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Effect of halotolerant rhizobacteria isolated from halophytes on the growth of sugar beet (Beta vulgaris L.) under salt stress

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One sentence summary: Investigation of the halotolerant plant-growth-promoting rhizobacteria from halophytes and the detection of their capacity to affect the salt tolerance of crops.

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ABSTRACT

Utilization of rhizobacteria that have associated with plant roots in harsh environments could be a feasible strategy to deal with limits to agricultural production caused by soil salinity. Halophytes occur naturally in high-salt environments, and their roots may be associated with promising microbial candidates for promoting growth and salt tolerance in crops. This study aimed to isolate efficient halotolerant plant-growth-promoting rhizobacterial strains from halophytes and evaluate their activity and effects on sugar beet (Beta vulgaris L.) growth under salinity stress. A total of 23 isolates were initially screened for their ability to secrete 1-aminocyclopropane-1-carboxylate deaminase (ACD) as well as other plant-growth-promoting characteristics and subsequently identified by sequencing the 16S rRNA gene. Three isolates, identified as Micrococcus yunnanensis, Planococcus rifietoensis and Variovorax paradoxus, enhanced salt stress tolerance remarkably in sugar beet, resulting in greater seed germination and plant biomass, higher photosynthetic capacity and lower stress-induced ethylene production at different NaCl concentrations (50–125 mM). These results demonstrate that salinity-adapted, ACD-producing bacteria isolated from halophytes could promote sugar beet growth under saline stress conditions.

Keywords: plant-growth-promoting rhizobacteria; 1-aminocyclopropane-1-carboxylate deaminase; ethylene; photosynthesis; salinity stress

INTRODUCTION

Soil salinity constitutes a major obstacle to agriculture, suppressing plant growth and yield in arid and semi-arid areas (Rozema and Flowers 2008). Taking sugar beet (Beta vulgaris L.) in China as an example, northwestern China is the major region of sugar beet production; however, 70% of the total area comprises saline soil, which often causes severe damage to sugar beet quality and productivity (Fan and Ma 2001; Steppuhn, Van Genuchten and Grieve 2005). Sustainable plant growth has become even more important because of the ever-increasing need for crop biomass (Nabti, Schmid and Hartmann 2015). As a relatively cost-efficient and readily utilisable strategy, application of plant-growth-promoting rhizobacteria (PGPRs) has been highlighted as a feasible way to help plants remain healthy when facing environmental stress (Nabti, Schmid and Hartmann 2015).
The exact mechanisms by which PGPRs improve plant stress resistance still remain largely speculative; however, possible explanations include solubilization of mineral phosphate, fixation of N₂ and production of siderophores and phytohormones (Ryan et al. 2008; Nabti, Schmid and Hartmann 2015). Recently, bacteria possessing 1-aminoacyclopropane-1-carboxylate deaminase (ACD) have been identified as a key factor in alleviating plant stress caused by salinity (Jha, Gontia and Hartmann 2012; Ali, Charles and Glick 2014). Habitat-adapted rhizobacteria have associated with their hosts over evolutionary timescales, and thus these bacteria could have contributed to plant adaptation to hostile environments (Rodriguez et al. 2008; Timmusk and Nevo 2011), suggesting that salt-adapted rhizobacteria have the potential to mitigate harmful effects of salt on plants.

Halophytes are distributed across saline environments worldwide. It has been demonstrated that salinity-adapted endophytic bacteria in halophytes can enhance host plant growth under conditions of salinity stress (Jha, Gontia and Hartmann 2012; Qin et al. 2014; Zhao et al. 2016). However, there have been no studies published describing the rhizobacterial communities associated with halophytes, and it is not known whether the native habitat-adapted rhizobacteria could be potential candidates for promoting crop growth and contributing to host salt tolerance. We speculated that halotolerant PGPRs associated with halophyte roots could improve the growth of inoculated crops under different salinity levels.

To test this hypothesis, the present study focused on the isolation of rhizobacteria from halophytes belonging to the family Chenopodiaceae collected from inland saline marshes in Xinjiang Province, northwestern China, and assessed the plant-growth-promoting activities of efficient ACD-producing strains under salinity stress. The capacity of these strains to improve the salinity tolerance of sugar beet (also belonging to the family Chenopodiaceae) was evaluated by inoculation experiments.

MATERIALS AND METHODS

Isolation of rhizobacteria

Three healthy, wild plant samples for each species of halophyte (seven species in total), collected from inland saline habitats at the edge of the Gurbantünggüt Desert (44°17′N 87°55′E), northwestern China, were used as sources for the isolation of bacteria. Rhizobacteria were isolated according to the procedures described by Timmusk et al. (2014) by using R2A medium and tryptic soy agar (TSA) (Zhao et al. 2015, 2016).

Screening for plant-growth-promoting traits in vitro

The presence of ACD activity was determined by the method described by Penrose and Glick (2003). The ACD activity was determined by estimating the amount of α-ketobutyrate (α-KB). Isolates with ACD activity above 1 μmol α-KB mg⁻¹ h⁻¹ were selected for further study.

Inorganic phosphate solubilization was determined according to the method of Nautiyal (1999). Siderophore production by the isolates was evaluated according to the method of Schwyn and Neillands (1987). An indole-3-acetic acid (IAA) assay was carried out using methods described by Patten and Glick (2002). Assay of the isolates for tolerance to salinity was performed according to the method of Zhao et al. (2016).

16S rRNA gene sequence analysis

PCR and identification of bacterial isolates were performed according to the procedures described by Zhao et al. (2015) using the EzTaxon-e database (Kim et al. 2012). The 16S rRNA gene was amplified with the bacterial universal primers 27f and 1492r (Weisburg et al. 1991). All sequences generated have been deposited in the GenBank database with the accession numbers (see Table 2).

Effects of inoculation on seed germination under salinity stress

Seed germination assays under five NaCl concentrations (50, 75, 100, 125 or 150 mM NaCl) were performed according to the method of Jha, Gontia and Hartmann (2012). Germination was conducted in a growth chamber at 28 ± 0.5 °C, and each treatment (50 seeds) was tested in four replicates. Percentage of germination was determined after 20 days.

Effects of inoculation on plant growth promotion under salinity stress

The three most efficient strains were selected from the seed germination test and further examined as bio-inoculants in a greenhouse. Surface-sterilized seeds were grown for 15 days in a sterilized seedbed. Seedlings with a similar phenotype were then transplanted into pots (9700 cm³) with 10 kg sterilized river sand mixed with 20% perlite. One-month-old plants were irrigated with 5 mL bacterial suspension of 10⁴ cells mL⁻¹. Control plants were treated with water without bacterial culture. Following bacterial infection, plants were allowed to grow for 24 h. Plants were then exposed to four different salt concentrations (50, 75, 100 or 125 mM NaCl). NaCl was dissolved in 1 L Hoagland’s nutrient solution (Song et al. 2006), and 200 mL of this solution was irrigated every day, which equalled the volume that was drained from the pots. Control plants were irrigated with 200 mL nutrient solution. Each treatment contained six plants and had four replicates.

At 12 weeks’ post-inoculation, half of the plants in each treatment were randomly selected and harvested to analyze height and dry weight. The fifteenth leaf of the remaining plants of each treatment was chosen to analyze chlorophyll fluorescence by using a pulse amplitude modulation fluorometer (PAM-2500, Walz, Effeltrich, Germany). Maximum photochemical yield (variable fluorescence (Fᵥ)/maximal fluorescence (Fₘᵥ)) was investigated in plants pre-adapted for 1 h in darkness. These plants were then harvested to analyze chlorophyll content and 1-aminoacyclopropane-1-carboxylate (ACC) and ethylene levels. Total chlorophyll was extracted from the fifteenth leaf in 80% (v/v) acetone and measured according to the method described by Shi, Lou and Li (2010). The ACC concentrations in plant roots were determined following the protocol described by Madhaiyan et al. (2006). Ethylene concentrations were calculated according to the methods of Siddique, Chauhan and Sa (2012).

Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS v21.0 for Windows (IBM SPS, Armonk, NY, USA), and means were compared using a least significant difference (LSD) method at the 5% probability level.
RESULTS

In the present study, 114 bacterial strains with ACD activity were isolated from seven plants. Of these, only 23 isolates exhibited relatively high ACD activity (>1 \( \mu \text{mol } \alpha \text{-KB } \text{mg}^{-1} \text{h}^{-1} \)) based on quantitative assays (Table 1). The highest ACD activity was observed in isolate TGT-R7 (18.52 \( \mu \text{mol } \alpha \text{-KB } \text{mg}^{-1} \text{h}^{-1} \)), followed by strains LH-T4 (14.53 \( \mu \text{mol } \alpha \text{-KB } \text{mg}^{-1} \text{h}^{-1} \)) and JM-R12 (11.08 \( \mu \text{mol } \alpha \text{-KB } \text{mg}^{-1} \text{h}^{-1} \)).

Among these 23 ACD-producing strains, 10 strains could produce IAA, and the levels of IAA ranged from 0.15 to 5.23 \( \mu \text{g} \text{ml}^{-1} \) (Table 1). Eleven strains showed the ability to solubilize inorganic phosphate, and nine strains were able to produce siderophores. Moreover, the 23 strains were able to tolerate at least 0.34 M NaCl (Table 1), and these strains were identified to 18 different bacterial genera (Table 2).

All 23 strains were tested for their effects on seed germination under salinity stress. Only strains TGT-R7, LH-T4 and NG-T6 could significantly promote seed germination under more than three NaCl levels (50–125 mM) (Table 3). At the highest NaCl concentration (150 mM), seedling growth was seriously inhibited. Thus, the roles of these three strains on sugar beet growth were further evaluated under moderate NaCl stress (50–125 mM).

After 12 weeks, it was observed that plant shoot length, root length and dry mass of inoculated plants were significantly increased (\( P < 0.05 \)) compared with those of the control plants at elevated NaCl concentrations (Figs 1 and 2). In the presence of 50 mM salt concentration, bacteria-inoculated plants showed significant increases in all growth parameters. At 75 mM salinity, the percentage significant (\( P < 0.05 \)) increase in shoot length in bacteria-inoculated plants was 15–17%, in taproot length it was 13%, in shoot dry weight it was 22%, and in taproot dry weight it was 22%. At 100 mM salt concentration, bacteria-inoculation significantly (\( P < 0.05 \)) increased shoot length by 16–19%, taproot length by 15%, shoot dry weight by 56–48%, and taproot dry weight by 20% as compared to the control plants. At 125 mM salinity, bacteria-inoculation significantly (\( P < 0.05 \)) increased shoot length by 21–24%, taproot length by 13%, shoot dry weight by 91–98%, and taproot dry weight by 33–42% as compared to the control plants. In addition, although the chlorophyll content and \( F_v/F_m \) were reduced with increasing salinity, when bacteria were present there were significant (\( P < 0.05 \)) increases in chlorophyll content and \( F_v/F_m \) regardless of salt concentration (Fig. 2).

Changes in ACC and ethylene content in sugar beet were shown in response to both NaCl and bacterial inoculation. Generally, salinity induced an increase in ACC concentration and ethylene synthesis in sugar beet tissue (Table 4); however, bacteria-inoculated plants showed clear decreases in both ACC concentration and ethylene synthesis (Table 4). Notably, under 50 mM NaCl stress, all strains significantly (\( P < 0.05 \)) decreased ACC by 11–23% and ethylene by 11–17% compared with the uninoculated control plants.

DISCUSSION

The present study revealed that inoculation with three ACD-possessing PGPR strains (\textit{Variovorax paradoxus} NG-T6, \textit{Micrococcus yunnanensis} TGT-R7 and \textit{Planococcus rifetensis} LH-T4) improved seed germination under salt stress. The plant-growth-promoting effects were consistent with the ACD activity level, with these three most promising strains displaying among the

| Isolate | Origin plant | NaCl-T\(^1\) | IPS\(^2\) | SD\(^3\) | IAA\(^4\) | ACD-A\(^5\) |
|---------|--------------|-------------|--------|--------|--------|--------|
| NG-T6   | \textit{Suaeda physophora} | 0–1.03 | + | + | 3.58 ± 0.71 | 10.24 ± 0.98 |
| NG-T9   | | 0–0.51 | + | ++ | − | 1.74 ± 0.53 |
| NG-T21  | | 0–0.34 | − | − | − | 1.32 ± 0.44 |
| NG-T29  | | 0–0.34 | − | − | − | 1.13 ± 0.31 |
| NG-R36  | | 0–0.85 | − | ++ | 4.46 ± 0.83 | 2.22 ± 0.17 |
| XY-T1   | \textit{Suaeda microphylla} | 0–0.51 | + | + | 0.53 ± 0.09 | 4.07 ± 0.99 |
| XY-T2   | | 0–2.56 | − | − | − | 1.82 ± 0.21 |
| JM-R5   | \textit{Halocnemum strobilaceum} | 0–1.71 | + | − | 3.03 ± 0.72 |
| JM-R12  | | 0–1.20 | ++ | − | 0.15 ± 0.03 | 11.08 ± 1.12 |
| JM-R34  | | 0–0.51 | − | − | − | 2.50 ± 0.46 |
| TGT-R3  | \textit{Nitraria tangutorum} | 0–2.56 | + | − | 1.90 ± 0.92 |
| TGT-R7  | | 0–0.51 | − | + | − | 18.52 ± 1.07 |
| TGT-R9  | | 0–0.51 | − | + | − | 7.57 ± 0.90 |
| TGT-T12 | | 0–1.54 | − | − | − | 3.45 ± 0.68 |
| TGT-T16 | | 0–2.2 | − | − | − | 4.69 ± 0.80 |
| XBL-T2  | \textit{Nitraria sibirica} | 0–0.51 | + | + | 2.65 ± 0.52 | 6.92 ± 0.86 |
| XBL-R8  | | 0–0.51 | − | − | − | 1.30 ± 0.33 |
| SM-T3   | \textit{Halostachys caspica} | 0–0.68 | ++ | − | 2.30 ± 0.18 | 2.81 ± 0.50 |
| SM-T10  | | 0–0.51 | − | − | − | 2.43 ± 0.12 |
| SM-T11  | | 0–1.37 | + | − | 5.23 ± 0.50 | 8.82 ± 1.01 |
| LH-T4   | \textit{Kaldium capsicum} | 0–0.68 | + | − | 1.01 ± 0.15 | 14.53 ± 1.16 |
| LH-T9   | | 0–0.34 | − | − | − | 2.14 ± 0.51 |
| LH-T10  | | 0–1.20 | − | + | 0.39 ± 0.08 | 5.78 ± 1.06 |

\(^{1}\)NaCl-T, NaCl tolerance (M).

\(^{2}\)IPS, inorganic phosphate solubilization: − no solubilization; + production of acid (change in colour from blue to yellow); ++ solubilization (a clear halo around the colony).

\(^{3}\)SD, siderophore secretion: − no production; + weak; ++ moderate or strong.

\(^{4}\)IAA, production of the plant hormone IAA (\(\mu\text{g} \text{ml}^{-1}\)); − negative; −\(\mu\)mean of lower than 0.1 \(\mu\text{g} \text{ml}^{-1}\).

\(^{5}\)ACD-A, ACD activity (\(\mu\text{mol } \alpha \text{-KB } \text{mg}^{-1} \text{h}^{-1}\)).

Mean values are based on three replicate observations ± standard deviation.

\(F_v/F_m\) were reduced with increasing salinity, when bacteria were present there were significant (\(P < 0.05\)) increases in chlorophyll content and \(F_v/F_m\) regardless of salt concentration (Fig. 2).

Changes in ACC and ethylene content in sugar beet were shown in response to both NaCl and bacterial inoculation. Generally, salinity induced an increase in ACC concentration and ethylene synthesis in sugar beet tissue (Table 4); however, bacteria-inoculated plants showed clear decreases in both ACC concentration and ethylene synthesis (Table 4). Notably, under 50 mM NaCl stress, all strains significantly (\(P < 0.05\)) decreased ACC by 11–23% and ethylene by 11–17% compared with the uninoculated control plants.

The present study revealed that inoculation with three ACD-possessing PGPR strains (\textit{Variovorax paradoxus} NG-T6, \textit{Micrococcus yunnanensis} TGT-R7 and \textit{Planococcus rifetensis} LH-T4) improved seed germination under salt stress. The plant-growth-promoting effects were consistent with the ACD activity level, with these three most promising strains displaying among the

Table 1. NaCl tolerance and plant growth-promoting traits of the isolates.
Table 2. Identification of the bacteria by 16S rRNA gene sequences.

| Isolate   | Nearest type strain (GAN)             | BR     | GAN     |
|-----------|---------------------------------------|--------|---------|
| NG-T6     | Variorovar paradoxus IAM 12373 (D88006) | 97.63  | KF844058 |
| NG-T9     | Streptomyces caereolatus GIMN4 (GQ529712) | 99.64  | KF844051 |
| NG-T21    | Ochrobactrum thiophenivorans DSM 7216 (AM490617) | 98.55  | KF844054 |
| NG-T29    | Pseudomonas sp. STM 307 (AY785325) | 99.35  | KF844047 |
| NG-R36    | Microbacterium oxydans DSM 20578 (Y17227) | 99.44  | KF844064 |
| XY-T1     | Frigoribacterium faeni 801 (Y18807) | 98.38  | KR476459 |
| XY-T2     | Bacillus vietnamicus 15-1 (AB099708) | 98.19  | KR476460 |
| JM-R5     | Myceligenans halotolerans XJEM 11063 (EU910872) | 99.07  | KF876906 |
| JM-R12    | Arthrobacter agilis DSM 20550 (X80748) | 97.99  | KF876903 |
| JM-R34    | Microbacterium arthrophila CC-VM-Y (FN870023) | 98.13  | KF876895 |
| TGT-R3    | Nesterenkonia sandararina YIM 70009 (AY588277) | 99.09  | KR476436 |
| TGT-R7    | Micrococcus yunnanensis YIM 65004 (FJ214355) | 99.72  | KR476438 |
| TGT-R9    | Micrococcus yunnanensis YIM 65004 (FJ214355) | 99.50  | KR476439 |
| TGT-T12   | Isopericola halotolerans YIM 70177 (AY789835) | 99.05  | KR476430 |
| TGT-T16   | Halomonas kenyensis AIR-2 (AY962237) | 97.08  | KR476432 |
| XBL-T2    | Frigoribacterium faeni 801 (Y18807) | 98.66  | KR476443 |
| XBL-R8    | Okibacterium frilliatum VKM Ac-2059 (AB042094) | 99.37  | KR476456 |
| SM-T3     | Rzizobium pusense NCIB 8070 (FJ969841) | 99.64  | KF876889 |
| SM-T10    | Bradyrhizobium japonicum USDA 110 (DQ825566) | 99.50  | KF876887 |
| SM-T11    | Seratia rubidae JCM 1240 (AB004751) | 97.29  | KF876890 |
| LH-T4     | Planococcus rifetensiae M8 (AY493659) | 99.24  | KF876875 |
| LH-T9     | Microbacterium saperdae IFO 15038 (AB004719) | 98.43  | KF876874 |
| LH-T10    | Streptomyces pactum NBRC 13453 (AB184398) | 99.23  | KF876876 |

BR: BLAST results (%); GAN: GenBank accession number.

Table 3. Effects of inoculation of ACD-producing strains on seed germination.

| Treatment | 50 mM | 75 mM | 100 mM | 125 mM | 150 mM |
|-----------|-------|-------|--------|--------|--------|
| Control   | 83% (+1.91) | 57% (+1.15) | 33% (+1.91) | 29% (+1.15) | 16% (+1.91) |
| NG-T6     | 87% (+1.15)* | 62% (+1.63)* | 49% (+3.42)* | 32% (+1.91) | 16% (+1.63) |
| NG-T9     | 82% (+1.63) | 56% (+1.63) | 32% (+1.63) | 28% (+1.63) | 15% (+1.15) |
| NG-T21    | 82% (+1.91) | 56% (+1.91) | 33% (+2.58) | 29% (+1.15) | 16% (+1.91) |
| NG-T29    | 86% (+1.63)* | 58% (+1.00) | 34% (+1.91) | 29% (+1.91) | 16% (+1.63) |
| NG-R36    | 83% (+2.52) | 57% (+1.15) | 33% (+3.00) | 28% (+3.00) | 15% (+2.52) |
| XY-T1     | 84% (+1.91) | 58% (+1.91) | 35% (+1.91) | 30% (+1.91) | 16% (+2.52) |
| XY-T2     | 82% (+2.31) | 57% (+1.91) | 34% (+2.52) | 29% (+1.15) | 15% (+1.91) |
| JM-R5     | 83% (+2.58) | 57% (+3.00) | 34% (+3.63) | 29% (+3.42) | 17% (+1.91) |
| JM-R12    | 85% (+1.91) | 59% (+3.42) | 35% (+3.42) | 31% (+2.52) | 17% (+2.58) |
| JM-R34    | 83% (+1.15) | 56% (+1.63) | 35% (+1.15) | 28% (+1.91) | 13% (+2.00) |
| TGT-R3    | 83% (+2.00) | 57% (+1.15) | 33% (+1.00) | 28% (+2.83) | 16% (+1.63) |
| TGT-R7    | 85% (+2.58) | 63% (+1.00)* | 52% (+2.83)* | 37% (+2.58)* | 17% (+1.91) |
| TGT-R9    | 87% (+3.00)* | 58% (+1.63) | 35% (+2.58) | 30% (+1.63) | 16% (+1.00) |
| TGT-T12   | 83% (+1.91) | 57% (+3.00) | 34% (+1.91) | 29% (+1.91) | 14% (+1.63) |
| TGT-T16   | 82% (+1.63) | 58% (+1.91) | 33% (+2.58) | 29% (+2.58) | 15% (+1.15) |
| XBL-T2    | 88% (+2.31)* | 59% (+2.58) | 38% (+3.42)* | 29% (+2.58) | 16% (+2.52) |
| XBL-R8    | 83% (+1.00) | 56% (+3.27) | 33% (+2.52) | 28% (+2.83) | 14% (+1.63) |
| SM-T3     | 83% (+1.15) | 58% (+1.91) | 34% (+1.63) | 27% (+3.42) | 17% (+2.52) |
| SM-T10    | 82% (+1.63) | 57% (+1.91) | 31% (+2.58) | 29% (+1.00) | 13% (+2.58) |
| SM-T11    | 90% (+1.91)* | 60% (+1.63)* | 36% (+2.52) | 30% (+1.91) | 17% (+1.91) |
| LH-T4     | 94% (+2.52)* | 63% (+2.58)* | 54% (+3.00)* | 37% (+1.91)* | 18% (+1.63) |
| LH-T9     | 83% (+1.91) | 57% (+2.00) | 33% (+2.58) | 29% (+1.15) | 15% (+1.15) |
| LH-T10    | 86% (+1.00)* | 58% (+1.91) | 35% (+1.91) | 30% (+3.42) | 16% (+2.83) |

Data (%-value means: germination rates of seeds) are represented as average of four replicates. Bold values with “*” are significantly higher compared with the control (P < 0.05).

highest ACD levels. However, an exceptional case was strain JM-R12, which showed higher ACD activity than NG-T6 but did not promote seed germination under salt stress, suggesting that growth promotion was not only dependent on this property, but also on other features as well (Islam et al. 2013). Moreover, strains TGT-R7 and TGT-R9 belonged to the same species, but only TGT-R7 exhibited the ability to stimulate plant growth, suggesting that the various mechanisms by which PGPRs affect plant growth may show variations at the strain level. All the three strains could tolerate at least 0.68 M NaCl. In earlier works
Table 4. Effect of inoculation with halotolerant bacteria on host ACC and ethylene biosynthesis.

| Treatment | 50 mM NaCl | 75 mM NaCl | 100 mM NaCl | 125 mM NaCl |
|-----------|------------|------------|-------------|-------------|
| ACC \( ^{\circ} \) Ethylene \( ^{\circ} \) | ACC \( ^{\circ} \) Ethylene \( ^{\circ} \) | ACC \( ^{\circ} \) Ethylene \( ^{\circ} \) | ACC \( ^{\circ} \) Ethylene \( ^{\circ} \) |
| Control   | 25.0 ± 0.9c| 259.7 ± 3.9c| 30.8 ± 4.0c | 38.8 ± 0.7c |
| NG-T6     | 19.2 ± 1.2a| 215.3 ± 5.4a| 29.6 ± 7.8b | 36.1 ± 1.3ab|
| TGT-R7    | 22.1 ± 1.3b| 231.1 ± 6.0b| 28.9 ± 5.3ab| 37.7 ± 0.8bc|
| LH-T4     | 21.9 ± 1.7b| 228.5 ± 7.0b| 26.8 ± 1.5a | 35.2 ± 1.3a |

The letters after each number represent the results of statistical analysis. The same letter indicates that no significant difference was observed at \( P = 0.05 \).

\(^{\circ} \text{ACC concentration is in nmol g}^{-1} \text{ fresh weight (FW).}\)

\(^{\circ} \text{Ethylene concentration is in pmol g}^{-1} \text{ FW h}^{-1}.\)

performed by other researchers, many PGPRs that withstand high salt concentration had been isolated from the rhizosphere of salt/alkaline-affected crop fields (Nautiyal et al. 2000; Nadeem et al. 2007). The salinity tolerance of these strains was probably due to their adaptation to saline habitats (Paul and Nair 2008). Interestingly, a few PGPRs showed the highest phosphate solubilization at 2.5% salt concentration followed by a sharp decrease at subsequent salt concentrations (Nautiyal et al. 2000; Banerjee et al. 2010), suggesting that the PGPRs might need a moderate salinity range for keeping high plant-growth-promoting characteristics. More studies to confirm this suggestion by comparison of PGPR characteristics under different salinity levels need to be conducted in the future.

The three isolates with high ACD activity also exhibited multiple plant-growth-promoting characteristics, including siderophore secretion, phosphate solubilization, and IAA production. The abilities of both phosphate solubilization and siderophore secretion are regarded as possible mechanisms by which PGPRs stimulate plant growth (Robin et al. 2008; Islam et al. 2013; Bergottini et al. 2015). However, it should be noted that sugar beet was supplied with nutrient solution in our pot experiment, which means that phosphate solubilization (affecting plant growth by facilitating P absorption) and siderophore activity (affecting plant growth by facilitating iron uptake) are unimportant factors for plant growth in the current study. Thus, solid evidence for proving that the growth-promotion activity of these isolates was solely attributable to the abilities of phosphate solubilization or siderophore secretion is needed by further repetitive inoculation experiments. One of the most promising strains, NG-T6, showed lower ACD activity but secreted a high level of IAA. Since IAA-like compounds produced by bacteria could stimulate root development and hence influence mineral uptake by plants (Qin et al. 2014), the promotion of seed germination by strain NG-T6 may be due to this mechanism. However, there may be additional traits employed by PGPRs in protecting plants against salt stress.

In addition to promoting seed germination, our pot experiment demonstrated that these three strains were able to facilitate sugar beet growth under salinity stress. Bacterial inoculation had a positive effect on plant growth by increasing plant shoot or root length and dry biomass. It should be noted that the inoculation with bacteria in the present study was performed before exposure to salt, and plants were given sufficient time to stabilize before salt was added; however, under real agricultural...
conditions, plants would probably be exposed to salt prior to inoculation. Thus, more research to confirm the potential of plant growth promotion by these isolates needs to be conducted in the future.

The chlorophyll contents and \( F_v/F_m \) values were significantly higher in all bacteria-inoculated plants as compared with control plants, suggesting that chloroplast activity was promoted. Our results were similar to those obtained by Shi, Lou and Li (2010), who found that the chlorophyll contents and \( F_v/F_m \) values of sugar beet plants were significantly enhanced upon inoculation with endophytic bacteria. Similar enhancement of chlorophyll content in plants following bacterial inoculation has been reported in other studies (Swedrzyńska and Sawicka 2000; Islam et al. 2013). The variation in chlorophyll contents and \( F_v/F_m \) values may be attributed to the increase in the excitation trapping rate and quantum efficiency for electron transport by photosystem II, or to the physiological modifications inflicted on the plants by the inoculated PGPRs (Swedrzyńska and Sawicka 2000; Shi, Lou and Li 2010; Islam et al. 2013).

The role of bacteria in reducing stress-induced ethylene biosynthesis in plants has been well documented (Glick 2004; Glick et al. 2007; Ali, Charles and Glick 2014). In response to saline stress, there is a significant rise in the concentration of endogenous ethylene (called ‘stress ethylene’) in plant roots (Mayak,
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