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Plant growth promotion properties of bacterial strains isolated from the rhizosphere of the Jerusalem artichoke (*Helianthus tuberosus* L.) adapted to saline–alkaline soils and their effect on wheat growth

Xiaolin Liu, Xiangyue Li, Yan Li, Runzhi Li, and Zhihong Xie

Abstract: The Jerusalem artichoke (JA; *Helianthus tuberosus*), known to be tolerant to saline–alkaline soil conditions, has been cultivated for many years in the Yellow River delta, Shandong Province coastal zone, in China. The aim of our study was to isolate nitrogen-fixing bacteria colonizing the rhizosphere of JA and to characterize other plant growth promotion properties. The ultimate goal was to identify isolates that could be used as inoculants benefiting an economic crop, in particular for improving wheat growth production in the Yellow River delta. Bacterial strains were isolated from the rhizosphere soil of JA on the basis of growth on nitrogen-free Ashby medium. Identification and phylogenetic analysis was performed after nucleotide sequencing of 16S rRNA gene. Plant-growth-promoting traits, such as nitrogen fixation activity, phosphate solubilization activity, indole-3-acetic acid production, were determined using conventional methods. Eleven strains were isolated and 6 of them were further examined for their level of salt tolerance and their effect on plant growth promotion. Inoculation of *Enterobacter* sp. strain N10 on JA and wheat led to significant increases in both root and shoot dry mass and shoot height. *Enterobacter* sp. strain N10 appeared to be the best plant-growth-promoting rhizobacteria to increase wheat productivity in future field applications.

Key words: Helianthus tuberosus, plant-growth-promoting rhizobacteria (PGPR), plant growth test, *Enterobacter*.

Résumé : Le topinambour (*Helianthus tuberosus*), reconnu pour sa tolérance à des conditions salines alcalines dans le sol, est cultivé depuis de nombreuses années dans le delta du fleuve Jaune situé dans la zone côtière de la province de Shandong en Chine. Notre étude visait à isoler des bactéries fixatrices d'azote colonisant la rhizosphère de topinambours et à caractériser d'autres attributs favorisant la croissance végétale. L'objectif final était de dénicher des isolats pouvant être employés à titre d'inoculants soutenant une culture industrielle, avec une attention particulière à l'amélioration de la production de blé dans le delta du fleuve Jaune. On a isolé des souches bactériennes de la rhizosphère du sol de topinambours d'après une croissance sur milieu Ashby sans azote. On a procédé à l'identification et à l'analyse phylogénétique après un séquençage nucléotidique du gène de l'ARNr 16S. Les traits favorisant la croissance végétale (FCV) comme l'activité de fixation de l'azote, l'activité de solubilisant du phosphate et la production d'acide indole-3-acétique ont été évalués au moyen de méthodes conventionnelles. Onze souches ont été isolées et on a analysé chez 6 d'entre elles le niveau de tolérance au sel et l'incidence sur la croissance végétale. Une inoculation de la souche N10 d'*Enterobacter* sp. sur du topinambour et du blé a donné lieu à une hausse significative du poids sec des racines et des pousses ainsi que de la taille des pousses. *Enterobacter* sp. N10 est la rhizobactérie FCV offrant le meilleur espoir d'augmenter la productivité du blé dans des applications ultérieures en champs. [Traduit par la Rédaction]

Mots-clés : Helianthus tuberosus, rhizobactéries favorisant la croissance végétale (RFCV), test de croissance végétale, *Enterobacter*.

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Introduction

Bacteria colonizing the plant rhizosphere that display a beneficial effect on plant productivity are referred to as plant-growth-promoting rhizobacteria (PGPR) (Kloepper and Schroth 1978). PGPR stimulate plant growth by various means, including biological nitrogen fixation, phosphate solubilization, phytohormone production (such as IAA; indole acetic acid) (Boddey and Döbereiner 1995; de Souza et al. 2015; Dobbelaere et al. 2003) and tolerate adverse stresses such as flooding, salt stress, and water deprivation (Yang et al. 2009).

Wheat is one of the most important cereals worldwide for the human diet. Although wheat is extensively cultivated in the area of the Yellow River delta, its area of cultivation is limited because of excessive salinity and alkalinity of soils. The Bohai Sea Granary is a program recently launched in China, the objective of which is to better exploit the saline–alkaline land for cereal cultivation. For this reason, the objective of our study is to isolate and identify bacterial strains from the rhizosphere of a salt-tolerant plant, the Jerusalem artichoke (JA) *Helianthus tuberosus* (Tassoni et al. 2010), and to determine if some of these isolates can benefit wheat growth. The choice of the JA is based on the following criteria.

(*i*) JA is a plant particularly well adapted to dry and poor soils and displays some tolerance to salinity excess. So JA is cultivated on a large scale in China, in particular in the Provinces of Shandong, Heilongjiang, Shanxi, and Jiangsu.

(*ii*) JA is considered to be an extremely efficient crop because of its low nutritional requirements (Matías et al. 2013). Current agricultural practice using 75 $kg \cdot ha^{-1}$ N and 30 $kg \cdot ha^{-1}$ P can generate up to 80 $tons \cdot ha^{-1}$ stem tubers and 40 $tons \cdot ha^{-1}$ straw (Long et al. 2005).

(*iii*) There exists a large amount of saline–alkaline soils in the Yellow River delta, China (Guan et al. 2001). JA, which has been cultivated for many years in the delta, may have favored, in its rhizosphere, the establishment of a unique microbial community structure with different genetic and physiological characteristics, including efficient PGPR strains.

(*iv*) Several recent reports deal with the diversity of rhizobacteria associated with JA with respect to nitrogen fertilization or soil salinity (Meng et al. 2012; Yang et al. 2016) as well as rhizobacteria associated with sunflower (*Helianthus annuus* L), another member of the Compositae family, of economic importance (Ambrosini et al. 2012; Forchetti et al. 2007).

We report here on the isolation of nitrogen-fixing bacteria from the rhizosphere of JA and on the determination of some of their PGP traits as well as on the effect on JA and wheat growth. Among the 11 strains isolated on the basis of colony formation on N-free medium, only 6 were unambiguously capable of fixing nitrogen as determined by the acetylene reduction test. Three strains belonging to the genus *Enterobacter* were found to increase JA and wheat growth significantly and therefore may be of importance as potential biofertilizer for wheat, to serve the local agriculture.

Materials and methods

Sample collection

Rhizosphere soil samples were collected September 2012 from the JA germplasm breeding test site in the coastal zone (37.60°N, 118.82°E). Five different plants were dug out and the rhizosphere soil was collected from their root surface and 1 g of each sample was immediately stored in a cooling box at 4 °C for further isolation and analysis. Available N, Olsen P, Olsen K, organic C, salinity, and pH of soil samples were determined (Kızılkaya 2008).

Isolation of rhizospheric bacteria

Each sample was aseptically transferred to an Erlenmeyer flask with 90 mL of sterile water and shaken at 150 $rev \cdot min^{-1}$ for 30 min. Then, the suspensions were serially diluted in a proportion of 1:10, up to 10^7, in sterile distilled water from each sample, and 100 μL of each dilution was coated on N-free Ashby medium agar plates in triplicate (Kızılkaya 2008). N-free Ashby medium agar plates contained 5 g of glucose, 5 g of mannitol, 0.1 g of $CaCl_2 \cdot 2H_2O$, 0.1 g of $MgSO_4 \cdot 7H_2O$, 5 mg of $Na_2MoO_4 \cdot 2H_2O$, 0.9 g of K_2HPO_4, 0.1 g of KH_2PO_4, 0.01 g of $FeSO_4 \cdot 7H_2O$, 5 g of $CaCO_3$, 15 g of agar in 1 L of distilled water; the final pH was adjusted to 7.3. Dilutions made with sterile water were used as a control treatment. Next, plates were incubated at 28 °C for 72 h. Single colonies were picked and purified on N-free Ashby medium, 3 times. The purified isolates were stored in 20% glycerol at −80 °C for further testing.

Acetylene reduction assay

Nitrogenase activity of the isolates was determined by the acetylene reduction assay (ARA) as follows. The bacterial isolates were grown first at 28 °C in NH_4-sufficient Ashby liquid medium with continuous agitation at 200 $rev \cdot min^{-1}$ in a gyratory shaker at 28 °C overnight. One millilitre of each bacterial culture was collected by centrifuging and washed twice with N-free Ashby liquid medium. Then the bacteria were suspended in 2 mL of N-free Ashby medium to an optical density at 600 nm (OD_{600}) of 0.2∼0.5 in a 20 mL vial sealed with a rubber septa and were grown under microaerobic conditions in an N_2 atmosphere containing 1% oxygen. After 16 h, up to 10% (*v*/*v*) acetylene was added to each vial (Burns 1972), and bacteria were incubated for 4 h at 28 °C. The amount of ethylene (C_2H_2) produced was measured using a gas chromatograph fitted with a flame ionization detector (Agilent 7890A). The protein content of the samples was determined by standard method (Bradford 1976). Uninoculated tubes served as the control. The results of the ARA were expressed as nmol $C_2H_2 \cdot (mg\ protein)^{-1} \cdot h^{-1}$.

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Determination of indolic compounds production

Strains were inoculated in triplicate in Erlenmeyer flasks containing 30 mL of sterilized combined carbon medium (Rennie 1981) supplemented with 100 mg·L^{-1} L-tryptophan and incubated at 200 rev·min^{-1} at 28 °C for 60 h. Uninoculated combined carbon medium was used as a control. After growth, 5 mL of the cell suspension was centrifuged (11 180*g*) to remove cells, and indolic compounds in the supernatant were revealed by the Salkowski colorimetric technique using the Pilet and Chollet reagent (12 g·L^{-1} $FeCl_3$ in 7.9 mol·L^{-1} H_2SO_4) (Glickmann and Dessaux 1995). The concentration of indolic compounds was determined spectrophotometrically by measuring the absorbance at 530 nm, using IAA as standard for calibration curves.

Detection of inorganic phosphate solubilization activity

Inorganic phosphate solubilization was assayed in 50 mL Erlenmeyer flasks filled with 25 mL of sterilized Pikovskaya medium (10 g glucose, 5 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)_2SO_4$, 0.2 g NaCl, 0.1 g $MgSO_4{\cdot}7H_2O$, 0.2 g KCl, 0.5 g yeast extract, 0.002 g $MnSO_4{\cdot}H_2O$, 0.002 g $FeSO_4{\cdot}7H_2O$) (Pikovskaia 1948). The strains were inoculated in triplicate and incubated for 10 days at at 200 rev·min^{-1} and 28 °C. Uninoculated Pikovskaya medium was used as a control. Afterwards, 5 mL of the cell suspensions were centrifuged (11 180*g*) to remove cells, and the supernatant was then used to measure the pH and to determine soluble P released into the solution by using the molybdate blue colorimetric method (Fiske and Subbarow 1925).

Genotypic characterization and identification of PGP strains

Bacterial strains were grown in Luria–Bertani (LB) medium. DNA was extracted using the Tiangen™ Bacterial Genomic DNA kit according to manufacturer's instructions. DNA concentration was determined with a Nanodrop ND-2000 spectrophotometer. For each selected strain, amplification and partial nucleotide sequencing of the 16S rDNA was performed for genotypic identification. The 16S rDNA was amplified by polymerase chain reaction (PCR) using *Taq* DNA polymerase and bacterial universal primers 27F (5′-GGTTACCTTGTTACGACTT-3′) and 1492R (5′-AGAGTTTGATCCTGGCTCAG-3′). The PCR products were identified by electrophoresis on 1.0% agarose gel and were commercially sequenced by Sangon Biotech. The resulting nucleotide sequences were compared with those deposited in the GenBank database. Using MEGA 5.0, a phylogenetic tree was constructed with the neighbour-joining method based on the 16S rDNA gene fragments and was evaluated by the bootstrap method with 1000 replicates (Tamura et al. 2004). Furthermore, the pairwise distance matrix of evolution was calculated using the Kimura 2-parameter distance model (Kimura 1980).

Determination of salt tolerance

Some PGPR, like *Enterobacter*, have ever been reported to have a maximal tolerance concentration of 7% (*m*/*v*) NaCl (Dastager et al. 2009). The salt tolerance of isolated strains was assayed by looking for growth in LB medium whose NaCl concentration was adjusted from 1% to 10% (*m*/*v*), respectively. The strains were inoculated at 37 °C for 16 h, prior measurement of OD at 600 nm. Standard LB broth was used as the control.

Plant growth assay

Selected small *H. tuberosus* tubers of roughly the same size and seeds of wheat were surface sterilized using 95% (*v*/*v*) ethanol for 30 s, followed by 0.2% (*v*/*v*) $HgCl_2$ for 5 min. Then, the tubers and seeds were washed 6 times with sterilized distilled water, transferred to sterilized beakers containing 0.3% water agar plate, and maintained for 2 days in a biochemical incubator at 28 °C for germination in the dark. The germinated seedlings were then transferred to pots containing sterilized vermiculite (1 seed per pot, 2 cm below the vermiculite surface) with low nitrogen–tricalcium phosphate nutrient mixture (0.075 g ferric citrate, 0.03 g $Ca(NO_3)_2$, 0.075 g KCl, 0.06 g $MgSO_4{\cdot}7H_2O$, 0.46 g $CaSO_4$, 1 mL trace salt). Trace salt contains (per 1 L) 2.86 g H_3BO_3, 1.81 g $MnSO_4$, 0.8 g $CuSO_4{\cdot}5H_2O$, 0.22 g $ZnSO_4$, 0.02 g H_2MoO_4. Each bacterial strain was grown overnight in LB liquid medium to an OD_{600} of 0.8–1.0, and 1 mL of bacterial culture was inoculated per plant. The experiment was performed in quintuplicate per isolate in a completely randomized block design. All pots were maintained in a controlled growth greenhouse adjusted to a 12 h photoperiod at 26 ± 2 °C and were watered appropriately with sterilized distilled water. Plants in pots were harvested 70 days after sowing. The entire plant was dried to a constant mass in an oven at 65 °C. Shoot and root mass and shoot height of every JA plant were measured, and every wheat plant was measured for the dry shoot biomass.

Statistical analysis

The results were subjected to analysis of variance (ANOVA). For multiple comparisons, the data were analyzed by Tukey's test using SPSS version 19 (IBM Corp., Armonk, New York). Differences were considered statistically significant at $P < 0.05$. Mean value and the standard errors were computed.

Results

Isolation of bacteria from the JA rhizosphere, and phylogenetic analysis

Plant rhizosphere is known as a carbon-rich nitrogen-limiting environment that that may provide an ecological niche favouring colonization of the root system by nitrogen-fixing bacteria (Döbereiner 1974). This is why, as a primarily screening strategy, the N-free Ashby medium was used to isolate putative nitrogen-fixing bacteria from the rhizosphere soil of the JA. Colonies appeared on the screening plates at dilutions from 10^{-1} to 10^{-4}, and

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Table 1. Assessment of chemical properties of the rhizosphere soil sample.

Property	Value
Available N	65 mg·kg^{-1}
Olsen P	9.5 mg·kg^{-1}
Olsen K	75.5 mg·kg^{-1}
Organic C	2.56 g·kg^{-1}
Salinity	2.0 g·kg^{-1}
pH	8.15

the viable cell count determination was in the range of 10^5 cells per gram of soil. A set of 11 colonies was purified by double streaking on solid agar and kept for further identification. The salinity of rhizosphere soil is 0.4% and pH is 8.15, making it of moderate salination. The chemical properties of soil samples are shown in Table 1. The soil quality in this plot of Yellow River delta is rather low.

BLAST was used to compare the nucleotide sequence of the 16S rDNA fragment obtained for each isolated strain with the 16S rDNA sequences in the GenBank database. Formal species identification will require further analysis, and at this stage we have only indicated the highest match observed (Table 2). The 11 isolates can be categorized into 4 main genera. The majority of the isolates (7 strains) were closely related to *Gammaproteobacteria* belonging to the genera *Enterobacter* and *Klebsiella*, and 2 strains were related to *Firmicutes* belonging to the *Bacillus* genus (Table 2 and Fig. 1), and 1 strain was related to the *Pseudomonas* genus. Among the first genus, 4 strains could be identified as members of the *Enterobacter* genus, a genus already reported as predominant in the rhizosphere of sunflower (*Helianthus annuus* L.) in Brazil (Ambrosini et al. 2012).

Determination of the nitrogen-fixating ability and other PGP traits

Colony formation on N-free medium is not sufficient per se to prove that the bacterial strain is nitrogen-fixating, since many non-nitrogen-fixing isolates can develop by scavenging nitrogen traces in the medium (Postgate 1982). Therefore, the ARA determined the nitrogen-fixating ability of the strains. Indeed, significant variability in the nitrogen-fixing ability was found among the strains. Only 6 strains, including 1 *Bacillus* sp. (N1), 2 *Klebsiella* sp. (N7, N8), and 3 *Enterobacter* spp. (N9, N10, N11), displayed an activity superior to 30 nmol C_2H_2·(mg protein)$^{-1}$·h^{-1} (Table 3). These 6 strains were considered as nitrogen fixers and were kept for further study. All exhibited inorganic phosphate solubilizing activity but only one of them, *Enterobacter* sp. strain N10, displayed tryptophan-dependent indolic compound production (Table 3). Meanwhile, it was checked that the growth of the 6 strains resulted in a notable acidification of the medium, and pH of the culture supernatant showed a significant decrease compared with the control decreased from 6 to 4.3~4.5.

Determination of salt (NaCl) tolerance

An excess of salt is deleterious for crop productivity. All the strains isolated in this work are indigenous of the rhizosphere soil of a plant growing in saline–alkaline soil. The chemical and physical characteristics of soil show heterogeneity depending on depth and plant root system. In particular, the rhizosphere differs from the bulk soil (Hinsinger et al. 2009). The salinity of the rhizosphere soil sample was moderate in the range of 0.4% with a pH of 8.15. *Enterobacter* was reported to have maximal tolerance concentration of 7% (*m*/*v*) NaCl (Dastager et al. 2009), so we design a series of salt concentrations, adjusted from 1% to 10% to evaluate the salt tolerance of these strains. The 6 nitrogen-fixing strains can tolerate much higher saline concentration, and all showed growth, which are similar to the growth when cultured in medium lower than the concentration of 4% NaCl (data not show). Although they all show growth in the concentration of 4% and 5% NaCl, none could grow in media containing 9% or more (Fig. 2). At a NaCl concentration of 7%, all the strains except N11 displayed significant growth ability. While the salt concentration increased to 8%, only N9 and N10 keep the state of growth. Overall, the *Enterobacter* sp. strains N9 and N10 seem to have the biggest potential to tolerate high salt concentration.

Effect of bacterial inoculation on plant growth of *H. tuberosus* and wheat

The PGP ability of the 6 strains was first assayed on JA (Fig. 3). A significant difference was observed in the plant growth of inoculated and uninoculated treatments. Among the inoculated treatments, only *Enterobacter* sp. N10 significantly improved the shoot dry mass and height, with an increase of 29.2% and 16.8%, respectively. With respect to the root dry biomass, a significant increase was observed after inoculation with *Bacillus* sp. strain N1, *Klebsiella* sp. strain N7, and *Enterobacter* sp. strains N10 and N11; N10 resulted in the maximum increase with an increase of 58.6%, followed by N1, N11, and N7. All the isolates except N9 improved the shoot-to-root ratio (S/R), and plants inoculated with N10 showed highest S/R. It appears that among the 6 nitrogen-fixing strains isolated from the rhizosphere of JA, 4 could benefit JA growth to some extent, with *Enterobacter* sp. N10 showing the best performance.

We incubated wheat with the 6 strains isolated from JA and tested their promoting effects on the growth of wheat. In terms of wheat, only N10 contributed to a statistically significant shoot mass increase compared with the control (Fig. 4). Strains N1 and N7 decreased shoot dry mass without any visible damage or eliciting symptoms of plant disease.

Discussion

Compared with the bulk soil, plant rhizosphere is abundant in nutrients because of the accumulation of various organic compounds released from roots by secre-

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Table 2. Genus identification and nitrogen fixation ability of the isolated strains.

Isolate	Genus	16S rRNA gene highest match	GenBank acc. No.	% Similarity	Nitrogenase activity*
N2	*Enterobacter*	*E. cowanii*	AJ508303	99.8	+
N3	*Enterobacter*	*E. ludwigii*	AJ853891	99.7	–
N11	*Enterobacter*	*E. ludwigii*	AJ853891	99.7	+
N9	*Enterobacter*	*E. cancerogenus*	Z96078	98.8	+
N10	*Enterobacter*	*E. cancerogenus*	Z96078	99.1	+
N4	*Klebsiella*	*K. oxytoca*	AF129440	99.4	–
N7	*Klebsiella*	*K. oxytoca*	AF129440	99.4	++
N8	*Klebsiella*	*K. oxytoca*	AF129440	99.3	++
N5	*Pseudomonas*	*P. lini*	AY035996	99.6	–
N1	*Bacillus*	*B. megaterium/B. aryabhattai*	D16273/EF114313	99.8	++
N6	*Bacillus*	*B. megaterium/B. aryabhattai*	D16273/EF114313	99.9	–

*Nitrogenase activity was determined by the acetylene reduction test (see Methods): ++, >130 nmol C_2H_2·(mg protein)$^{-1}$·h^{-1}; –, ≤4 nmol C_2H_2·(mg protein)$^{-1}$·h^{-1}; +, intermediate levels.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing positions of the 6 isolates (N1, N7, N8, N9, N10, N11) and relative type strains of species in different genera. The accession numbers in NCBI database are indicated after the bacterial names. Bootstrap analyses were made with 1000 cycles and only bootstrap values >50% are shown at the branch points. Scale bar indicates 0.005 changes per site.

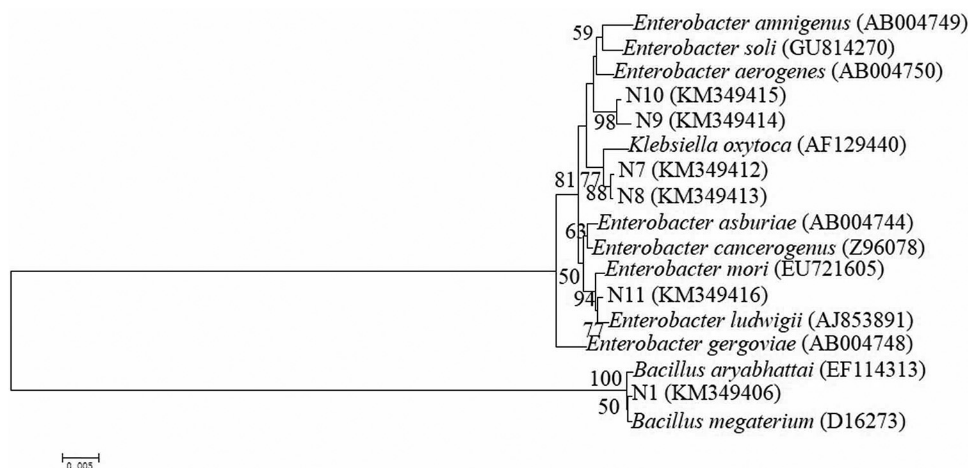

Table 3. In vitro screening results for plant-growth-promoting rhizobacteria traits.

Strain name	P-solubilizing activity (mg·L^{-1})	Final pH of medium	IAA production (μg·mL^{-1})	Acetylene reduction assay (nmol C_2H_2·(mg protein)$^{-1}$·h^{-1})
Bacillus sp. N1	330.80±43.97	4.38±0.02	0	251.27±21.22
Klebsiella sp. N7	229.05±3.18	4.40±0.04	0	134.19±20.55
Klebsiella sp. N8	213.12±12.10	4.36±0.83	0	229.92±24.94
Enterobacter sp. N9	230.14±6.06	4.32±0.03	0	33.83±4.02
Enterobacter sp. N10	254.58±51.70	4.29±0.10	1.10±0.18	83.76±2.23
Enterobacter sp. N11	339.31±27.23	4.48±0.30	0	31.63±6.56
Control	0.00±0.00	7.49±0.08	0	0.00±0.00

Note: The values are the means of 3 replicates, and the results are expressed as means ± standard deviation.

tion, exudation, and deposition (Curl and Truelove 1986), which forms a preferential niche for a variety of microorganisms in the soil. In this study, 6 culturable nitrogen-fixing bacterial isolates were characterized from the rhizosphere soil of JA, which were subdivided into 3 genera on the basis of their 16S rRNA gene sequences: *Enterobacter* (3 strains), *Klebsiella* (2 strains), *Bacillus* (1 strain). These genera are commonly found in the rhizosphere of other plants.

Bacillus species are common rhizobacteria. They have been isolated from various agricultural crops, such as tomato (Almaghrabi et al. 2013), rice (Beneduzi et al. 2008*b*), soybean (Park et al. 2005), wheat (Beneduzi et al. 2008*a*), maize and crabgrass (Habibi et al. 2014), and were reported as predominant in sunflower (Goes et al. 2012). *Klebsiella* strains have also been described as PGPR. Roesch et al. (2008) analyzed the biodiversity of diazotrophic bacteria within the soil, root, and stem of field-grown maize,

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Fig. 2. The growth of each bacteria under different salt (NaCl) concentrations. The same letters above the bars indicate no significant difference at $P < 0.05$. Data are expressed as means ± SD (n = 3) of 3 replicates of bacteria grown in media. The values of the control are zero (data not shown).

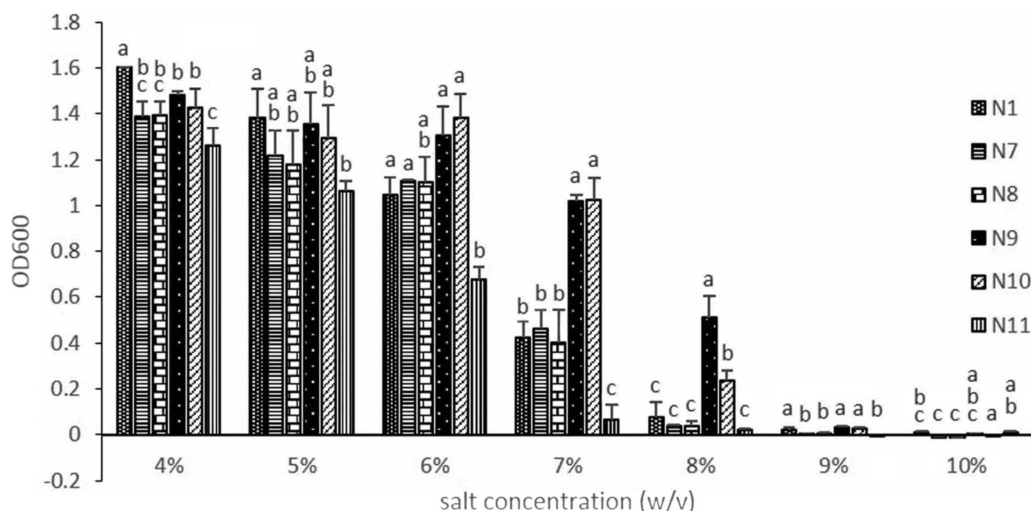
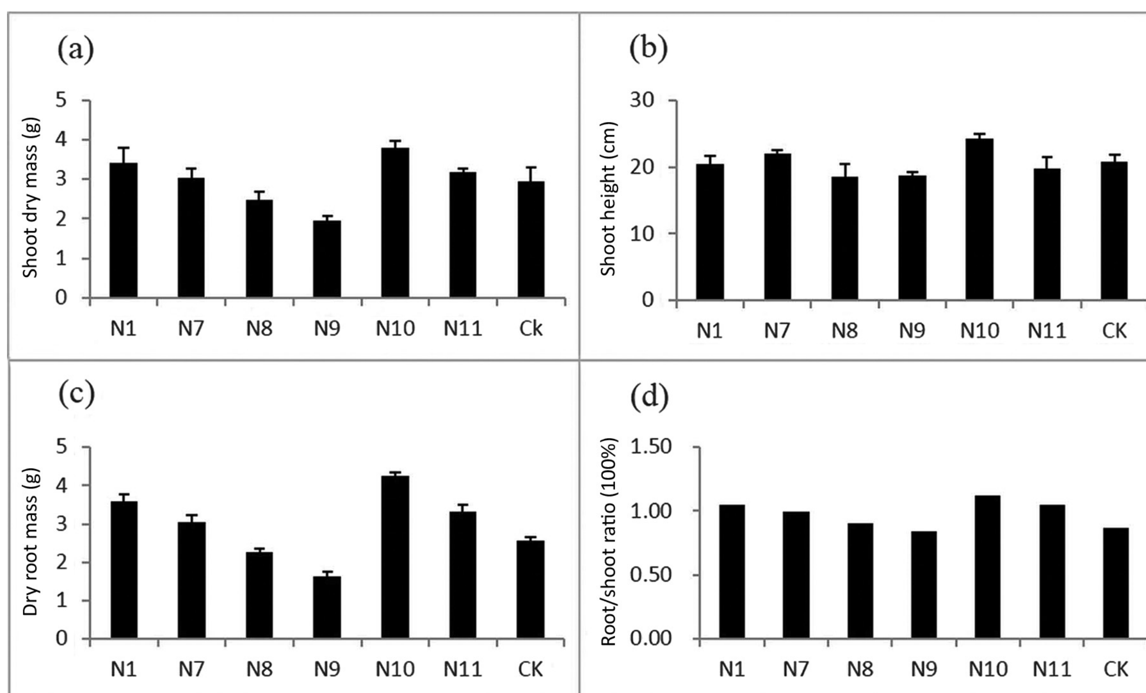


Fig. 3. Summary of the effect of inoculation by each isolate on the growth of Jerusalem artichoke. (*a*) Shoot dry mass; (*b*) shoot height; (*c*) dry root mass; (*d*) root/shoot ratio. Data are expressed as means ± SD (n = 3) of 5 replicates of plants grown in vermiculite in a greenhouse. CK, control.



noting that *Klebsiella* appeared to be a plant endophyte but was much rarer in soil. Nevertheless, de Souza et al. (2013) also isolated *Klebsiella* bacteria from the rhizospheric soil of rice. The occurrence of *Enterobacter* in the rhizosphere of a variety of plants is well documented, such as maize (Hinton and Bacon 1995), citrus (Araújo et al. 2001), wheat (Joshi and Bhatt 2011), soybean (Kuklinsky-Sobral et al. 2004), sugarcane (Ferrara et al. 2012; Magnani et al. 2010), pine (Ribeiro and Cardoso 2012), cotton and sweet corn (McInroy and Kloepper 1995). Meanwhile, *Enterobacter* was found to be one of the most abundant putative nitrogen-fixing and PGP rhizobacteria of rice and sunflower (Ambrosini et al. 2012; de Souza et al. 2013).

In a recent report of wheat inoculation, strains of *Bacillus* spp. and *Pseudomonas* spp. were preferentially used over *Enterobacter* sp., which was not found to be the best performer (Nadeem et al. 2013). However, in this present work, we observed that the isolated *Enterobacter* strains performed better than the *Bacillus* strains.

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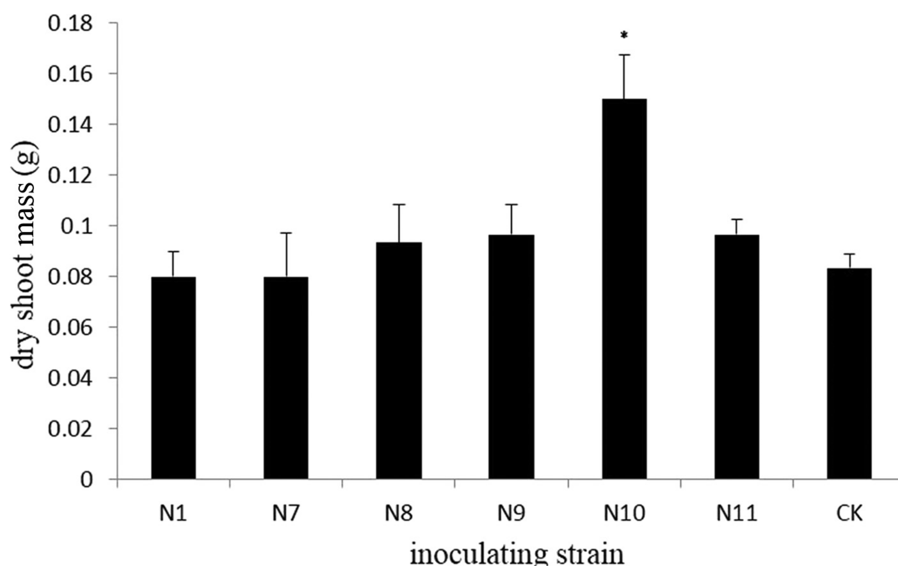
Fig. 4. Summary of the effect of inoculation by each isolate on dry shoot mass in the wheat growth test. Data are expressed as means ± SD (n = 3) of 5 replicates of plants grown in vermiculite in greenhouse. *, indicates a significant difference from that of the control, as analyzed by SPSS using Tukey's test (P < 0.05).



PGP traits, salt tolerance, as well as the effect on plant growth are greatly variable among different species and even among strains of the same species. Since most soils are nitrogen and phosphorus deficient, nitrogen-fixing and P-solubilizing indigenous soil bacteria are thought to play a role in soil fertilization and plant growth promotion by PGPR (do Amaral et al. 2014; Richardson 2001; Rodriguez and Fraga 1999). *Bacillus* and *Enterobacter* were reported to be the efficient phosphate-solubilizing genera (Shahid et al. 2012), and we found that the *Klebsiella* isolated during this work also has the ability. *Bacillus* sp. strain N1 and *Klebsiella* sp. strains N7 and N8 isolated in this work are the best performers, in vitro, in terms of nitrogen-fixation efficiency and phosphate solubilization. However, none of them resulted in a significant increase in promoting the growth of wheat compared with the control. Thus, there was no correlation between these PGP traits assayed in vitro and the effect on plant growth. In the case of the nitrogen-fixing *Azospirillum*, it was reported that inoculation of maize, sorghum, and *Setaria* grass did not show substantial N_2 fixation in greenhouse studies (Okon and Labandera-Gonzalez 1994; Dobbelaere et al. 2003).

IAA production is another important trait of PGPR. Increasing evidence has indicated that IAA secreted by the diazotrophic bacteria plays an important role in promoting plant growth (Dobbelaere et al. 2003). In the infertile soil, nutrient availability is poor and not sufficient to meet the optimal growth of the plant. Thus, it is a critical issue for plants to absorb nutrient efficiently from soil by developing their root system (Richardson et al. 2009). Indeed, IAA-producing rhizobacteria can promote the growth of plants by increasing the length of roots and enhancing total root surface and root volume, resulting in a better nutrition of the plant having access to more essential nutrients from the soil (Kapulnik et al. 1985; Okon and Labandera-Gonzalez 1994; Dobbelaere et al. 2003; Shaharoona et al. 2008). Considering the conditions used for the plant tests, where the levels of available N and Olsen P in vermiculite soil are significantly lower than that reported in fertile soils, it appears that *Enterobacter* sp. N10, which is performing best with plants, is also the best IAA producer. Strain N10, in addition, displayed relatively high nitrogen-fixation and phosphate-solubilizing activities, which is in agreement with its higher performance on plant growth. This might imply that relatively higher nitrogen-fixing and phosphate-solubilizing activities established richer nutrient conditions, in which IAA stimulated the growth of the roots and enabled the plants to access more essential nutrients needed for plant growth.

Excessive soil salinity is an important stress factor known to limit plant growth and crop yield (Yang et al. 2016), but halotolerant rhizobacteria play a role in alleviating the negative effects of salinity (Ahmad et al. 2011). This is why tolerance to salt was an important criterion to identify appropriate rhizobacteria to implement the inoculation assay of wheat in the Yellow River delta. *Enterobacter* sp. N9 and N10 displayed the highest halotolerance among the assayed strains. It has been reported that plant growth regulators, including IAA, can considerably promote plant growth under salt conditions (Egamberdieva 2009). This is why it is expected that *Enterobacter* sp. N10, which showed significant IAA production ability, appears as the most promising for future plant assays under saline conditions. Similarly, ACC-deaminase activity (Rajput et al. 2013) and exopolysaccharide production (Upadhyay and Singh 2015) were reported to play a role in the tolerance of salt and these properties should be further studied with *Enterobacter* sp. N10.

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In conclusion, *Enterobacter* was the dominant genus and *Enterobacter* sp. N10 appears as the best PGPR for *H. tuberosus* and wheat. Meanwhile, if it appears important to select strains presenting PGP traits, there is no guarantee that isolated strains showing these desirable traits will benefit plant growth in greenhouse experiments. Nitrogen-fixing activity and phosphate-solubilizing activity may not be as pivotal as we previously expected. In contrast, more attention should be paid to the production of IAA, which may well play a more significant role as a vital regulator in promoting plant growth. It is also of importance to ensure that the isolated strains properly colonize the root system under the plant growth conditions utilized. Furthermore, an essential plant growth test and a salt tolerance test cannot be ignored. Notably, *Enterobacter* sp. N10 showed prominent PGP traits in both in vitro assay and in pot experiment and contributed to a considerable increase on the growth of *H. tuberosus* and wheat. Furthermore, N10 showed the strongest salt tolerance. As the PGP effect is highly variable and dependent on environmental conditions (Boddey et al. 1991), further studies are required. In particular it is important to perform field experiments to evaluate the promoting effect in natural conditions to develop an inoculant for wheat in future field applications.

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