Marinobacter Sp. Zp-2095 Enhances *Tomato* and *Maize* to Salt Tolerance for Potential Application in Saline Soil Reclamation

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Abstract. Strain zp-2095, a moderately halophilic bacteria, was isolated from the rhizosphere soil of *Suaeda glauca* plant, in the Aiding lake, Xinjiang, China. Molecular identification result indicated strain zp-2095 is a member of *Marinobacter*. It could dissolve organophosphorus and produce amylase and cellulase. Strain zp-2095 has the function of fixing nitrogen which is helpful for plant through maintaining nitrogen in the soil. Siderophore, which can enhance the plant's absorption of iron, was produced by strain zp-2095. The results of petri dish growth promotion experiments indicated strain zp-2095 markedly increased the lateral root number of tomato under varying saline-alkali stress ($p<0.0001$), and the fresh weight of treated plants significantly surpassed that of the control group ($p<0.001$ or $p<0.0001$). In a pot experiment, at 0‰ NaCl maize treated with strain zp-2095 showed increases of 16.8% in above-ground and 37.0% in below-ground fresh weight. At 6‰ NaCl, the weights of above-ground and below-ground increased by 29.8% and 30.5%, respectively, showing significant growth improvement ($p<0.0001$). Overall, this strain plays an important role in promoting plant growth in saline soils.

1 Introduction

Soil salinization represents a significant global challenge that impedes agricultural productivity and undermines sustainability. The accumulation of a large amounts of water-soluble salt ions, such as Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+} and SO_4^{2-} et. al, leads soil salinization [1], which features poor soil organic matter content [2] and a limited water potential [1]. That can lead to leaf senescence, poor root development, decreased leaf chlorophyll content (SPAD) and photosynthesis, as well as insufficient nutrient absorption [3], ultimately effecting the growth of plants.

Faced with these stressors, plants have developed diverse regulatory strategies to enhance resistance, encompassing hormone synthesis, salt-responsive signaling pathways, ion homeostasis and reactive oxygen species (ROS) [4]. Plant growth-promoting rhizobacteria (PGPR), which can colonize in the plant rhizosphere, can improve the plant rhizosphere environment inhibit plant pathogens and finally promote plant growth [5]. Previous studies have shown that PGPR can improve the growth of plants through multiple pathways, including producing ACC deaminaseethylene accumulation in plants [4], and regulating the antioxidant enzymatic activities to enhance the salt tolerance of Arabidopsis Thaliana [6]. PGPR has been reported to improve the growth of others plants such as pepper [7], canola [8], cereals and legumes [9]. Therefore, the plant microbiome is considered the second genome of plants [10].

Xinjiang has a unique climate environment, which results in breeding rich microbial resources. In this study, a *Marinobacter* strain was isolated from the rhizospheric soil of *Suaeda glauca.* Based on present reports there is little research on the role of members of the genus *Marinobacter* in promoting plant growth. This article discovered a beneficial bacteria belonging to genus *Marinobacter,* named zp-2095. It possesses multiple excellent properties and promotes the growth of tomato and maize under the salt stress. It is considered as a bacteria source in the saline-alkali land management and plant growth promotion.

2 Result

2.1 Phenotypic, physiological and biochemical characteristics of strain zp-2095

Strain zp-2095 was isolated from the rhizosphere soil of *Suaeda glauca* plant, in the Aiding lake, Xinjinag, China. The modified Luria-Bertani (mLB) medium (with final 3% NaCl, pH=9.0) was used to aerobically incubated this strain. The optical microscope (CX-22, Olympus) and scanning electron microscope (SEM, Philips XL30, Netherlands) were used to observed its gram stain and morphology. NaCl tolerance were determined on the mLB medium with different concentrations of NaCl ranging from 0 to 20% (w/v) at 1% intervals.

The colony of this strain was white, circular, ridgy and smooth surface on mLB solid medium. Its cell was rod-shaped, single, about 5 μm×1 μm in size under the

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SEM (Fig. 1). Oxidase and catalase reactions were positive and the gram staining was negative. Growth was observed in the range of 2%-16% NaCl concentrations (optimum 6%, w/v) in mLB medium. Unique carbon source experiments indicated that strain zp-2095 can not use glucose, sucrose, xylose, fructose, galactose, arabinose. Hydrolysis of aesculin, gelatin and reduction of nitrate to nitrite were negative.

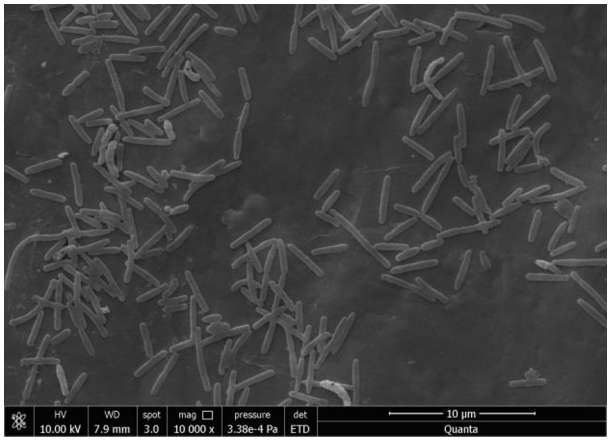

Fig. 1. The morphology of strain zp-2095 under scanning electron microscope.

2.2 Phylogenetic analysis

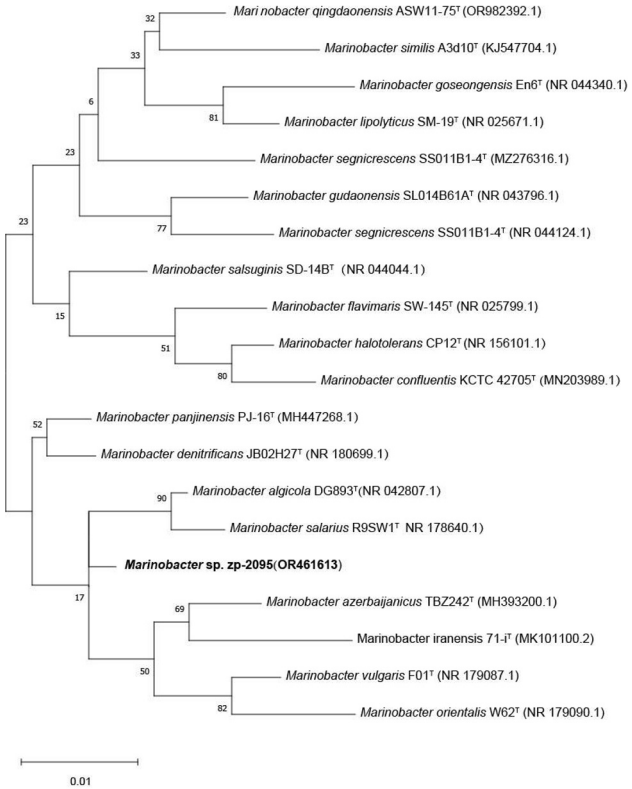

Fig. 2. Phylogenetic tree of strain zp-2095 and other type strains in the genus Marinobacter based on the maximum-likehood method.

The 16S rRNA gene of strain zp-2095 was amplified by universal primers (27F and 1492R) and sequenced in the Sangon Biotech (Shanghai) Co., Ltd. The sequence was registered in National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) (GenBank: OR461613).

The 16S rRNA gene sequence (1348bp) was uploaded the EZbiocloud database for comparison. Strain zp-2095 and *M. lipolytus* SM19^T, *M. salinus* Hb8^T, *M. alexandrii* LZ-8^T had a closer relationship, suggesting it was a member of the genus *Marinobacter*. But the similarities with these type species lower than the suggested threshold (98.90%) for distinguishing between two procaryotic species. The phylogenetic tree based on the 16S rRNA gene sequences was constructed using the maximum-likelihood method (Fig. 2) and neighbor-joining method (Fig. 3), both of which showed strain zp-2095 form an independent branch, indicating that it may be a new strain of the genus *Marinobacter*.

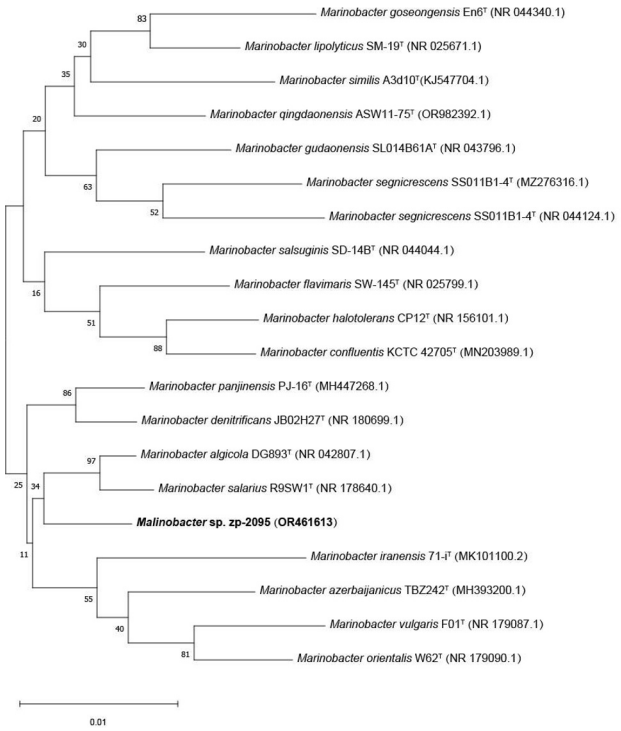

Fig. 3. Phylogenetic tree of strain zp-2095 and other type strains in the genus *Marinobacter* based on the neighbor-joining method.

2.3 Characterization of growth promoting function of strains

Strain zp-2095 showed positive siderophore production by the emerging of an orange hallow zone on CAS medium. It could grow on Ashby's Nitrogen-Free medium, which suggested that strain zp-2095 has nitrogen-fixing function. Strain zp-2095 could produce the hallow zone on Pikovskayas agar, which showed it could solubilize inorganic phosphorus. No hallow zone was produced on Mongina agar, so it could not solubilize inorganic phosphorus. The Salkowaski reagent did not change color after adding the fermentation liquor of strain zp-2095, showing no IAA was produced. Strain zp-2095 could form dissolving zone on the solid medium of starch and cellulose, indicating the secretion of

amylase and cellulase. The results were shown in Fig. 4 and Table 1.

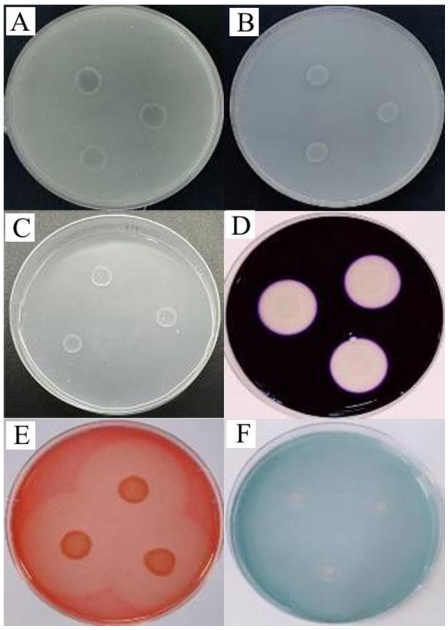

Fig. 4. The growth promoting characteristics of strain zp-2095; A: Organophosphorus decomposability diagram; B: Diagram of ability to dissolve inorganic phosphorus; C: Nitrogen fixing capacity; D: Ability to produce amylase; E: Cellulase production capacity; F:Iron carrier capability rendering.

Table 1. Summary of growth promoting characteristics of strain zp-2095.

Growth promoting characteristics of strain zp-2095	Result
Organophosphorus hydrolysis	positive
Dissolved inorganic phosphorus	negative
Nitrogen fixation	positive
Amylase	positive
Cellulase	positive
IAA	negative
Siderophore	positive

2.4 Effect of zp-2095 inoculation on tomato growth under saline-alkali stress

Tomato seedlings growing evenly were transfered to modified 1/2 MS medium with differential saline-alkali stress. Strain zp-2095 (OD_{600nm}=0.6-0.8) was in inoculated 1cm below the petri dish as shown in Fig. 5.

Growth of tomato treated with strain zp-2095 was evidently superior to control group (Fig. 4). The fresh weight of tomato seedings, root length and lateral root number were counted (Fig. 5). Strain zp-2095 significantly enhanced ($p<0.0001$) the lateral root number of tomato under the differential saline-alkali stress. The fresh weight treated with strain zp-2095 significantly ($p<0.001$ or $p<0.0001$) outbalanced the control group (Fig. 6).

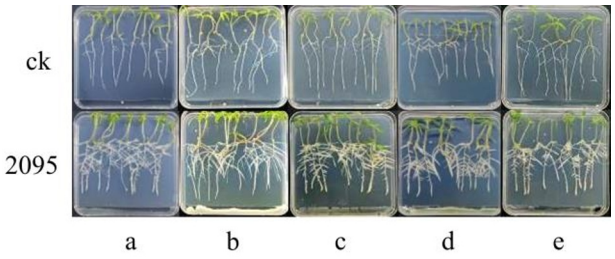

Fig. 5. Effect of strain zp-2095 on tomato growth promotion (a: pH 5.8; b: pH 8; c: pH 8+2 mM$NaHCO_3$; d: pH 8+3‰ NaCl; e: pH 8+2 mM $NaHCO_3$+3‰ NaCl).

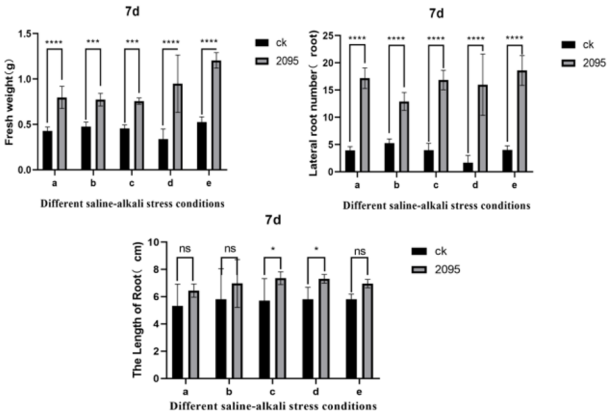

Fig. 6. Statistical diagram of growth promotion effect of strain zp-2095 on tomato; a: pH 5.8; b: pH 8; c: pH 8+2 mM$NaHCO_3$; d: pH 8+3‰ NaCl; e: pH 8+2 mM $NaHCO_3$+3‰ NaCl.

2.5 Effect of zp-2095 inoculation on maize growth under salt stress

The vernalized corn seeds were seeded in soil with an equal ratio of vermiculite and nutrient soil, and the soil salt conditions were 0‰ and 6‰ (NaCl) (w/v). The seeds of the experimental group were soaked with bacterial suspension with OD_{600nm} value of 0.6-0.8 for 5 minutes, and the control group was soaked with aquae sterilisata. After 14 days of culture, the root soil of corn was cleaned and the growth index of corn was measured.

The sesults of the pot experiment showed that strain zp-2095 had a beneficial effect on maize growth (Fig. 7). Fresh weigh of above and below ground increased by 16.8% and 37.0% in maize treated with strain zp-2095 at a salt stress of 0‰ (NaCl,w/v). At a salt stress of 6‰ (NaCl, w/v), the fresh weight of above and below ground of the inoculating group increased by 29.8% and 30.5%, respectively, which showed strain zp-2095 significantly improved ($p<0.0001$) the growth of maize (Fig. 8).

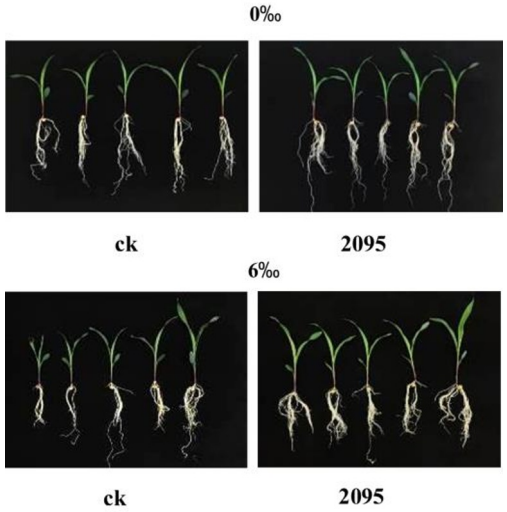

Fig. 7. Growth promotion effect of strain zp-2095 on maize**.**

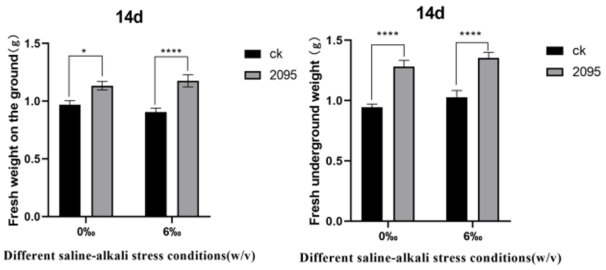

Fig. 8. Statistical diagram of growth promotion effect of strain zp-2095 on maize.

3 Conclusion

Strain zp-2095, a moderately halophilic bacteria, was isolated from the rhizosphere soil of *Suaeda glauca* plant, in the Aiding lake, Xinjinag, China. Molecular identification result indicated strain zp-2095 is a member of *Marinobacter*, and it is a potential novel species based on the alignment of 16S rRNA sequence. It possesses excellent and multiple growth promoting characteristics, which lay a foundation for promoting plant growth.

The strain could significantly promote the growth of tomato under different salt and alkali conditions. At the same time, strain zp-2095 enhanced maize growth significantly, increasing above-ground fresh weight by 16.8% at 0‰ NaCl and by 29.8% at 6‰ NaCl. Below-ground fresh weight increased by 37.0% at 0‰ NaCl and by 30.5% at 6‰ NaCl. This strain will be an important resource for promoting plant growth. It is meaningful to explore the growth promoting mechanism of strain zp-2095 in the future.

Acknowledgments

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