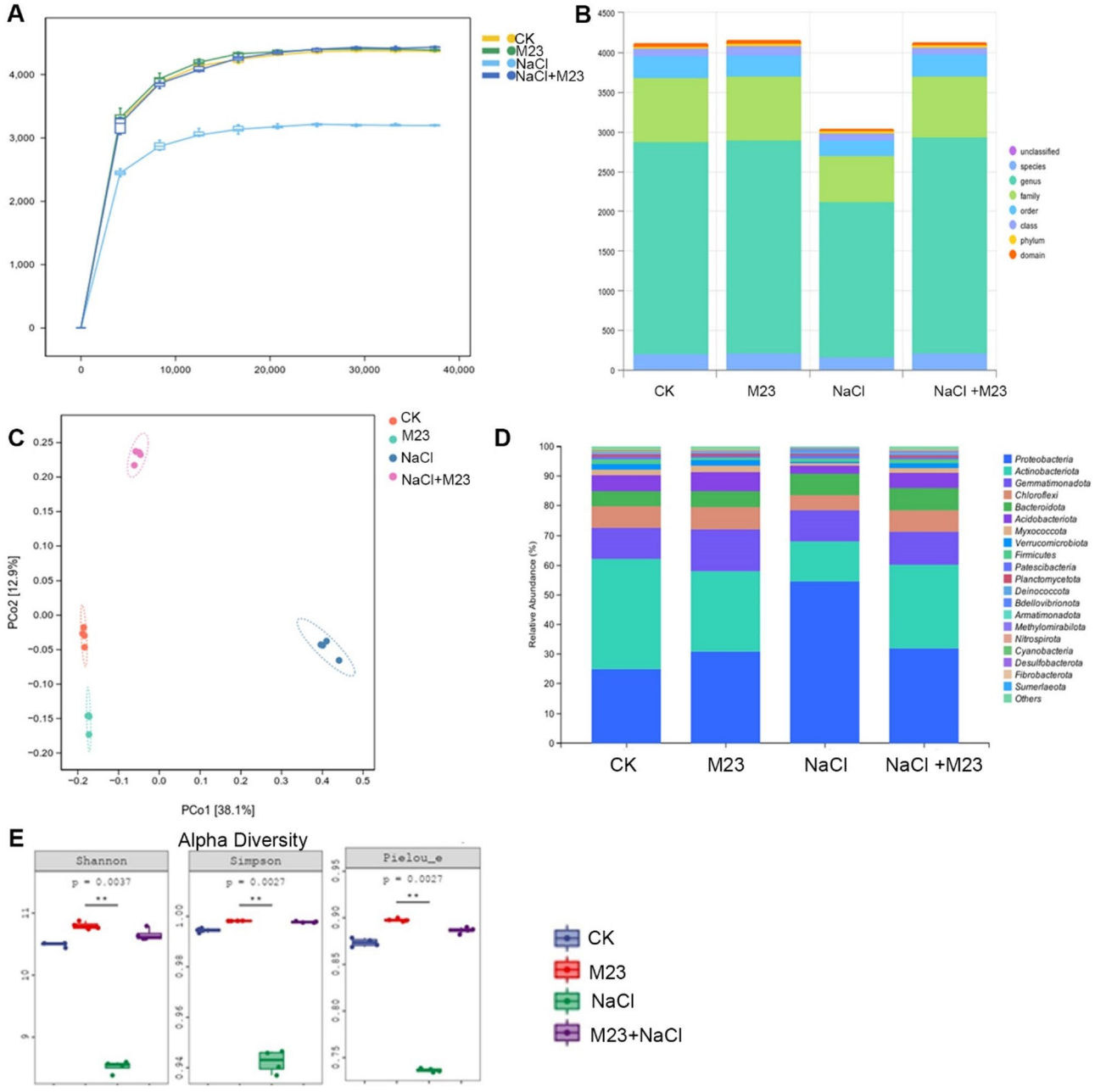

Fig. 5 The sparse curve analysis, taxonomic unit count of species, PCoA analysis, the species composition analysis, and the α diversity analysis indices of the bacteria in rhizosphere soil of the maize under normal and salt stress in soil culture condition with and without M23. **A**, The sparse curve analysis. **B**, The species composition analysis (Taxonomic unit count of species). **C**, The β diversity analysis (PCoA analysis). D, The species composition analysis (The relative abundances of bacterial communities at phylum level). **E**, The α diversity analysis indices

the medium. Furthermore, the K^+ content in the medium also increased slightly. Therefore, we speculate that M23 can absorb environmental Na^+ to alleviate ionic toxicity in plants under salt stress. M23 is a salt-tolerant strain capable of tolerating 14% NaCl. The salt tolerance of the M23 strain may also play a crucial role in its interaction with plants.

Additionally, we analyzed the expression of ion transporter-related genes in plant leaves. Under salt stress, M23 induced the upregulation of several ion homeostasis-related genes, including Na^+/H^+ antiporter genes (*NHX5*, *NHX8*, *NHX9*, and *NHX14*) and high-affinity potassium transporter genes (*HAK2*, *HAK3*, *HAK4*, *HAK7*, *HAK8*). The increased expression of these genes significantly alleviated ionic stress in plants, enhancing their salt tolerance. This may represent another mechanism by which M23 mitigates salt-induced ionic stress in maize.

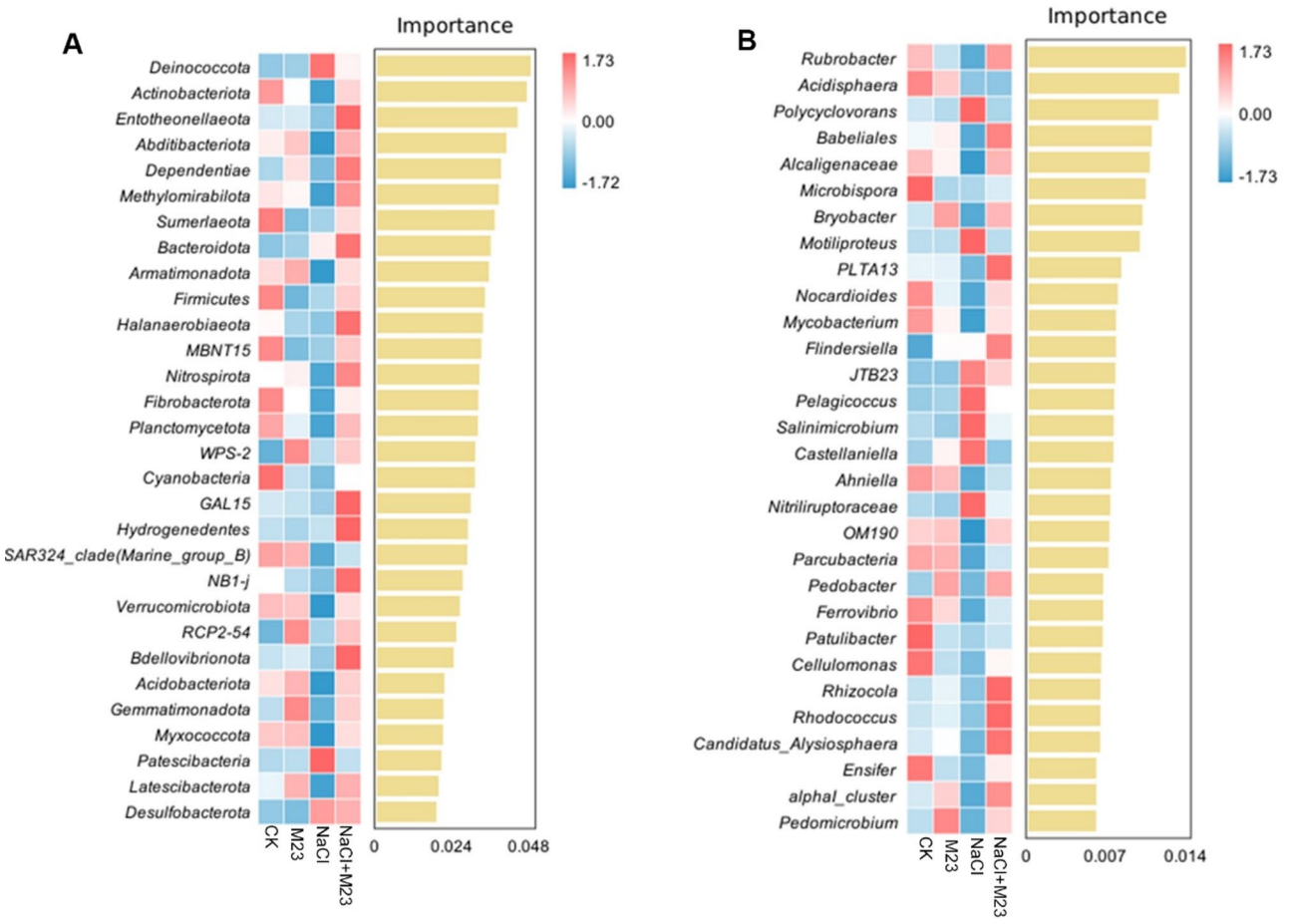

Fig. 6 The heatmap of the top 30 phylum (**A**), Genus (**B**) in the rhizosphere soil of bacteria in rhizosphere soil of maize

M23 may enhances maize salt tolerance by secreting beneficial amino acids that support osmotic regulation, stress signaling, and hormonal balance

Salt-tolerant bacteria adapt to high salt stress through various methods. One of the most common strategies is the accumulation of compatible solutes, such as sugars, amino acids, and glycine betaine. These molecules serve as compatible solutes, protecting cells from damage while maintaining normal cellular functions [22]. Under salt stress, amino acids such as Glutamine, Glutamic acid, Proline, tryptophan and L-Citrulline significantly increase in strain M23. These amino acids are the main compatible substances that the salt-tolerant plant-promoting bacteria use to alleviate salt stress [22]. These amino acids can act as compatible solutes to help M23 adapt to salt stress. Proline, as an osmoprotectant, can be absorbed by plants to help maintain cellular osmotic balance, mitigating dehydration caused by salt/drought stress [23]. Glutamate not only serves as a proteinogenic amino acid but also acts as a signaling molecule, participating in various physiological processes. Under normal conditions, it regulates seed germination and root development. Under stress conditions, it contributes to plant pathogen resistance and abiotic stress responses (e.g., salinity, low/high temperature, and drought) [24]. Thus, M23-secreted glutamate may promote root development and enhance stress resistance in plants. Tryptophan, a precursor of phytohormones (e.g., auxin), can be absorbed by plants to stimulate auxin synthesis [25], potentially improving plant growth under stress. These findings suggest that M23 may enhance maize salt tolerance by secreting beneficial amino acids that support osmoregulation, stress signaling, and hormonal balance.

Moreover, M23 can produce auxin IAA, which is essential for cell division and elongation in plants responding to salt stress [26]. Auxin (IAA), an essential plant hormone, is produced by numerous endophytic bacteria colonizing halophytes. IAA exerts its functions by enhancing plant seed germination, root proliferation, and cellular permeability to water, as well as by reducing cell wall pressure [27].

M23 may alleviate oxidative damage induced by salt stress

Plants under salt stress often produce excessive reactive oxygen species (ROS), which can be harmful to proteins, DNA, and lipids, leading to membrane damage, enzyme

inhibition, and chlorophyll degradation. Therefore, ROS scavenging is crucial for the survival and growth of plants under salt stress [28]. Excessive ROS are scavenged by antioxidants in plants, such as antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione peroxidase (GPX), ascorbate peroxidase (APX), etc.) [29]. Under salt stress, M23 strain increases the activity of antioxidant enzymes such as POD, CAT, GST, and APX. These enzymes can effectively scavenge ROS produced by plants to mitigate the oxidative damage under salt stress, thereby enhancing the salt tolerance of plants. The observed changes in the activity of antioxidant enzymes suggest that M23 may help alleviate oxidative stress, and we will further investigate how M23 enhances plant salt tolerance by alleviating oxidative stress, by determining the levels of reactive oxygen species (ROS) and oxidative status in different parts of plants under salt stress in the future.

M23 may enhance salt tolerance in plant by promoting ABA synthesis

Physiological and biochemical assays further confirmed a significant increase in ABA content in maize leaves after M23 treatment. ABA, as a stress-resistant hormone, can significantly enhance many stresses tolerance in plants [30]. Furthermore, we analyzed the gene expression related to ABA synthesis, and signaling pathways in maize leaves treated with M23 under both normal and salt stress conditions and found that M23 treatment significantly induced the expression of the genes involved in ABA biosynthesis and ABA signaling pathways. These results demonstrate that M23 may enhance the salt tolerance in plant, at least in part, by promoting ABA synthesis. And in the future, we will use the ABA synthesis and ABA signaling pathway defective mutants to further verify whether M23 enhances plant salt tolerance through ABA synthesis and the ABA signaling pathway.

M23 may enhance salt tolerance in maize by changing the rhizosphere bacterial community

To investigate the mechanism of M23 in improving plant salt tolerance, we also assessed its impact on soil microbial communities. Long-term salt stress affects the structure of soil microbial communities. Our research also showed that the abundance of soil microorganisms significantly decreased after salt stress. However, inoculating with M23 altered the composition of microorganisms in rhizosphere soil. Under salt stress, most species were significantly enriched in saline soil treated with M23 at the phylum level. At the genus level, *Rubrobacter*, *Babeliales*, *Alcaligenaceae*, *Bryobacter*, *PLTA13*, *Nocardioides*, *Mycobacterium*, *Flindersiella*, *Pedobacter*, *Rhizocola*, *Rhodococcus*, *Candidatus_Alysiosphaera*, *Ensifer*, *alphaI_cluster*, and *Pedomicrobium* were enriched. Among them, *Rubrobacter* has a close positive correlation with soil organic carbon, pH, available phosphorus, ammonia nitrogen (N), and nitrate nitrogen under drought conditions [31], and it is significantly enriched under salt stress [32]. *Bryobacter* is also a plant growth-promoting bacterium that can recruit many beneficial microorganisms associated with organic matter decomposition, plant growth promotion, and pathogen suppression, thereby promoting plant growth [33]. *Bryobacter* [34], *Nocardioides* [35], *Micromonosporaceae* [36] also belong to plant growth-promoting bacteria. We will conduct additional experiments to quantify the specific contributions of the altered bacterial community to maize salt tolerance and to compare the effectiveness of different bacterial strains. In the future, we would isolate these key salt-tolerant bacteria and investigate the potential synergistic interactions between M23 and the key microbial taxa.

All experimental results presented in this paper were obtained under laboratory conditions. In the next phase, we will apply for patent protection for this bacterium and seek permission to conduct field experiments to further confirm the growth-promoting effects of M23 on plants under saline-alkali conditions.

Conclusion

A salt-tolerant plant growth-promoting bacterium, *Halomonas alkaliantarcticae M23* (M23), was isolated from the rhizosphere soil of the salt-tolerant plant *Suaeda salsa*. M23 can tolerate up to 14% NaCl, produce auxin, and exhibit the ability to absorb Na^+ and accumulate K^+ under salt stress. This study also measured amino acid production by M23 under different salinity conditions and found that M23 could mainly produce glutamic acid (Glu), glutamine, proline, and lysine, with their contents significantly increasing as salinity rises. And M23 inoculation may improve the salt tolerance of maize by coordinately increasing the K^+/Na^+ ratio, improving the antioxidant levels, and regulating its ABA levels in maize. Additionally, inoculating with strain M23 not only increases soil diversity but also alters the composition of bacterial communities in the maize rhizosphere soil. Some salt-tolerant plant growth-promoting bacteria such as *Bryobacter*, *Nocardioides*, and *Micromonosporaceae* were significantly enriched in the maize rhizosphere soil inoculating with strain M23 which could also increase the salt resistance of plants. This study demonstrates that M23 has great potential in promoting plant growth in saline-alkali soils (Fig. 7).

Materials and methods

Bacteria and soil

Halomonas alkaliantarcticae M23 (NCBI accession number: PP864102) was isolated from the rhizosphere soil of

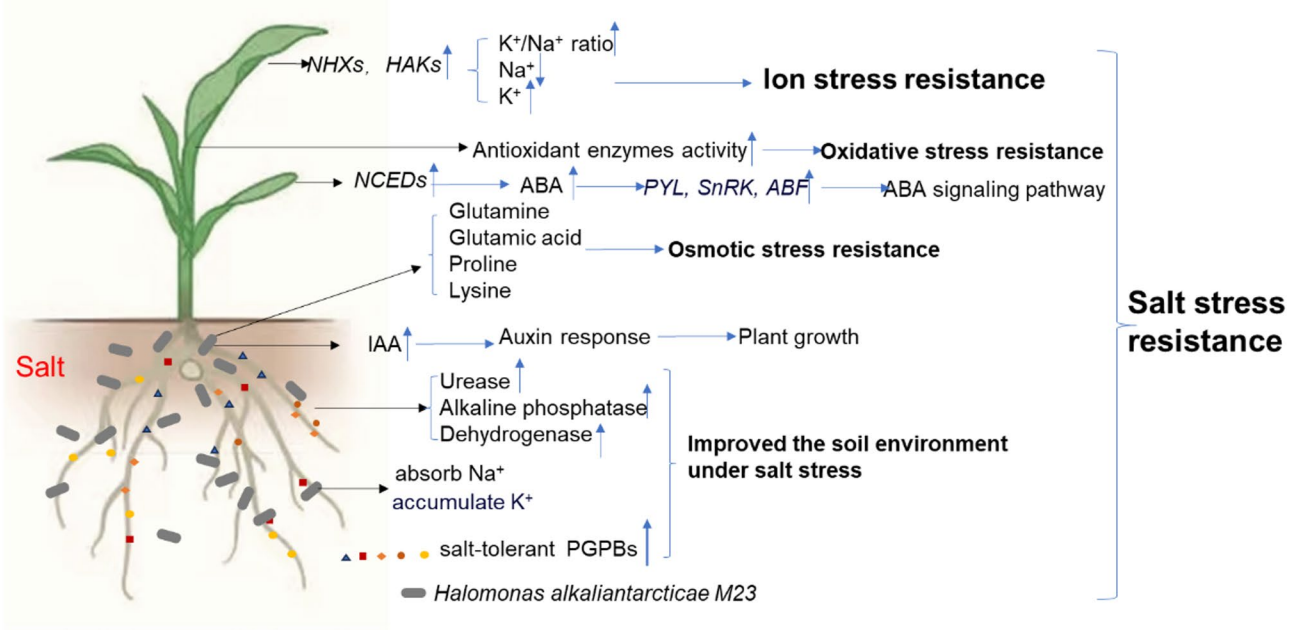

Fig. 7 Proposed model for the role of M23 on maize under salt stress

halophyte salineponde growing in the soil of saline-alkali soil in Shandong Province of China.

The basic properties of the soil are: pH 7.05; organic matter, 20.7 g/kg; CEC, 18.4 cmol/kg; available P, 80.8 mg/kg; available K, 271 mg/kg; NH_4^+-N, 7.1 mg/kg; NO_3^--N, 60.4 mg/kg.

Isolation and identification of salt-tolerant microorganisms

Soil samples were collected from the rhizosphere soil of *Suaeda salsa* from Dongying, Shandong Province (37.46 N, 118.49 E, North China) and stored at 4°C. The 2.0 g rhizosphere soil sample was weighed and placed in a conical bottle filled with 100mL double steam water, sealed with a sealing film, and shaken in a 200 rpm shaking table at 30℃ for 24 h to obtain soil suspension. The soil suspension was diluted with 1×10^6 and coated on a plate containing a Luria Bertani (LB) medium with 10% NaCl, and the single colony with good growth was selected to obtain salt-tolerant strains. Different strains were selected from each plate and purified by a streaking method on a LB medium with 10% NaCl. After purification, the strains were stored at 4°C using glycerol preservation. For molecular identification, the 16S rRNA gene was amplified by the universal primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The final volume of the amplification reaction solution was 25 μL containing 10×buffer (2.5 μL), dNTP (2 μL), of primers (each 0.5 μL), Taq polymerase (0.15 μL), ddH_2O (18.35 μL) and a template solution (1 μL). The conditions of the PCR were as follows: the first step of 95 °C for 5 min followed by 40 cycles of 95 °C for 10 s, 58 °C for 40 s, and 72 °C for 90 s, and the final extension at 72 °C for 10 min. The obtained sequences of the 16 S rRNA gene were compared with sequences of the NCBI database (https://www.ncbi.nlm.nih.gov/) using BLAST. And a phylogenetic tree was constructed by multiple alignments using the MEGA (Molecular Evolutionary Genetics Analysis) software (version 7.0) and the neighbor-joining method.

Determination of auxin production capacity of salt-tolerant bacteria

IAA production of the isolates was quantitatively determined based on the method of Gordon and Weber [37]. The isolate was cultured in 10 mL nutrient broth supplemented with different concentrations of 0, 1, 2, 3, 4, and 5% NaCl and 100 mg/l tryptophan, and then incubated in a constant temperature (30℃) shaker. After 48 h, 4 mL of bacteria supernatants was mixed with 4 mL Salkowski reagent, and kept in the dark for 30 min. The optical density was then measured at the absorbance of 530 nm, and the IAA production of strain M23 was calculated by IAA standard curve.

Determination of K^+ and Na^+ in M23 supernatant

The activated bacteria were inoculated into LB medium containing 0, 1.5, and 3% NaCl, and cultured at 28℃ for 48 h, and centrifuged at 4℃ for 12,000 g for 15 min to collect the supernatant. The content of Na^+ and K^+ ions in supernatant was determined by flame photometer.

Determination of free amino acids in M23 supernatant

The activated bacteria were inoculated in LB medium with 0, 3, 5 and 10% NaCl concentration for 48 h, and 50

mL of cell fluid of the bacteria treated with different NaCl was collected by centrifugation. 50 mL of cell bacteria solution was pre-cooled in ice bath, and then centrifuged at 6000 rpm at 4℃ for 15 min to collect bacteria. The precipitated bacteria were washed twice with isotonic NaCl solution (with sterile NaCl solution of 0, 3, 5, 10%), centrifuged and collected again, then dissolved with 5 mL pre-cooled 0.25 mol /L perchloric acid, and then left for 10 min after mixing evenly. Then the supernatant was refrigerated and centrifuged for 15 min to determine the content of free amino acids using LC-MS.

The promotion test on plant salt tolerance

The 3-leaves seedlings were watered using the solution with M23 and NaCl. And the control group was only watered with NaCl solution.

Maize inbred line KN5585 (KN5585, a maize inbred line cultivated by the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences (CAAS), boasts outstanding agronomic traits and robust combining ability, rendering it ideal as parental material for hybrid breeding programs.) was seeded into the pots(diameter:10 cm, height:10 cm). M23 was grown in a sterilized liquid LB medium, until OD600 reached 0.8. Afterwards, M23 fluid was collected and resuspended in sterile deionized water. The absorbance was adjusted to an optical density of 0.8 at 600 nm. When the maize reached the stage of three leaves, furrows (1–2 cm deep) were made around the roots of the maize, and bacterial suspension (100 mL per pot) was added to the furrows, while non-bacterial inoculation was used as the control. After inoculation for 7 days, some of the pots were grown under normal conditions, and some pots were used for salt stress treatment. The treatment group was irrigated with water solution containing 250 mM NaCl + M23 suspension (OD600 = 0.8), and the control group was irrigated with water solution containing 250 mM NaCl every 2 d for 3 times, the experiment lasted for 20 days and the maize plants were grown in the greenhouse (temperature 25 ± 3° C, relative humidity 70%, 16 h light, 8 h dark). The biomass of the maize was measured after 20 days (the final soil salinity was 8.0 dS/m).

Determination of ABA, enzyme activity in maize tissue and rhizosphere soil

The ABA content was measured following the reported method [38]. The total peroxidase (POD) activity was determined spectrophotometrically by measuring the oxidation of guaiacol at 470 nm, using the kit (ADS-W-KY003, Jiangsu Jingmei Biological Technology Co.,Ltd). The activity of catalase (CAT) was assayed by measuring the disappearance of H_2O_2 [39]. The activity of glutathione S-transferase (GST) and ascorbic peroxidase (APX) was detected using the kits (ADS-W-G005 and ADS-W-VC005, Jiangsu Jingmei Biological Technology Co.,Ltd). The activities of the alkaline phosphatase, dehydrogenase, urease and catalase in rhizosphere soils were detected according to the reported methods [40, 41]. Three biological replicates were performed.

The measurement of K^+ /Na^+ ratio of maize leaves

After 20 days of culture, maize leaves and roots were washed and dried, and the contents of nutrients and ions were determined. Maize leaves were ground to a fine powder, 0.2 g FW of the sample was weighed and added to a digestive tube of 1 mL distilled water. 5 mL H_2SO_4 was added to the mixture and 2 mL hydrogen peroxide was added twice. After intense reaction, the mixture was dissolved in a digestion furnace, and the heating was stopped when the solution turns brown. After cooling slightly, added 10 drops of H_2O_2 and continue heating until the solution was colorless or clear. Continue heating for 5 min and removing the excess H_2O_2. K^+ and Na^+ were determined by flame photometry (FP640, China) according to the protocol described by Wolf [42]. The K^+ /Na^+ ratio was calculated based on K^+ and Na^+ concentrations.

Bacterial community analysis of maize rhizosphere soil

The soils tightly bound to the roots (served as rhizosphere soils) were collected and analyzed for the composition of microbial community. Total bacterial genomic DNA samples were extracted using OMEGA Soil DNA Kit (D5625-01, USA) This experiment was performed in triplicate. Amplification and high-throughput sequencing of 16s rDNA of soil bacteria in maize rhizosphere were performed as described by Wang et al. [43]. PCR amplification of the bacterial 16S rRNA genes V3–V4 region was conducted using the forward primer (5'-ACTCCTACGGGAGGCAGCA-3') and the reverse primer (5'-GGACTACHVGGGTWTCTAAT-3'). High-throughput sequencing was performed using Illlumina MiSeq platform with MiSeq Reagent Kit v3 at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China). The presence of differences at different taxa, including phylum, class, order, family and genus, between groups were analyzed using Metastats. Nonmetric multidimensional scaling (NMDS) was performed on distance matrices. The 2D graphical outputs were then drawn using the coordinates.

Statistical analysis

All data have at least three biological replicates. The data were presented as the mean ± standard deviation (SD). The statistical analysis was performed using T-test and Duncan's tests of one-way ANOVAs in SPSS (version 22.0.0.0). Significant differences were indicated by asterisks, $^*p < 0.05$; $^{**}p < 0.01$.

Abbreviations

M23	Halomonas alkaliantarcticae M23
Glu	Glutamic acid
ROS	Reactive oxygen species
PGPB	Plant growth promoting bacteria
VOC	Volatile organic compounds
EPS	Extracellular polysaccharide
CAT	Catalase
POD	Peroxidase
APX	Ascorbic peroxidase
GST	Glutathione S-transferase
IAA	Indole acetic acid

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions

JL and HZM wrote the main manuscript text and performed the experiments and analyzed the data. HZM and CBW designed the research project and wrote this manuscript. HZM and JL prepared Figs. 1, 2, 3, 4, 5, 6 and 7. XHZ, YQN, MW and BH assisted in the determination of some physiological indexes and bacterial community analysis. All authors reviewed the manuscript.

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Data availability

All datasets generated for this study are included in the article/Supplementary Materials.The data of 16s rDNA from maize rhizosphere soil bacterial were deposited in the figshare database: https://figshare.com/articles/dataset/16s_rDNA_from_maize_rhizosphere_soil_bacterial/20549364.

Declarations

Ethics approval and consent to participate

There is no ethics approval and consent to participate in this manuscript.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Yang Y, Guo Y. Elucidating the molecular mechanisms mediating plant salt-stress responses. New Phytol. 2018;217(2):523–39.
2. Fita A, Rodríguez-Burruezo A, Boscaiu M, Prohens J, Vicente O. Breeding and domesticating crops adapted to drought and salinity: A new paradigm for increasing food production. Front Plant Sci. 2015;6:978.
3. Kumawat KC, Sharma B, Nagpal S, Kumar A, Tiwari S, Nair RM. Plant growth-promoting rhizobacteria: salt stress alleviators to improve crop productivity for sustainable agriculture development. Front Plant Sci. 2022;13:1101862.
4. Prittesh P, Avnika P, Kinjal P, Jinal HN, Sakthivel K, Amaresan N. Amelioration effect of salt-tolerant plant growth-promoting bacteria on growth and physiological properties of rice (Oryza sativa) under salt-stressed conditions. Arch Microbiol. 2020;202(9):2419–28.
5. Park HJ, Kim WY, Yun DJ. A new insight of salt stress signaling in plant. Mol Cells. 2016;39(6):447–59.
6. Li Z, Zhu L, Zhao F, Li J, Zhang X, Kong X, Wu H, Zhang Z. Plant salinity stress response and Nano-Enabled plant salt tolerance. Front Plant Sci. 2022;13:843994.
7. Yang Y, Guo Y. Unraveling salt stress signaling in plants. J Integr Plant Biol. 2018;60(9):796–804.
8. Kamran M, Parveen A, Ahmar S, Malik Z, Hussain S, Chattha MS, Saleem MH, Adil M, Heidari P, Chen JT. An overview of hazardous impacts of soil salinity in crops, tolerance mechanisms, and amelioration through selenium supplementation. Int J Mol Sci 2019, 21(1).
9. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochemistry: PPB. 2010;48(12):909–30.
10. Apse MP, Aharon GS, Snedden WA, Blumwald E. Salt tolerance conferred by overexpression of a vacuolar Na+/H + antiport in Arabidopsis. Sci (New York NY). 1999;285(5431):1256–8.
11. Shi H, Lee BH, Wu SJ, Zhu JK. Overexpression of a plasma membrane Na+/H + antiporter gene improves salt tolerance in Arabidopsis thaliana. Nat Biotechnol. 2003;21(1):81–5.
12. Møller IS, Gilliham M, Jha D, Mayo GM, Roy SJ, Coates JC, Haseloff J, Tester M. Shoot Na + exclusion and increased salinity tolerance engineered by cell type-specific alteration of Na + transport in Arabidopsis. Plant Cell. 2009;21(7):2163–78.
13. Etesami H, Maheshwari DK. Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: action mechanisms and future prospects. Ecotoxicol Environ Saf. 2018;156:225–46.
14. Hou Y, Zeng W, Ao C, Luo Y, Wang Z, Hou M, Huang J. Bacillus atrophaeus WZYH01 and Planococcus soli WZYH02 improve salt tolerance of maize (Zea mays L.) in saline soil. Front Plant Sci. 2022;13:891372.
15. Kumar A, Patel JS, Meena VS, Srivastava R. Recent advances of PGPR based approaches for stress tolerance in plants for sustainable agriculture. Biocatal Agric Biotechnol. 2019;20:101271.
16. Tirry N, Kouchou A, Laghmari G, Lemjereb M, Hnadi H, Amrani K, Bahafid W. El Ghachtouli N: improved salinity tolerance of Medicago sativa and soil enzyme activities by PGPR. Biocatal Agric Biotechnol. 2021;31:101914.
17. Kumar A, Singh S, Gaurav AK, Srivastava S, Verma JP. Plant Growth-Promoting bacteria: biological tools for the mitigation of salinity stress in plants. Front Microbiol. 2020;11:1216.
18. Ali S, Charles TC, Glick BR. Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant Physiol Biochemistry: PPB. 2014;80:160–7.
19. Ha-Tran DM, Nguyen TTM, Hung SH, Huang E, Huang CC. Roles of plant Growth-Promoting rhizobacteria (PGPR) in stimulating salinity stress defense in plants: A review. Int J Mol Sci 2021;22(6).
20. Lucke M, Correa MG, Levy A. The role of secretion systems, effectors, and secondary metabolites of beneficial rhizobacteria in interactions with plants and microbes. Front Plant Sci. 2020;11:589416.
21. Hanin M, Ebel C, Ngom M, Laplaze L, Masmoudi K. New insights on plant salt tolerance mechanisms and their potential use for breeding. Front Plant Sci. 2016;7:1787.
22. Shi L, Zhu X, Qian T, Du J, Du Y, Ye J. Mechanism of salt tolerance and plant growth promotion in Priestia megaterium ZS-3 revealed by cellular metabolism and Whole-Genome studies. Int J Mol Sci 2023, 24(21).
23. Ali S, Akhtar MS, Siraj M, Zaman W. Molecular communication of microbial plant biostimulants in the rhizosphere under abiotic stress conditions. Int J Mol Sci 2024, 25(22).

24. Qiu XM, Sun YY, Ye XY, Li ZG. Signaling role of glutamate in plants. Front Plant Sci. 2019;10:1743.
25. Mashiguchi K, Tanaka K, Sakai T, Sugawara S, Kawaide H, Natsume M, Hanada A, Yaeno T, Shirasu K, Yao H, et al. The main auxin biosynthesis pathway in Arabidopsis. Proc Natl Acad Sci USA. 2011;108(45):18512–7.
26. Egamberdieva D, Wirth S, Bellingrath-Kimura SD, Mishra J, Arora NK. Salt-Tolerant plant growth promoting rhizobacteria for enhancing crop productivity of saline soils. Front Microbiol. 2019;10:2791.
27. Hoffman MT, Gunatilaka MK, Wijeratne K, Gunatilaka L, Arnold AE. Endohyphal bacterium enhances production of indole-3-acetic acid by a foliar fungal endophyte. PLoS ONE. 2013;8(9):e73132.
28. Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ. 2010;33(4):453–67.
29. Jiang S, Lan Z, Zhang Y, Kang X, Zhao L, Wu X, Gao H. Mechanisms by Which Exogenous Substances Enhance Plant Salt Tolerance through the Modulation of Ion Membrane Transport and Reactive Oxygen Species Metabolism. *Antioxidants (Basel, Switzerland)* 2024;13(9).
30. Ma H, Li P, Liu X, Li C, Zhang S, Wang X, Tao X. Poly-γ-glutamic acid enhanced the drought resistance of maize by improving photosynthesis and affecting the rhizosphere microbial community. BMC Plant Biol. 2022;22(1):11.
31. Duan D, Jiang F, Lin W, Tian Z, Wu N, Feng X, Chen T, Nan Z. Effects of drought on the growth of Lespedeza davurica through the alteration of soil microbial communities and nutrient availability. J fungi (Basel Switzerland) 2022, 8(4).
32. Cao C, Tao S, Cui Z, Zhang Y. Response of soil properties and microbial communities to increasing salinization in the meadow grassland of Northeast China. Microb Ecol. 2021;82(3):722–35.
33. Gu J, Guo F, Lin L, Zhang J, Sun W, Muhammad R, Liang H, Duan D, Deng X, Lin Z, et al. Microbiological mechanism for production while remediating in Cd-contaminated paddy fields: A field experiment. Sci Total Environ. 2023;885:163896.
34. Liu C, Xia R, Tang M, Liu X, Bian R, Yang L, Zheng J, Cheng K, Zhang X, Drosos M, et al. More microbial manipulation and plant defense than soil fertility for Biochar in food production: A field experiment of replanted ginseng with different Biochars. Front Microbiol. 2022;13:1065313.
35. Cao Y, Du P, Li Z, Xu J, Ma C, Liang B. Melatonin promotes the recovery of Apple plants after waterlogging by shaping the structure and function of the rhizosphere Microbiome. Plant Cell Environ. 2024;47(7):2614–30.
36. Matsumoto A, Kawaguchi Y, Nakashima T, Iwatsuki M, Ōmura S, Takahashi Y. Rhizocola Hellebori gen. Nov., Sp. Nov., an actinomycete of the family Micromonosporaceae containing 3,4-dihydroxydiaminopimelic acid in the cell-wall peptidoglycan. Int J Syst Evol MicroBiol. 2014;64(Pt 8):2706–11.
37. Gordon SA, Weber RP. Colorimetric Estimation of indoleacetic acid. Plant Physiol. 1951;26(1):192–5.
38. Ma H, Liu C, Li Z, Ran Q, Xie G, Wang B, Fang S, Chu J, Zhang J. ZmbZIP4 contributes to stress resistance in maize by regulating ABA synthesis and root development. Plant Physiol. 2018;178(2):753–70.
39. Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121–6.
40. Tabatabai MA, Bremner JM. Assay of urease activity in soils. Soil Biol Biochem. 1972;4(4):479–87.
41. Dick RPJSSSA. Methods of Soil Enzymology. 2011.
42. Wolf B. A comprehensive system of leaf analyses and its use for diagnosing crop nutrient status. Commun Soil Sci Plant Anal. 1982;13(12):1035–59.
43. Wang X, Dong G, Liu X, Zhang S, Li C, Lu X, Xia T. Poly-γ-glutamic acid-producing bacteria reduced cd uptake and effected the rhizosphere microbial communities of lettuce. J Hazard Mater. 2020;398:123146.

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