Characteristics of *Azotobacter* sp. strain AC11 and their effects on the growth of tomato seedlings under salt stress

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**ABSTRACT**

This study was aimed to investigate the potential of *Azotobacter* sp. strain AC11 in promoting growth and enhancing resistance to salinity stress in tomato seedlings. In this study, we measured the ability of strain AC11 to fix nitrogen and solubilize phosphorus and potassium, as well as its production of indole-3-acetic acid (IAA) and siderophores. A greenhouse pot experiment was conducted to investigate whether strain AC11 promoted tomato seedlings’ growth and enhanced their salt resistance. The results showed that strain AC11 produced IAA and siderophores, fixed nitrogen, and solubilized potassium and phosphorus. In pot trials, strain AC11 increased the shoot height, root length, and dry and fresh weights of tomato seedlings, and also increased their chlorophyll, soluble protein, and soluble sugar content. Furthermore, the bacteria induced the activities of superoxide dismutase (SOD; EC: 1.15.1.1), peroxidase (POD; EC: 1.11.1.7), and catalase (CAT; EC: 1.11.1.6), while it reduced the malondialdehyde (MDA) content and rate of O$_2^-$ generation in tomato seedlings under salt stress. In summary, *Azotobacter* sp. strain AC11 promoted the growth of tomato seedlings and induced resistance to salt stress by producing IAA and siderophores, promoting the activities of antioxidant enzymes, and increasing the content of osmotic adjustment substances as well as enhancing the availability of the macronutrients N, P, K, and Fe$^{3+}$ in the soil.

**Keywords:** *Azotobacter* sp. strain AC11; Plant growth promotion; Tomato; Salt stress; Antioxidant enzyme

**INTRODUCTION**

Vegetable cultivation in solar greenhouses makes it possible to produce vegetables during winter without additional heating and lighting in some areas of northern China. Owing to the high profitability of vegetable cultivation in greenhouses, the land area used for solar greenhouse production has rapidly increased over the past several decades (Guo et al., 2012; Li, 2014). Tomato is one of the greenhouse vegetables cultivated in some northern areas of China, which is nutrient-rich and very popular with consumers (Padayachee et al., 2017). Therefore, its cultivation area has continued to expand in recent years. In efforts to increase tomato yield, the over-application of inorganic fertilizers has become very common. However, overuse of fertilizers and unsuitable irrigation in a solar greenhouse lead to the accumulation of salts in the soil, and further result in a series of other problems, including the degradation of soil properties and groundwater pollution (Chen et al., 2004; Shi et al., 2009; Shen et al., 2010; Singh, 2015). High soil salinity is one of the most important forms of soil degradation (Chen et al., 2004; Shi et al., 2009; Shen et al., 2010). Soil salinity caused ion toxicity, disruption of ion equilibrium, and osmotic stress in plants, which finally destroyed the normal structure and function of cell membranes, inhibited chlorophyll production and antioxidant enzyme activities, and finally affected plant growth and crop productivity (Hasanuzzaman et al., 2013; Qi and Zhao, 2013). In order to overcome this problem, microbial fertilizers have been used over the past decade (Mayak et al., 2004; Kim et al., 2014; Palaniyandi et al., 2014; Hahm et al., 2017). Microbial fertilizers are environmentally friendly alternatives to conventional fertilizers to promote the sustainable development of agriculture.

Microbial fertilizers are promising alternatives to chemical fertilizers that could provide an important means of alleviating the dependence of agricultural production on classical fertilizers (Aseri et al., 2008; Adesemoye et al., 2009). Some studies have reported that microbial fertilizers alleviated the
damage caused by salt stress in plants and increased vegetable yield (Mayak et al., 2004; Kim et al., 2014; Palaniyanid et al., 2014; Hahn et al., 2017). Palaniyanid et al. (2014) reported that fertilization with bacteria of *Streptomyces* sp. strain PGPA39 significantly increased plant biomass and chlorophyll content and reduced the proline content in tomato plants under salt stress. In addition, Mayak et al. (2004) showed that *Achromobacter piechaudii* significantly increased the fresh and dry weights of tomato seedlings grown in the presence of up to 172 mM NaCl, and slightly increased the plants’ uptake of phosphorous and potassium, which may contribute in part to the activation of processes involved in the alleviation of salt stress effects on seedlings. Moreover, Kim et al. (2014) showed that *Enterobacter* sp. EJ01 alleviated the deleterious effects of salt stress on tomato plants through producing 1-aminoclopropane-1-carboxylate (ACC) deaminase and IAA and increasing the expression of salt stress-responsive genes. In sum, exogenous microbiota alleviated the damage of salt stress on plant growth by the synthesis of various enzymes and phytohormones (Wu et al., 2012; Mohite, 2013; Kim et al., 2014), the production of siderophore and 5-aminolevulinic acid (Qi and Zhao, 2013; Nunkaew et al., 2014), and nutrient adjustment of nitrogen, phosphorus and potassium levels (Mayak et al., 2004; Leangvutiviroj et al., 2010; Taurian et al., 2013; Zhang and Kong, 2014). From the abovementioned reports, it appears likely that the application of exogenous microbiota could increase plant resistance to salt stress, and ultimately improve vegetable yields. In addition, microbial fertilizers have many advantages due to their environmental compatibility and safety. Applying exogenous microbiota and researching their growth-promoting mechanisms in plants has very important theoretical and practical significance to the sustainable development of agriculture. Accordingly, in this study *Azotobacter* sp. strain AC11 was applied to tomato seedlings, and was found to mitigate the adverse effects of salt stress on them. The growth-promoting and detoxification mechanisms of *Azotobacter* sp. strain AC11 on salt-stressed tomato seedlings were also analyzed by measuring the metabolic products of *Azotobacter* sp. strain AC11 and the growth and physiological characteristics of the tomato seedlings under salt stress. The present study provides further understanding of the mechanisms of salt stress alleviation on plants by bacteria of the strain AC11, and provides the basis for the application of *Azotobacter* sp. strain AC11 as a microbial fertilizer.

**MATERIALS AND METHODS**

*Azotobacter* sp. strain AC11 and culture conditions

*Azotobacter* sp. strain AC11 was isolated from tomato roots using a nitrogen-free liquid medium (containing 5.0 g mannitol, 0.5 g KH₂PO₄, 0.2 g MgSO₄·7H₂O, 0.5 g NaCl, 5 g CaCO₃, and 1.0 L distilled water, with a pH of 7.0), to which 3% NaCl was added, and then strain AC11 was deposited in the Microbiology Laboratory of Zhourkou Normal University, China. Cells of strain AC11 were mass propagated in the same nitrogen-free liquid medium and cultured for 2 days at 30°C on a rotary shaker at 180 rpm. The concentration of *Azotobacter* sp. strain AC11 in the medium was measured using a UV-Vis spectrophotometer (HP-8453E, Hewlett-Packard Company, USA) at 660 nm, and the medium was diluted to a concentration of 10⁸ CFU mL⁻¹ using sterile water.

**Characterization of Plant growth-promoting *Azotobacter* sp. strain AC11**

To analyze plant growth properties of *Azotobacter* sp. strain AC11, we measured the metabolic characteristics of strain AC11, such as its production of IAA and siderophores, and its ability to fix nitrogen and solubilize potassium and phosphate. IAA production was measured using the method of Glickmann and Dessaux (1955). Siderophore production was estimated using the method of Shin et al. (2001). The nitrogen-fixing, phosphate- and potassium-solubilizing ability of strain AC11 was measured according to the method of Ge and Zhang (2018).

**Tomato seedling growth and treatment with *Azotobacter* sp. strain AC11 and salt**

Tomato seeds were disinfected in 70% ethanol for 1 min and in 1% sodium hypochlorite solution for 10 min, and then washed with distilled water 3-4 times. Surface-sterilized seeds were allowed to germinate in Petri dishes containing wet filter paper at 28°C for 4 days. Afterwards, tomato seedlings was sown in pots (25 cm in diameter × 20 cm in height) containing 850 g of air-dried soil and 25 mL of 10⁸ CFU mL⁻¹ AC11 inoculant. For controls treatment, seeds were sown in pots containing only 850 g of air-dried soil and 25 mL of the nitrogen-free liquid medium. Salinity treatment was started by adding 4 g of NaCl per kg of dry soil. There were four experimental groups [control, AC11-inoculated (AC11), AC11-inoculated and treated with NaCl (AC11 + salt), and treated with NaCl but uninoculated (salt)], with three replicates each. The seedlings were placed under a climate-controlled chamber with a temperature of 28°C, 12 h day⁻¹ light regime, and air humidity of 70%. After 14 days of treatment, the shoot height, root length, fresh weight, and dry weight of each tomato seedling was measured.

**Analysis of physiological and biochemical indices of tomato seedlings treated with *Azotobacter* sp. strain AC11 and salt**

After 14 days of treatment with strain AC11 and salt, antioxidant enzyme activities, O₂ production rate, and malondialdehyde (MDA) content (an indicator of oxidative
Data analyses
The data were averaged across the three replicates for each treatment. We analyzed the differences among the different treatments using Duncan’s Multiple Range Test at $P < 0.05$. All analyses were performed with SPSS 16.0 software.

RESULTS AND DISCUSSION

Plant growth-promoting characteristics of *Azotobacter* sp. strain AC11
Soil salinization increases the concentration of Na\(^+\) in soil, and Na\(^+\) interferes with the plant’s K\(^+\) uptake (Hasanuzzaman et al., 2013). In addition, the availability of micronutrients in saline soils is dependent on their solubility (Hasanuzzaman et al., 2013), so the correction of nutrient imbalances is also one of the growth- and detoxification-promoting mechanisms of bacteria on salt-stressed plants (Mayak et al., 2004; Qi and Zhao, 2013; Ge and Zhang, 2018). Siderophores could chelate ferric iron from the soil, which promotes the uptake and utilization of Fe\(^{3+}\) by the plant (Loper and Buyer, 1991). In addition, bacteria with the ability to perform nitrogen fixation and P and K solubilization can mediated the availability of nitrogen, phosphorus, and potassium to plants in the soil (Taurian et al., 2013; Zhang and Kong, 2014). Furthermore, Mohite (2013) demonstrated IAA plays a major role in promoting plant growth. Therefore, we analyzed the growth-promoting mechanisms of *Azotobacter* sp. strain AC11 on salt-stressed tomato seedlings by measuring its metabolic products consisting of secretion of IAA and siderophores, and its ability to fix nitrogen and solubilize potassium and phosphate. The results of the present study showed that strain AC11 secreted IAA (Fig. 1A).

The highest concentration of IAA in the culture of strain AC11 reached to 21.89 mg mL\(^{-1}\) after 6 days of incubation. Strain AC11 formed orange halos around the paper discs on blue agar, suggesting that strain AC11 could also produce siderophores (Fig. 1B). In addition, strain AC11 could form colonies on the nitrogen-free medium, showing that AC11 had the ability to fix nitrogen (Fig. 1C). Moreover, Strain AC11 had potassium- and phosphorus-solubilizing abilities, as transparent zones formed on both the potassium-dissolving medium and Pikovskaya medium (Fig. 1D, E). These results indicated that strain AC11 had plant growth-promoting potential including the secretion of IAA and siderophores, as well as nutrient adjustment of the macronutrients N, P, K, and Fe\(^{3+}\).

Effects of *Azotobacter* sp. strain AC11 on the growth and total chlorophyll content of tomato seedlings under salt stress
The effects of strain AC11 to the growth of tomato seedlings under salt stress are shown in Table 1. Salt stress inhibited the growth of tomato seedlings. The shoot height, root growth, and fresh and dry weights of salt-treated tomato seedlings decreased significantly by 16.51, 20.45, 23.11, and 33.64%, respectively, in comparison to those of control plants ($P < 0.05$). However, AC11-inoculated seedlings growing under salt stress showed better growth than the uninoculated salt-stressed tomato seedlings. The seedlings treated with AC11 + salt had shoot heights, root growths, and fresh and dry weights that were greater by 16.39, 24.03, 20.62, and 33.80%, respectively, than those of the uninoculated salt-stressed tomato seedlings ($P < 0.05$). These results demonstrated that the strain AC11 significantly promoted the growth of tomato seedlings. Strain AC11 had nitrogen-fixing, siderophore-producing abilities, and potassium- and phosphorus-solubilizing abilities, suggesting that strain AC11 can correct nutrient imbalances in tomato plants by enhancing the availability of the macronutrients N, P, K, and Fe\(^{3+}\) in the soil, and further promoting plant growth. This is supported by the

![Fig 1. The growth-promoting characteristics of strain AC11. Determination of IAA production (A) siderophore production (B), nitrogen-fixing (C) potassium- (D) and phosphorus-solubilizing abilities (E).](image-url)
The reduced total chlorophyll content in plants under salt stress is a typical symptom of oxidative stress, and the total chlorophyll content was thus used as a sensitive indicator of the physiological state of the plant cells (Chutipajit et al., 2011). In this study, salt treatment significantly decreased the chlorophyll content of tomato seedlings by 43.64% compared to that in the control plants \((P < 0.05)\) (Table 1). However, exogenously applied strain AC11 increased the chlorophyll content of the seedlings in comparison to that of the non-inoculated groups treated both with and without salt \((P < 0.05)\). Tomato seedlings treated with AC11 + salt had a 104.84% higher total chlorophyll content compared to that in uninoculated salt-stressed plants \((P < 0.05)\), which was in agreement with the findings of Silini et al. (2016), who found that Azotobacter chroococcum AZ6 reduced the negative effects of salt stress on plant growth parameters, such as root length, plant height, fresh shoot and root weight, and dry shoot and root weight by improving the chlorophyll content in plants. Strain AC11 increased the total chlorophyll content of the seedlings both with and without salt stress, and ultimately enhanced the tomato seedlings’ growth, as confirmed by higher shoot height and root growth as well as more fresh and dry weight of the seedlings after AC11 treatment.

### Effects of Azotobacter sp. strain AC11 on O\(_2\) production, MDA content and antioxidant enzyme activities in tomato seedlings under salt stress

Salt stress significantly increases the production of reactive oxygen species (ROS) (Hasanuzzaman et al., 2013; Oukarroum et al., 2015). In addition, excessive ROS accumulation can cause severe cellular damage through the peroxidation and deestification of membrane lipids, ultimately resulting in the elevation of MDA levels (Ahmad et al., 2010). Superoxide (O\(_2^−\)) is an important ROS in plants. To block the damage inflicted on plants by ROS, plants increase the activities of antioxidant enzymes that scavenge excessive ROS. So, we analyzed whether treatment with strain AC11 induced tomato resistance to salt stress by measuring rates of O\(_2^−\) generation and the MDA content of the seedlings, as well as SOD, CAT, and POD activities. Results showed that salt stress increased the rate of O\(_2^−\) production and the MDA content in tomato seedlings by 48.16% and 30.18%, respectively, compared to that in control plants \((P < 0.05)\) (Fig. 3A, B). However, after treatment with AC11, both the seedlings treated with and without salt stress showed lower O\(_2^−\) production rates and the MDA content. AC11-inoculated seedlings growing under salt stress had 31.22% lower O\(_2^−\) production rates and 13.24% lower the MDA content compared to those of the uninoculated salt-stressed plants \((P < 0.05)\) (Fig. 3A, B). Furthermore, AC11 treatment significantly increased the activities of SOD, CAT and POD in the seedlings treated with salt by 39.32, 65.85, and 62.36%, respectively, in comparison to those in the uninoculated salt-stressed plants \((P < 0.05)\) (Fig. 2). From the above results, it can be concluded that AC11 enhanced the salt stress resistance of the seedlings by increasing the soluble sugar and soluble protein content. The accumulation of the soluble sugar and soluble protein increased plants osmotic adjustment ability, as a result of aiding in the maintenance of water balance in these plants (Hasanuzzaman et al., 2013). Supported to this study, previous studies also demonstrated the beneficial effects of the accumulation of soluble sugars and proteins induced by exogenous microbiota on salt tolerance in cucumber and pepper (Qi and Zhao, 2013; Hahm et al., 2017; Ge and Zhang, 2018).

### Effects of Azotobacter sp. strain AC11 on the soluble sugar and protein content of tomato seedlings under salt stress

Osmotic adjustment substances, such as soluble sugars and proteins, adjust cell osmotic pressure, protect membrane integrity, and modulate some antioxidative enzyme activities (Hoekstra et al., 2001). In addition, the accumulation of osmolytes by exogenous microbiota is a mechanism that allows plants to defend against salt stress (Hasanuzzaman et al., 2013; Qi and Zhao, 2013). After AC11 treatment, the soluble sugar and soluble protein content in the leaves of inoculated tomato seedlings under salt stress were increased by 47.31 and 46.56%, respectively, in comparison to those of uninoculated salt-stressed plants \((P < 0.05)\) (Fig. 2). From the above results, it can be concluded that AC11 enhanced the salt stress resistance of the seedlings by increasing the soluble sugar and soluble protein content. The accumulation of the soluble sugar and soluble protein increased plants osmotic adjustment ability, as a result of aiding in the maintenance of water balance in these plants (Hasanuzzaman et al., 2013). Supported to this study, previous studies also demonstrated the beneficial effects of the accumulation of soluble sugars and proteins induced by exogenous microbiota on salt tolerance in cucumber and pepper (Qi and Zhao, 2013; Hahm et al., 2017; Ge and Zhang, 2018).

### Table 1: Effects of strain AC11 on the growth and total chlorophyll content of tomato seedlings under salt stress

| Treatment  | Shoot height (cm) | Root length (cm) | Plant fresh weight (g) | Plant dry weight (g) | Chlorophyll content (mg g\(^{-1}\) FW) |
|------------|-------------------|------------------|------------------------|---------------------|---------------------------------------|
| AC11       | 22.38±1.46a       | 18.85±1.36a      | 22.86±1.16a            | 3.74±0.23a          | 2.36±0.16a                            |
| AC11+salt  | 19.60±1.38b       | 16.26±1.15c      | 18.78±1.21b            | 2.85±0.16b          | 1.27±0.15b                            |
| Salt       | 16.84±1.05d       | 13.11±1.03e      | 15.57±1.24f            | 2.13±0.32g          | 0.62±0.15h                            |
| Control    | 20.17±1.22b       | 16.48±1.12c      | 20.25±1.57b            | 3.21±0.18h          | 1.10±0.28i                            |

Table values represent means of three replicates ± SD. Numbers in the same column marked with different lowercase letters significantly differed at \(P<0.05\)
levels by promoting the activities of antioxidant enzymes that scavenge excessive ROS. This is supported by the findings of previous studies that exogenous microbiota induced antioxidant enzymes in plant seedlings to mitigate the oxidative damage caused by ROS under salt stress (Qi and Zhao, 2013; Ge and Zhang, 2018; Zhang et al., 2016).

CONCLUSION

In summary, our study revealed that *Azotobacter* sp. strain AC11 promoted growth and enhanced resistance to salinity stress in tomato seedlings. Strain AC11 increased the shoot height, root length, and dry and fresh as well as the total chlorophyll of tomato seedling, which could be correlated with the production of IAA and siderophores, as well as nitrogen-fixing, potassium- and phosphorus-solubilizing abilities. Moreover, strain AC11 enhanced tomato resistance to salt stress by increasing the content of soluble proteins and sugars, inducing the activities of SOD, POD, and CAT, and reducing the MDA content and \( \text{O}_2^- \) generation rates in tomato seedling. These findings may lead to the potential application of strain AC11 as a microbial fertilizer in agriculture.

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Author contributions

Honglian Ge designed and performed the experiments, analyzed the data and wrote the paper; Zhonghua Liu analyzed the data.

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