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## Screening of Plant Growth-Promoting Bacteria from Giant Juncao Roots and Their Growth-Promoting Effects (Postprint)

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### Abstract

This study employed *Pennisetum giganteum* roots as the primary material to screen for plant growth-promoting bacteria and assess their growth-promoting efficacy. Using phosphate solubilization, nitrogen fixation, and IAA production as selection criteria, the growth-promoting capabilities of initially isolated strains were evaluated. Strain YB-07, which demonstrated the most significant growth-promoting effects, was taxonomically classified and identified via morphological observation, physiological and biochemical characterization, and 16S rDNA sequence homology analysis. Following individual assessment of their growth-promoting capacities, eleven highly effective strains were selected for pot experiments. The effects on wheat growth were determined through pot experiments involving both single-strain inoculation and multi-strain co-inoculation. A total of 101 plant growth-promoting bacterial strains were isolated from *P. giganteum* roots. Taxonomic analysis revealed that strain YB-07 belonged to the genus *Rhizobium*, exhibiting phosphate solubilization of  $20.1 \text{ mg} \cdot \text{L}^{-1}$ , IAA production of  $23.7 \text{ mg} \cdot \text{L}^{-1}$ , and concurrent ammonia production. Results from pot experiments demonstrated that multi-strain co-inoculation enhanced wheat plant height, dry weight, fresh weight, and chlorophyll content by 24.49%, 31.84%, 28.06%, and 34.14%, respectively, compared to the control. Single-strain inoculation increased these parameters by 13.54%, 20.45%, 16.84%, and 35.19%, respectively, relative to the control. The selected strains exhibited excellent growth-promoting activities and represent valuable germplasm resources for constructing *P. giganteum* growth-promoting bacterial consortia.

## Full Text

### Screening of Growth-Promoting Bacteria Associated with *Pennisetum sinense* Roots and Their Growth-Promoting Effects

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**Abstract:** This study utilized *Pennisetum sinense* roots as the primary research material to screen for growth-promoting bacteria and evaluate their growth-promoting effects. Using phosphate solubilization, nitrogen fixation, and indole-3-acetic acid (IAA) production as screening criteria, we assessed multiple growth-promoting capabilities of initially selected strains. The most effective strain, YB-07, was classified and identified through morphological observation, physiological and biochemical characterization, and 16S rDNA sequence homology analysis. After measuring their growth-promoting abilities, eleven strains with strong growth-promoting effects were selected for pot experiments. The growth-promoting effects on wheat were evaluated through both single-strain inoculation and multi-strain mixed inoculation pot experiments. A total of 101 growth-promoting bacterial strains were isolated from *P. sinense* roots. Identification results showed that strain YB-07 belongs to the genus *Rhizobium*, with a phosphate solubilization capacity of  $20.1 \text{ mg} \cdot \text{L}^{-1}$ , IAA production of  $23.7 \text{ mg} \cdot \text{L}^{-1}$ , and concurrent ammonia production capability. Pot experiment results demonstrated that multi-strain mixed inoculation increased wheat plant height, dry weight, fresh weight, and chlorophyll content by 24.49%, 31.84%, 28.06%, and 34.14% respectively compared to the control group. Single-strain inoculation increased plant height, dry weight, fresh weight, and chlorophyll content by 13.54%, 20.45%, 16.84%, and 35.19% respectively compared to the control. The selected strains exhibit excellent growth-promoting effects and can provide valuable germplasm resources for further construction of *P. sinense* growth-promoting bacterial consortia.

**Keywords:** *Pennisetum sinense*, growth-promoting bacteria, screening, growth-promoting effect, pot experiment

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## Introduction

*Pennisetum sinense*, a perennial grass belonging to the genus *Pennisetum* in the family Poaceae, is suitable for growth and cultivation in tropical, subtropical, and temperate regions. Introduced to China from South Africa between 2005 and 2007 by the Juncao Research Institute of Fujian Agriculture and Forestry

University, this variety was initially named “giant juncao” due to its exceptionally tall growth habit in its native region. Subsequent identification revealed slight differences from other *Pennisetum* species in China, and it is currently undergoing evaluation for new variety certification. As a typical C4 plant, *P. sinense* features tall plants generally reaching 3–5 m in height, strong stress resistance, high yield, and high contents of crude protein and sugar (Lin et al., 2013). Plant endophytes have occupied an increasingly important position in modern plant microbiology and applied microbiology research, largely attributable to their beneficial biological characteristics. Endophytic bacteria can promote plant growth through various mechanisms, including enhanced phosphorus solubilization (Wang et al., 2015), secretion of indole-3-acetic acid (IAA) (Luo et al., 2011), and nitrogen fixation (Webster et al., 1997). To date, *P. sinense* has been utilized for cultivating various edible and medicinal fungi such as *Lentinus edodes* and *Auricularia auricula* (Wang et al., 2015), yet research on this species has primarily focused on cold resistance, alkali tolerance, salt tolerance, and drought resistance (Lin et al., 2013; Wang et al., 2015).

Since the implementation of the “Grain for Green” project in the Yan’ an region, the livestock farming model has shifted from traditional free-range grazing to intensive pen feeding due to afforestation measures. Consequently, forage shortage has become a bottleneck constraining the development of animal husbandry in Yan’ an. To address this forage deficit, reduce costs, and improve soil conditions for the “Gully and Land Consolidation Project,” researchers from Yan’ an University successfully introduced *P. sinense* from the National Juncao Engineering Research Center to Yan’ an City in 2012. Following several years of trials and demonstrations, the cultivation area has successfully expanded to 30,000 mu, promoting local livestock development. However, current research remains in its infancy, with few reports on *P. sinense* endophytes. In light of this, the present study utilized *P. sinense* as experimental material to screen for endophytic bacterial strains with growth-promoting effects. Through single-strain and multi-strain mixed inoculation pot experiments, we measured the growth-promoting effects of the selected strains to provide germplasm resources for further development of plant growth-promoting bacteria and references for efficient standardized management and demonstration promotion of *P. sinense* in the Yan’ an region.

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## 1 Materials and Methods

### 1.1 Materials

#### 1.1.1 Collection and Preliminary Processing of *P. sinense* Root Samples

Root samples were collected from the *P. sinense* demonstration base at Cuiyuan, Yan’ an University using a five-point sampling method. Eighty healthy *P. sinense* plants with good growth characteristics and no disease symptoms were selected, and their root samples were collected, packaged in sterilized bags, and trans-

ported to the laboratory. Samples were stored at 4 °C and processed within 48 hours.

**1.1.2 Source and Processing of Pot Experiment Soil** Soil for pot experiments was collected from the back hill of Yan' an University, placed in sterilized bags, and transported to the laboratory. The soil was passed through a 5 mm sieve to remove large soil particles, gravel, plant roots, and incompletely decomposed leaves and branches. The processed soil was placed in plastic pots (12 cm diameter) at 1.5 kg per pot for subsequent use.

### 1.1.3 Culture Media

1. **LB medium:** Peptone 10 g, NaCl 10 g, yeast extract 5 g, distilled water 1000 mL, pH 7.0.
2. **PDA medium:** Potato 200 g, glucose 20 g, agar 15-20 g, distilled water 1000 mL, natural pH.
3. **Ashby medium:** Glucose or mannitol 10.0 g,  $\text{KH}_2\text{PO}_4$  0.2 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.2 g, NaCl 0.2 g,  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  0.1 g,  $\text{CaCO}_3$  5.0 g, agar 15-20 g,  $\text{H}_2\text{O}$  1000 mL, pH 7.0.
4. **Phosphate-solubilizing bacteria medium:** NaCl 0.3 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.3 g,  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  0.03 g, KCl 0.3 g,  $(\text{NH}_4)_2\text{SO}_4$  0.5 g,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.03 g,  $\text{Ca}_3(\text{PO}_4)_2$  5.0 g, sucrose 10 g, agar 15-20 g,  $\text{H}_2\text{O}$  1000 mL, pH 7.0-7.5.
5. **NA medium:** Beef extract 3 g, peptone 10 g, NaCl 5 g, sucrose 10 g, agar 15 g, distilled water 1000 mL, pH 7.0.
6. **IAA production medium:**  $\text{KH}_2\text{PO}_4$  0.5 g, yeast extract 1 g, mannitol 10 g,  $\text{MgSO}_4$  0.2 g, NaCl 0.1 g, tryptophan 100 mg, distilled water 1000 mL, pH 6.8-7.2.

## 1.2 Methods

**1.2.1 Surface Sterilization of Samples** *P. sinense* roots were washed with tap water to remove residual soil from the surface, then blotted dry with absorbent paper. The root samples were soaked in 75% ethanol for 2-3 minutes, rinsed three times with sterile  $\text{ddH}_2\text{O}$ , and blotted dry with sterilized absorbent paper. Subsequently, samples were soaked in 0.1%  $\text{HgCl}_2$  solution for 3 minutes, then immediately rinsed six times with sterile  $\text{ddH}_2\text{O}$  to ensure complete removal of disinfectant. The final rinse water was plated onto culture media to verify complete surface sterilization (Wang et al., 2014).

**1.2.2 Screening of Growth-Promoting Bacterial Strains** Surface-sterilized root samples were cut into approximately 1 cm segments and placed in screening media including PDA, IAA production medium, Ashby medium, and phosphate-solubilizing medium. The samples were incubated statically at  $28 \pm 1$  °C for 3-5 days. When visible colonies appeared, they were transferred to corresponding media for purification, followed by colony morphology

description, numbering, and storage at 4 °C. The final rinse water was plated as a control to verify surface sterilization effectiveness.

### 1.3 Determination of Growth-Promoting Abilities

**1.3.1 Spectrophotometric Determination of Indole-3-Acetic Acid Content** Selected strains were inoculated into PDA medium and cultured on a shaker at 180 r/min and 28 °C for 48 hours. Three milliliters of each culture were centrifuged at 10,000 r · min<sup>-1</sup> for 8 minutes. One milliliter of supernatant was mixed with 2 mL of Salkowski' s reagent (10.8 mol · L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> containing 4.5 g FeCl<sub>3</sub>) and reacted in darkness for 30 minutes. The OD<sub>540 nm</sub> was measured, and a standard curve was prepared using standard solutions to calculate IAA concentration. Deionized water served as the control (Tan et al., 2005).

**1.3.2 Molybdenum Blue Colorimetric Determination of Phosphorus Content** Phosphorus standard solutions (50 µg · mL<sup>-1</sup>) were prepared at volumes of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 mL, placed in 20 mL stoppered test tubes, and diluted to 6 mL with distilled water. Two milliliters of 1 mol · L<sup>-1</sup> sulfuric acid and 2 mL of iron-molybdate reagent were added, mixed, and allowed to stand for 15 minutes before measuring absorbance at OD<sub>660 nm</sub>. A standard curve was prepared to calculate phosphorus content in samples (Zhang, 2008).

**1.3.3 Ammonia Production Capacity Determination** Test strains were inoculated into NA medium and cultured on a shaker at 180 r · min<sup>-1</sup> and 28 °C for 48 hours. One hundred microliters of culture were inoculated into 5 mL of Ashby liquid medium with three replicates per treatment, using sterile water as control. After 7 days of incubation at 28 °C, turbidity was compared between test and control groups, with obvious turbidity indicating positive nitrogen fixation activity (Wang, 2010; Zahoor et al., 2017). For ammonia production, test strains were inoculated into peptone broth (10 g · L<sup>-1</sup>) with 0.5 mL Nessler' s reagent added; yellow-brown precipitate indicated positive NH<sub>3</sub> production (Wang et al., 2009).

### 1.4 Strain Identification

**1.4.1 Cultural Characteristic Observation** Strain YB-07 was streaked onto NA medium and incubated at 28 °C for 1-2 days. Colony characteristics were observed and recorded.

**1.4.2 Physiological and Biochemical Identification** Strain YB-07 was subjected to Gram staining, methyl red test, V-P test, starch hydrolysis, indole test, lipid hydrolysis, and gelatin hydrolysis. Methods and preliminary identification followed *Microbiology Experiments* (Cai and Huang, 2010).

**1.4.3 16S rRNA Sequencing and Phylogenetic Analysis** After determining the growth-promoting abilities of initially screened strains, the superior strain YB-07 with multiple growth-promoting effects was selected for identification. The 16S rDNA gene fragment of strain YB-07 was amplified by PCR and sequenced. After obtaining the GenBank accession number, MEGA 6.06 software was used to construct a phylogenetic tree using the Neighbor-Joining method for phylogenetic analysis (Zhang et al., 2017; Zhang, 2017).

## 1.5 Pot Experiments

**1.5.1 Wheat Seed Treatment** Wheat seeds (variety: Shanyou 225) were used for pot experiments. Seeds were pre-soaked in cold water for 4-6 hours, then immersed in 52-55 °C warm water for 1-2 minutes to reach 50 °C, transferred to 56 °C water for 5 minutes, cooled with cold water, and air-dried before sowing.

**1.5.2 Compatibility Testing Among Growth-Promoting Strains** Cross-streaking methods were used to test for antagonistic effects between strains. Selected growth-promoting strains were cross-inoculated pairwise on PDA medium and incubated at 28 °C for 2-3 days. Normal growth at cross-streak intersections indicated no antagonism, permitting preparation of composite bacterial suspensions.

**1.5.3 Preparation of Inoculants** Selected strains were inoculated into LB liquid medium and cultured at 25 °C and 180 r · min<sup>-1</sup> for 48 hours. Cultures were centrifuged at 10,000 r · min<sup>-1</sup> for 10 minutes, supernatants were discarded, and bacterial suspensions were prepared by resuspending pellets in 1 mL physiological saline. According to the design in Table 1 (where mixed bacterial groups included nitrogen fixation + phosphate solubilization, phosphate solubilization + IAA production, and nitrogen fixation + phosphate solubilization + IAA production), 1.2 mL of each suspension ( $2.0 \times 10^9$  cells · mL<sup>-1</sup>) was inoculated into sterilized LB liquid medium and cultured at 28 °C and 160 r · min<sup>-1</sup> for 2-3 days to obtain inoculants.

**1.5.4 Inoculation Treatment** Five uniform, plump wheat seeds were sown per pot at five evenly distributed points, 3-5 cm below the soil surface. Each treatment had three replicates and was cultured under natural light. During germination and weekly thereafter, 2 mL of bacterial suspension ( $2.0 \times 10^9$  cells · mL<sup>-1</sup>) was applied to each treatment, with the control receiving equal volumes of sterile water. Soil moisture was maintained at 60%. After 15 days, wheat seedling height, root length, fresh weight, chlorophyll content, and other indices were measured for statistical analysis.

## 2 Results and Analysis

### 2.1 Strain Screening Results

Using multiple culture media, 101 strains were isolated and purified from P. sinense root samples. After determining their growth-promoting abilities, 11 strains with excellent growth-promoting effects were selected for subsequent experiments. Among these, 8 strains exhibited ammonia production capability, 6 showed phosphate-solubilizing ability, and 3 demonstrated IAA production capacity (Table 2).

### 2.2 Determination of Growth-Promoting Abilities

The growth-promoting indices of 11 selected strains were measured (Table 3). Strain YB-07 possessed all three growth-promoting capabilities: ammonia production, IAA production, and phosphate solubilization. Strain NP-01 showed the highest phosphate solubilization at  $45.1 \text{ mg} \cdot \text{L}^{-1}$ . Among the three IAA-producing strains, production levels were similar, with strain N-01 producing the maximum at  $26.5 \text{ mg} \cdot \text{L}^{-1}$ . Due to its multiple growth-promoting abilities, strain YB-07 was selected as the representative strain for further study.

### 2.3 Compatibility Testing Results Among Growth-Promoting Strains

Cross-streaking tests showed that strains N-02 with NP-01, N-01 with NP-01, and YB-07, YB-08, YB-09 with YB-10 could grow at intersection points, indicating no antagonistic effects among these strains and their suitability for mixed inoculant preparation.

### 2.4 Identification Results of Strain YB-07

**2.4.1 Cultural Characteristics** After 2 days of culture on NA medium, strain YB-07 formed nearly circular, viscous, translucent colonies with neat edges, slightly convex surfaces, and glossy appearance (Figure 1 [Figure 1: see original paper]).

**2.4.2 Physiological and Biochemical Characteristics** Results for strain YB-07 are shown in Table 4.

**2.4.3 16S rRNA Sequencing and Phylogenetic Analysis** NCBI Blast analysis revealed that strain YB-07 showed 99.67% similarity with *Rhizobium* species, confirming its classification in the genus *Rhizobium* (GenBank accession number: KY852244). The phylogenetic tree is shown in Figure 2 [Figure 2: see original paper].

### 2.5 Pot Experiment Results

After 15 days of cultivation, morphological parameters of wheat under different treatments were measured (Table 5). Overall, multi-strain mixed inoculation

showed superior growth-promoting effects compared to single-strain inoculation for plant height, root length, dry weight, and fresh weight, with significant differences between treatments N+P, mixed groups PY, NPY, N+P and single-strain treatments ( $P < 0.05$ ). All inoculation treatments, both single and mixed, significantly outperformed the control group ( $P < 0.05$ ).

For plant height, single-strain inoculation increased values by 8.13%-31.69% compared to control, while multi-strain mixed inoculation increased height by 21.08%-40.46%, all showing significant differences ( $P < 0.05$ ).

For dry weight, single-strain inoculation increased values by 10.20%-41.33%, while mixed inoculation increased dry weight by 28.06%-52.55%, with significant differences ( $P < 0.05$ ).

For fresh weight, single-strain inoculation increased values by 0%-27.27%, while mixed inoculation increased fresh weight by 20.45%-65.91%, with significant differences ( $P < 0.05$ ).

For chlorophyll content, single-strain inoculation increased values by 16.13%-35.19%, while mixed inoculation increased chlorophyll by 17.19%-34.14%, with significant differences ( $P < 0.05$ ).

Overall, both single and multi-strain inoculations promoted wheat growth, with multi-strain mixed inoculation demonstrating significantly superior effects. However, certain single-strain treatments showed higher effectiveness than some mixed treatments. For example, strain N3 increased wheat chlorophyll content by 38.0%, exceeding the 36.2% increase from mixed treatment PY, with similar results for other indices. All growth-promoting indices of N3 were significantly higher than other single-strain treatments ( $P < 0.05$ ). Wheat growth status is shown in Figure 3 [Figure 3: see original paper].

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### 3 Discussion and Conclusion

This study screened 11 strains with strong growth-promoting capabilities from *P. sinense* root samples, including 6 phosphate-solubilizing strains and 3 IAA-producing strains. Pot experiments demonstrated that IAA production and phosphate solubilization capacity significantly promoted wheat plant height, root length, fresh weight, dry weight, and chlorophyll content. Wheat growth indices showed positive correlations with bacterial ammonia production, phosphate solubilization, and IAA production capabilities, indicating that inoculation with growth-promoting bacteria is an effective approach for increasing plant biomass with positive implications for agricultural production.

Research has shown that endophytic bacteria promote plant growth through phosphate solubilization (Paul & Sundararao, 1971), phytohormone secretion (Luo et al., 2011), and enhanced mineral element absorption (Shi, 2005). Numerous studies have reported application effects of growth-promoting

bacteria in production. For instance, Deng et al. (2012) reported that plant growth-promoting rhizobacteria increased maize plant height, root length, and dry weight by 30.14%, 81.10%, and 33.33% respectively compared to controls. Chang et al. (2016) found that wheat seed treatment with strains HN1202, HP1218, and HK1216 significantly increased wheat seedling root length by 12.6%, 20.4%, and 17.5%, and plant height by 11.8%, 13.2%, and 8.8% respectively. Zhu et al. (2007) reported that highly effective phosphate-solubilizing bacteria increased maize seedling height and fresh weight by 33.7% and 97.7% respectively.

In this study, pot experiments with 11 screened strains showed that multi-strain mixed inoculation increased wheat plant height, dry weight, fresh weight, and chlorophyll content by 24.49%, 31.84%, 28.06%, and 34.14% respectively compared to controls. Overall, multi-strain mixed inoculation was significantly superior to single-strain inoculation, with synergistic interactions among strains in the mixed consortium enhancing growth-promoting effects, consistent with findings by Chang et al. (2016) and Zhu (2007). However, certain single-strain treatments outperformed some mixed treatments, possibly due to inhibitory substances produced in mixed cultures that suppressed growth-promoting factors. The factors influencing endophytic bacterial promotion of wheat growth are complex, involving phosphorus solubilization, IAA secretion, nitrogen fixation, nutrient absorption, and secretion of vitamins, gibberellins, and cytokinins, as well as interactions among these factors (Pang et al., 2016).

The strains screened in this study possess multiple growth-promoting abilities (including nitrogen fixation, IAA production, and phosphate solubilization). Pot experiments confirmed the significant growth-promoting effects of strains N-01, N-02, YB-07, and P-03 on wheat. However, further validation is needed regarding colonization ability, growth-promoting capacity, and dominant strains in composite consortia under field conditions. Additionally, since single-strain inoculation was less effective than multi-strain mixed inoculation, further research is required to determine optimal scientific and rational combinations of these strains for better performance in field trials.

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