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Research Article 研究报告

Characterizations of three diazotrophic *Paenibacillus* spp. and their effect on Chinese pakchoi yield and soil enzyme activities

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Abstract: [Objective] Nitrogen-fixing (diazotrophic) bacteria are considered possible alternative to nitrogen fertilizers for promoting plant growth and yield. This study was to run a phylogenetic analysis and determine the PGPR traits of the isolated diazotrophic bacteria and characterize the effect of diazotrophic bacteria on Chinese pakchoi yield and soil enzyme activities. **[Methods]** We isolated 30 diazotrophic bacteria from rhizosphere of wheat (11 isolates), Chinese pakchoi (16 isolates), and lotus (3 isolates) on N-free medium plates. Based on 16S rRNA sequence analysis, the dominant diazotrophic bacteria of wheat, Chinese pakchoi, and lotus belonged to the genus *Paenibacillus*. **[Results]** Three multi-function diazotrophic bacteria designated as *Paenibacillus* spp. P-4, W-7 and L-3 were screened from these 30 diazotrophic. The nitrogenase activities of P-4, W-7 and L-3 were higher than the that of *Azotobacter chroococcum* used as the control. Strains P-4, W-7 and L-3 can inhibit the growth of two or three plant pathogens of *Sclerotinia sclerotiorum*, *Gibberella zeae* and *Verticillium dahliae*. Strain W-7 has also the phosphate solubilizing ability. Inoculation with *Paenibacillus* spp. W-7 or L-3 significantly increased the shoot fresh weight of Chinese pakchoi and changed the activity of soil sucrase, phosphatase, and catalase under field conditions, whereas inoculation with *Paenibacillus* sp. P-4 had no significant effect on plant growth or enzyme activity. **[Conclusion]** *Paenibacillus* sp. W-7 and L-3 have good potential to promote plant yield and improve soil quality.

Keywords: diazotrophic bacteria, PGPR characterizations, *Paenibacillus*, plant promotion, soil enzyme

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Nitrogen(N)-fixing (diazotrophic) bacteria performed fundamental processes that contribute to nutrient cycling, healthy root growth, and plant growth promotion[1]. The community of diazotrophic bacteria is mainly affected by soil type and physico-chemical properties as well as by plant species and the growth stage[2–4]. Numerous N-fixing bacteria have been isolated from the rhizosphere of various crop species such as rice, wheat, soybean, maize, sugarcane, coffee, and so on[3,5–6]. These N-fixation bacteria belong to the genera *Azotobacter*, *Burkholderia*, *Agrobacterium*, *Pseudomonas*, *Gluconacetobacter*, *Achromobacter*, *Paenibacillus*, *Enterobacter*, *Herbaspirillum*, *Klebsiella* and *Serratia*[6–8]. Many N-fixing bacteria are plant growth promoting rhizobacteria (PGPR) that are known for their beneficial effects on plants through various mechanisms, including N-fixing[9] (Xie *et al.*, 2003), phosphate solubilization[10], phytohormone production[11], pathogens suppression[12], stress reduction and ethylene control[13–14]. Therefore, the interest in the isolation of N-fixing bacteria and identification of their PGPR traits has been increased. Most studies have focused on a single plant species, while only a few compared the communities of N-fixing bacteria from different plant species[7–9,13]. The objectives of this study were to (i) isolate diazotrophic bacteria from the rhizosphere of wheat, Chinese pakchoi and lotus; (ii) run a phylogenetic analysis and determine the PGPR traits of the isolated diazotrophic bacteria; and (iii) characterize the effect of diazotrophic bacteria on Chinese pakchoi yield and soil enzyme activities.

1 Materials and Methods

1.1 Isolation of N-fixing bacteria and phenotypic characterization

Rhizosphere soil samples were collected from three crop species, namely wheat (*Triticum aestivum*) grown in Yakeshi, Inner Mongolia Autonomous Region, China; Chinese pakchoi (*Brassica campestris* L.) and lotus (*Nelumbo nucifera* Gaertn.) both grown in Chaoyang District, Beijing, China. We confirm that the field studies did not involve any endangered or protected species, and no specific permissions were required for these locations or activities. To isolate N-fixing bacteria, 10 g of soil samples were shaken (160 r/min) for 30 min with 100 mL ACCC55 N-free medium (10 g/L sucrose, 0.5 g/L $K_2HPO_4·3H_2O$, 0.2 g/L NaCl, 1 g/L $CaCO_3$, 0.2 g/L $MgSO_4·7H_2O$; pH 7.0–7.2) at 28 °C[15]. Serial dilutions (10^{-4}–10^{-6}) were made and 100 μL aliquots were spread on plates containing ACCC55 with 1.8% water-washed agar. The plates were incubated at 28 °C until single colonies appeared. Each colony was purified and sub-cultured for several generations to ensure its diazotrophic nature. Pure isolates were stored temporally in ACCC55 medium at 4 °C or as glycerol suspensions (15% *W*/*V*) at –80 °C for long-term conservation.

1.2 PCR amplification of 16S rRNA gene

The genomic DNA of each isolate was extracted from a 3 d culture in nitrogen-free liquid medium by the CTBA protocol[16] and used as template in the PCR amplification. Sequences of the 16S rRNA gene were obtained from PCR products amplified using the universal forward primer 27F and 1492R[17]. The PCR reactions contained 1 μL genomic DNA, 25 μL 2×mix *Taq*, 1 μL of each primer and ultrapure sterile water to a final volume of 50 μL. The reaction conditions were, 5 min at 94 °C, 30 cycles of 1 min at 94 °C, 1 min at 55 °C, and 1min at 72 °C, a final step of 5 min at 72 °C. The PCR products of partial 16S rRNA genes were obtained and sequenced by the Shengong Biological Engineering Technology Service (Shanghai, China). The nucleotide sequences of 16S rRNA genes were deposited in the GenBank database. Sequence analyses and reference sequences were performed and obtained in the EzTaxon (http://eztaxon-e.ezbiocloud.net/). Phylogenetic analysis was performed using the software package MEGA 5.0 after multiple alignments of the sequence data with Clustal X[18]. Phylogenetic trees based on the neighbour-joining method[19].

1.3 Acetylene reduction assay (ARA)

The N-fixing activity of bacterial cultures was examined by ARA[20]. Pure cultures were transferred in 25 mL test tubes containing 4 mL of solid ACCC55 N-free medium. The air was replaced by injecting acetylene gas into the capped tubes at a final concentration of 10% (*V*/*V*) and incubated at 28 °C for 72 h. Ethylene concentrations were measured by a GC9790II gas chromatograph (Fuli Analytical Instruments Co., Zhejiang, China) as described previously using *Azotobacter chroococcum* (ACCC11104) as a positive control. The nitrogenase activity was expressed in nmol C_2H_4 mg^{-1} protein h^{-1}.

1.4 ACC deaminase activity

ACC deaminase activity was determined as described by Donna and Bernard[21], The number of mmol of a-ketobutyrate produced by this reaction is determined by comparing the absorbance at 540 nm of a sample to a standard curve of a-ketobutyrate ranging between 0.1 and 1.0 mmol. ACC deaminase activity (μmol·h)/mg=the amount of a-ketobutyrate (μmol)/protein levels (mg)/response time (h)[21].

1.5 Phosphate solubilization

P solubilization was determined qualitatively as described by Chen *et al.*[22] in inorganic phosphate solubilization medium (pH 7.0) containing 10 g/L glucose, 0.5 g/L $(NH_4)_2SO_4$, 0.3 g/L NaCl, 0.03 g/L KCl, 0.03 g/L $FeSO_4·7H_2O$, 0.3 g/L $MgSO_4·7H_2O$, 0.03 g/L $MnSO_4·4H_2O$, 10 g/L $Ca_3(PO_4)_2$ and 15 g/L agar. For organic phosphate solubilization, $Ca_3(PO_4)_2$ was replaced by 0.2 g lecithin. Pure bacterial culture was grown in ACCC55 medium at 28 °C for 3 d. Then, an aliquot of 10 μL (optical density of 1.2 at 600 nm) was transferred into plates containing organic or inorganic phosphate solubilization medium and incubated at 30 °C for 7 d until a solubilization zone around the colonies was observed. Each isolate was studied in triplicate. The halo zone (z) and colony diameter (n) were measured, and the ratio of z to n (z/n) was calculated[23].

1.6 Evaluation of antagonism against plant pathogens

N-fixing bacteria were tested *in vitro* for antagonism against the phytopathogenic fungi *Verticillium dahliae* (ACCC 30308), which causes verticillium wilt in over 300 woody and herbaceous plant species; *Sclerotinia sclerotiorum* (ACCC 36019), which causes white mold in hundreds of plant species growing under adequate conditions; and *Gibberella zeae* (ACCC 31053), which causes fusarium head blight in wheat, barley and tomato[24]. The analysis was performed by dual culture (DC) assay on potato dextrose agar (PDA) as previously described. 12 plates containing PDA were inoculated with a single pathogen and used as control. Each experiment was conducted in triplicate. The antifungal index was calculated by the following formula[25]: Antifungal index (%)=[1–(D_a–0.5)/(D_b–0.5)]×100%, where D_a is the diameter (cm) of growth zone in the test plate, and D_b is the diameter (cm) of growth zone in the control plate.

1.7 Plant growth and inoculation in the field

The *Paenibacillus* spp. W-7, P-4 and L-3 isolated from wheat, Chinese pakchoi and lotus, respectively, were used for further evaluation under field conditions. The soil used in this study was collected from a greenhouse located in Xuzhou, Jiangsu Province, China. The soil of this field had the following properties: pH 7.3, soil was irrigated to 30%–50% of maximum field capacity, total organic carbon 10.7 g/kg, total nitrogen 1.03 g/kg, NO_3^- 62.1 mg/kg, total potassium 13.57 g/kg, available potassium 108.31 mg/kg, total phosphorus 0.77 g/kg and available phosphorus 3.2 mg/kg. The experiment was conducted with four treatments: CK, *Paenibacillus* spp. W-7, P-4 and L-3. Each treatment contained three replicates. Chinese pakchoi seeds were sown manually on ridges using a dibbler. Row-to-row and plant-to-plant distances were 10 cm and 25 cm, respectively, while the pot size was 1 m^2. Fertilizer was applied at a concentration of 25 kg/ha

as basal dose. Pure bacterial cultures were grown in ACCC55 medium at 28 °C. After two weeks, Chinese pakchoi seedlings were inoculated with 10 mL of single strain suspensions or 10 mL of ACCC55 medium that used as control. Shoot fresh weights were determined 40 d after inoculation.

1.8 Soil characteristics analysis

Soil pH was measured in a 1:2.5 (*V*:*V*) soil:water ratio using a S220 K pH meter (Mettler-Toledo Interna-tional Inc., China). Soil total organic carbon (SOC) was analyzed by wet digestion with H_2SO_4-$K_2Cr_2O_7$, and total N (TN) was determined by semimicro Kjeldahl digestion using Se, $CuSO_4$ and K_2SO_4 as catalysts. Determination of available nitrogen using Alkali N-proliferation method; Determination of soil total phosphorus using $HClO_4$-H_2SO_4 boiled-molybdenum blue colorimetric method; Determination of soil available phosphorus using the Olsen improvement method; Determination of soil total potassium by flame photometric method for the determination of sodium hydroxide melt; Determination of soil available potassium using flame spectrophotometry.

1.9 Soil enzyme activities

After field experiment, samples from each plot were collected from the rhizosphere of five Chinese pakchoi. Samples were air-dried and passed through a 1-mm mesh. Sucrase, urease, and phosphatase activities were measured as described by Guan, Shen and Tabatabai[26–27], while catalase activity by the titration method[28].

1.10 Statistical analysis

Data were analyzed using SPSS (IBM SPSS, Chicago, IL, USA), and differences were considered significant at $P<0.05$ as determined by Duncan's test.

2 Results

2.1 Isolation and phylogenetic analysis of diazotrophic bacteria

A total of 30 diazotrophic bacteria (11 from wheat, 16 from Chinese pakchoi, and 3 from lotus) were isolated on N-free medium plantes. Phylogenetic analysis of 30 diazotrophic bacteria on 16S rRNA sequences and the topologies of phylogenetic tree built using the maximum-likelihood (Figure 1). The 11 diazotrophic bacteria isolated from wheat belonged to the following genera: *Paenibacillus* (3), *Arthrobacter* (3), *Bacillus* (2), *Brevibacillus* (1), *Phyllobacterium* (1) and *Chitinophaga* (1). The 16 diazotrophic bacteria isolated from Chinese pakchoi belonged to the following genera: *Paenibacillus* (4), *Bacillus* (3), *Ensifer* (2), *Rhizobium* (2), *Brevibacillus* (1), *Variovorax* (1), *Flavobacterium* (1), *Pseudomonas* (1) and *Stenotrophomonas* (1). The three diazotrophic bacteria isolated from lotus belonged to the genera *Paenibacillus* (2) and *Bacillus* (1). The dominant diazotrophic bacteria of wheat, Chinese pakchoi and lotus belonged to the genus *Paenibacillus*.

2.2 N fixation

The nitrogenase activities of diazotrophic bacteria compared with *A. chroococcum* that used as control are presented in Table 1. The nitrogenase activities of *Paenibacillus* spp. W-7 (27.36±11.06 nmol C_2H_4/mg protein h), P-4 (30.66±5.82 nmol C_2H_4/mg protein h), P-16 (38.65±30.40 nmol C_2H_4/mg protein h), and L-3 (31.50±12.18 nmol C_2H_4/mg protein h), and *Bacillus* sp. L-2 (28.16±6.26 nmol C_2H_4/mg protein h) were significantly higher ($P<0.01$) than that of *A. chroococcum* ACCC11104 (9.76±0.19 nmol C_2H_4/mg protein h).

2.3 Phosphate solubilization

Clear zones were observed only around the colonies of *Paenibacillus* sp. W-7 with a z/n ratio of 1.10 on organic phosphate medium and 1.14 on inorganic phosphate medium, while no clear zones were observed around the colonies of the other 29 strains on any medium (Table 1).

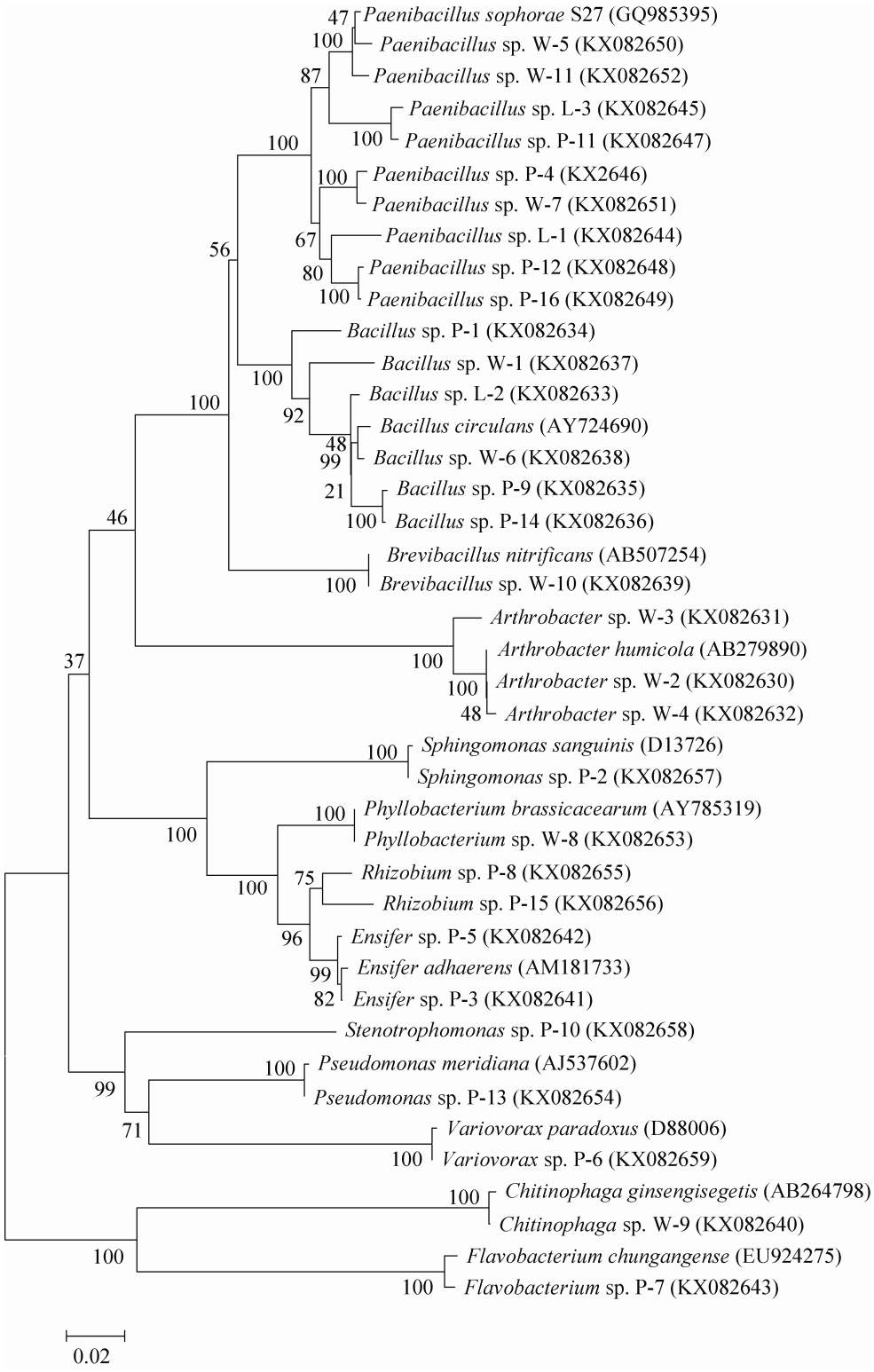

Figure 1. Neighbour-joining trees showing the phylogenetic relationships of 30 diazotrophic bacteria and similar microorganisms based on the 16S rRNA gene sequences. Bar, 0.02 substitutions per nucleotide position. Bootstrap analyses were determined based on 1000 resampling; values>50% were shown at the nodes.

Table 1. Plant growth-promoting traits of 30 diazotrophic bacteria

Isolated rhizobacteria	N-fixing ability/ (nmol/mg protein h)	ACC deaminase activity/ (μmol/mg protein h)	Antifungal index/%			Phosphate solubilization (z/n ratio)	
			1	2	3	Organic	Inorganic
Arthrobacter sp. W-3	10.07±1.43	2.53±0.23a	−	−	−	−	−
Arthrobacter sp. W-4	2.01±0.31	−	−	−	−	−	−
Paenibacillus sp. W−5	12.29±2.07	−	−	−	−	−	−
Bacillus sp. W-6	1.83±0.71	−	−	−	−	−	−
Paenibacillus sp. W-7	27.36±11.06*	−	56.6	58.0	47.2	1.10	1.14
Phyllobacterium sp. W-8	3.40±2.16	−	−	−	−	−	−
Chitinophaga sp. W-9	9.34±5.46	−	−	−	−	−	−
Brevibacillus sp. W-10	6.76±4.13	−	−	−	−	−	−
Paenibacillus sp. W-11	5.90±1.98	−	−	−	−	−	−
Bacillus sp. P-1	1.74±0.61	−	−	−	−	−	−
Sphingomonas sp. P-2	6.837±0.391	−	−	−	−	−	−
Ensifer sp. P-3	3.74±1.45	11.71±0.28bc	−	−	−	−	−
Paenibacillus sp. P-4	30.66±5.82*	−	−	58.4	49.2	−	−
Ensifer sp. P-5	13.53±7.86	−	−	−	−	−	−
Variovorax sp. P-6	1.158±0.313	−	−	−	−	−	−
Flavobacterium sp. P-7	3.588±1.966	−	−	−	−	−	−
Rhizobium sp. P-8	9.58±5.74	−	−	−	−	−	−
Bacillus sp. P-9	9.97±3.85	−	−	−	−	−	−
Stenotrophomonas sp. P-10	3.34±2.22	−	−	−	−	−	−
Paenibacillus sp. P-11	9.34±6.08	−	−	−	−	−	−
Paenibacillus sp. P-12	10.49±8.36	15.64±4.84cd	−	−	−	−	−
Pseudomonas sp. P-13	8.05±0.56	−	−	−	−	−	−
Bacillus sp. P-14	6.04±2.28	−	−	−	−	−	−
Rhizobium sp. P-15	6.05±3.18	−	−	−	−	−	−
Paenibacillus sp. P-16	38.65±30.40*	−	−	−	−	−	−
Paenibacillus sp. L-1	1.47±0.22	−	−	−	−	−	−
Bacillus sp. L-2	28.16±6.26*	1.45±0.47a	−	−	−	−	−
Paenibacillus sp. L-3	31.50±12.18*	−	48.1	47.3	26.4	−	−
ACCC111103	9.76±0.19						

1: *Sclerotinia sclerotiorum*; 2: *Gibberella zeae*; 3: *Verticillium dahlia*. z/n, the ratio of halo zone (z) to colony diameter (n).

2.4 ACC deaminase activity

Among the 30 strains, four strains that scored positive for the production of ACC deaminase belonged to different genera (Table 1). The ACC deaminase activities of *Arthrobacter* sp. W-3 isolated from wheat, *Ensifer* sp. P-3 and *Paenibacillus* sp. P-12 isolated from Chinese pakchoi, and *Bacillus* sp. L-2 isolated from lotus were 2.53±0.23, 11.71±0.28, 15.64±4.84 and 1.45±0.47 μmol/mg protein h, respectively.

2.5 Antagonistic activity

The growth of *S. sclerotiorum*, *G. zeae*, and *V. dahliae* was inhibited as indicated by the presence of clear zones around the colonies of *Paenibacillus* spp. W-7, P-4 and L-3, and *Bacillus* sp. W-1 isolated from wheat (Figure. 2). The antifungal indices of *S. sclerotiorum*, *G. zeae* and *V. dahliae* are presented in Table 1. *Paenibacillus* spp. W-7 and L-3, and *Bacillus* sp. W-1 showed antagonistic activity against *S. sclerotiorum*, *G. zeae* and *V. dahlia,* while *Paenibacillus* sp. P-4 against *G. zeae* and *V. dahlia*.

2.6 Effects of inoculation on the growth of Chinese pakchoi under field conditions

N-fixing bacteria of the genus *Paenibacillus* were the dominant in the rhizosphere of wheat,

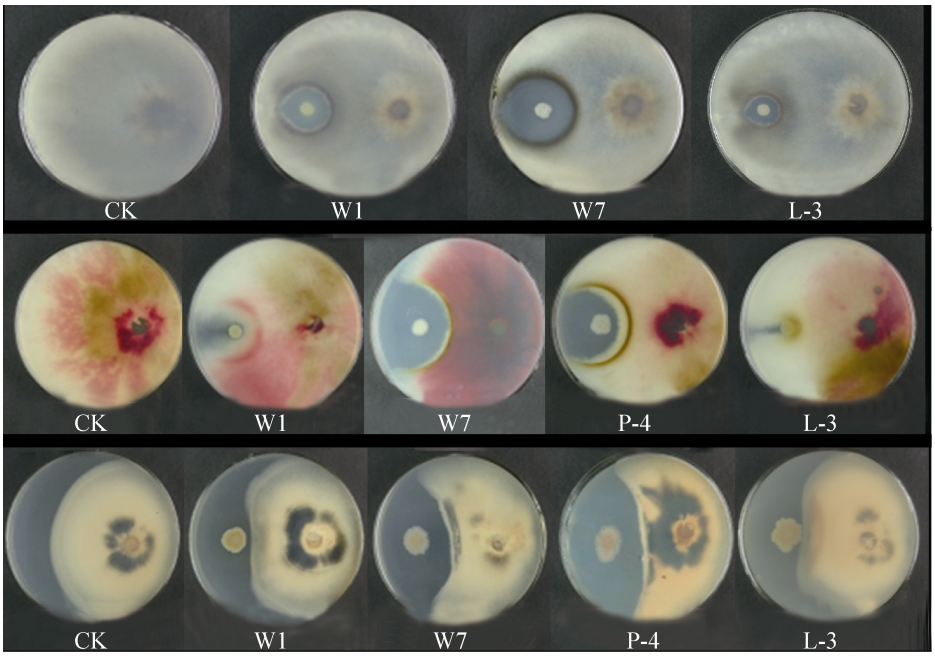

Figure. 2. Inhibition of *Paenibacillus* spp. W-7, P-4, and L-3, and *Bacillus* sp. W-1 to (A) *Sclerotinia sclerotiorum*, (B) *Gibberella zeae*, and (C) *Verticillium dahliae* in plates containing potato dextrose agar. CK, pure culture. W-1 and W-7, isolated from wheat; P-4, isolated from Chinese pakchoi; L-3, isolated from lotus. Plates were incubated for 7 d at 28 °C.

Chinese pakchoi, and lotus. *Paenibacillus* spp. W-7, P-4 and L-3 had various PGP traits. The shoot fresh weight of Chinese pakchoi at 40 d after inoculation with *Paenibacillus* spp. W-7 (7.359±0.011 kg/m^2) or *Paenibacillus* spp. L-3 (7.355±0.013 kg/m^2) was significantly higher (P<0.05) compared with the control (6.035±0.386 kg/m^2), while no significant differences were observed after the inoculation with *Paenibacillus* spp. P-4 (6.292±0.569 kg/m^2).

2.7 Soil enzyme activities

The activities of soil sucrase, urease, phosphatase and catalase are presented in Table 2. Inoculation with *Paenibacillus* sp. W-7 significantly changed (P<0.05) the activity of sucrase, phosphatase and catalase compared with the control, but had no effect on urease; inoculation with *Paenibacillus* sp. P4 had no significant effect on the activity of soil enzymes; while inoculation with *Paenibacillus* sp. L-3 significantly increased (P<0.05) the activity of all the enzymes.

Table 2. Soil enzyme activities after inoculation with *Paenibacillus* spp. W-7, P-4 or L-3

Enzyme (activity unit)	W7	P4	L3	Control
Urease [mg/(10 g·3h)]	8.83±0.31	9.04±0.00	14.85±0.10*	8.94±0.52
Phosphatase [mg/(10 g·d)]	165.82±1.49*	130.97±1.00	164.58±1.99*	144.91±0.75
Sucrase [mg/(g·d)]	15.73±0.05*	13.05±0.02b	25.60±0.04*	14.54±0.04
Catalase [mL/(g·30 min)]	0.30 ± 0.00*	0.25 ± 0.00	0.31 ± 0.01*	0.26 ± 0.00

Control: no inoculation. W-7: isolated from wheat; P-4: isolated from Chinese pakchoi; L-3: isolated from lotus. *: significance at P<0.05. Each value is the mean of three replications.

3 Discussion

The diazotrophic community can respond differentially under different environmental conditions[1,4]. The prominent culturable dizaotorphic bacteria were incompletely similar in the rhizosphere and endophytic of rice, wheat, soybean, maize, sugarcane, and so on[29–30]. In this study, we selected three crop species wheat, Chinese pakchoi and lotus) to identify diazotrophic bacteria and study their PGP traits. Wheat is a major renewable resource for food, animal feed and industrial raw material, and is the most widely grown crop in the world[31]. Chinese pakchoi is one of the most important vegetable crops in East Asia, and lotus is widely planted in China for its high starch, sugar, protein and phenolic contents[20,32]. We isolated 30 diazotrophic bacteria with different morphotypes: 11 from wheat, 16 from Chinese pakchoi and three from lotus. These isolates from wheat and Chinese pakchoi had a variety of morphotypes and belonged to six and nine different genera, respectively, while isolates from lotus had three morphotypes and belonged to two genera. The dominant diazotrophic bacteria of wheat, Chinese pakchoi and lotus belonged to the genus *Paenibacillus*. The genus *Paenibacillus* includes gram-positive, spore-forming, rod-shape and facultative aerobic bacteria found in the soil, water or in association with plants[33]. Previous studies have shown that the genus *Paenibacillus* is prominent in the wheat fields of South Brazil, cold deserts[11].

Paenibacillus spp. had multiple PGP traits such as N fixation, phosphate solubilization, indole-3-acetic acid production, and bio-control ability[11,33]. In this study, we screened three *Paenibacillus* spp. isolated from wheat (W-7), Chinese pakchoi (P-4) and lotus (L-3) for N fixation, phosphate solubilization, ACC-deaminase activity, and antagonistic activity against three plants' pathogenic fungi (*S. sclerotiorum*, *G. zeae* and *V. dahliae*). The nitrogenase activities of *Paenibacillus* spp. W-7 (27.36±11.06 nmol C_2H_4/mg protein h), P-4 (30.66±5.82 nmol C_2H_4/mg protein h), and L-3 (31.50±12.18 nmol C_2H_4/mg protein h) were relatively high, demonstrating the potentially important role of these bacteria in N-fixation. *Paenibacillus* sp. W-7 also contributed to phosphate solubilization, improving the supply of P to the plant. In the soil, a large portion of P is immobilized after application and becomes unavailable to plants[34]. Therefore, P-solubilizing bacteria are important for enhancing the efficiency of applied fertilizers. In addition, it is known that many *Paenibacillus* strains are able to suppress a wide range of plant pathogens[32,35]. In this study, the antagonistic abilities of *Paenibacillus* spp. W-7, P-4 and L-3 against *S. sclerotiorum*, *G. zeae* and *V. dahliae* showed their potentially important role as bio-control agents.

To evaluate the effects of *Paenibacillus* spp. W-7, P-4 and L-3 on plant growth and yield, Chinese pakchoi was used as a model plant due to its small size and rapid life cycle, which allows a time-efficient analysis[36]. In this study, the shoot fresh weight of Chinese pakchoi increased significantly (P<0.05) at 40 d after inoculation with *Paenibacillus* spp. W-7 or L-3, while inoculation with *Paenibacillus* sp. P-4 had no significant effect, this may be related to the changes in soil enzyme activities after inoculation with *Paenibacillus* spp. W-7, P-4 or L-3. These results were in agreement with previous studies, in which *Paenibacillus* spp. increased both plant growth and yield in a pot experiment revealed that inoculation with PGPR containing ACC-deaminase activity significantly increased the root and shoot growth, and fresh and dry weights[10,35,37–38]. It has been reported that certain microorganisms contain an enzyme ACC deaminase that hydrolyzed the ACC into NH_3 and α-ketobutyrate and reduced the inhibitory effects of ethylene and increased the root-shoot length and fresh and dry weights[39], which are consistent with this study, what's more, these isolates can inhibit the growth of two or three plant pathogens of *Sclerotinia sclerotiorum*, *Gibberella zeae* and *Verticillium dahliae*.

Soil enzymes are essential soil components that act as biological catalysts to facilitate different reactions and metabolic processes[40]. Among the different enzymes, urease, sucrase, phosphatase and catalase play important roles in the transformation of different plant nutrients and organic matters within the soil ecosystem[41]. The activities of soil enzymes were used to evaluate the effects of *Paenibacillus* spp. inoculation on soil microbial activities. In this study, *Paenibacillus* sp. W-7 or L-3 isolated from wheat and lotus, respectively, significantly (P<0.05) changed the activities of sucrase, phosphatase and catalase, while *Paenibacillus* sp. P4 isolated from Chinese pakchoi had no significant effect on any enzyme. The changes in soil enzyme activity were consistent with those in the biomass of Chinese pakchoi, indicating that inoculation with *Paenibacillus* sp. W-7 or L-3 could increase soil microbial activities and plant yield. In conclusion, these isolates have the potential to promote plant growth and protect Chinese pakchoi from devastating diseases.

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三株固氮类芽孢杆菌的特点及其对中国青菜的产量和土壤酶活性的影响

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摘要：【目的】本研究分析三株固氮菌 PGPR 性状特征及其对中国青菜产量和土壤酶活的影响。【方法】氮(N)-修复(固氮)细菌被认为是一种能够促进植物生长和增产的施氮方式。在本研究中，我们用无氮培养基分离出了 30 株根际固氮细菌：11 株来自小麦根际，16 株来自中国青菜根际和 3 株来自莲花根际。基于 16S rDNA 序列分析，对小麦、中国青菜和莲花等植物根际中属于类芽孢杆菌属的主要固氮细菌进行研究。【结果】本研究从这 30 株固氮菌中筛选出三株属于类芽孢杆菌属(*Paenibacillus*)的细菌，分别命名为 P-4、W-7 和 L-3，它们的固氮酶活性不但高于对照组(圆褐固氮菌)，而且可以有效抑制两种或三种植物病原菌的生长，即核盘菌(*Sclerotinia sclerotiorum*)、玉蜀黍赤霉(*Gibberella zeae*)和棉花黄萎病菌(*Verticillium dahliae*)。菌株 W-7 还具有溶解难溶磷的能力，中国青菜在接种菌株 W-7 和 L-3 后，其鲜重显著增加，同时改变了田间土壤蔗糖酶、磷酸酶和过氧化氢酶的活性；而接种了菌株 P-4 对植物的生长和酶活性没有显著的影响。【结论】土壤蔗糖酶、磷酸酶和过氧化氢酶活性与中国青菜的生物量呈正相关。同时，菌株 W-7 和 L-3 具有促进植物产量和提高土壤质量的良好潜力。

关键词：固氮细菌，PGPR 特征，类芽孢杆菌，植物促生，土壤酶

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