

Original Research

# Enhanced Plant Growth and Pathogen Inhibition by *Bacillus amyloliquefaciens* Strain YB1701: A Novel PGPR from Extreme Environments

Xiaoqing Yi, Shuting Jia, Yaqi Zuo, Qingxue Guo, Yuzhu Dong, Yueying Li, Lanlan Wang, Xuemei Li\*\*, Lianju Ma\*

College of Life Science, Shenyang Normal University, Shenyang 110034, China

Received: 20 August 2024

Accepted: 13 October 2024

## Abstract

Bacteria that thrive in extreme environmental conditions possess unique abilities to promote plant growth and enhance disease resistance. In this study, a plant growth-promoting rhizobacteria (PGPR) was isolated and purified from the rhizosphere soil collected at the Red Beach of Panjin, designated as strain YB1701. Strain YB1701 was identified as *Bacillus amyloliquefaciens*, a gram-negative *Bacillus*, measuring between 1.6  $\mu\text{m}$  to 3.1  $\mu\text{m}$  in length and 0.9  $\mu\text{m}$  to 1.1  $\mu\text{m}$  in width. The optimal pH for strain YB1701 growth was determined to be between 7.0 and 8.0, and the strain exhibited a strong ability to degrade starch. The indole-3-acetic acid (IAA) content produced by the strain was 137.58  $\mu\text{g}\cdot\text{mL}^{-1}$ , and it showed 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity of 3.03  $\mu\text{mol}\cdot\text{mg}^{-1}\cdot\text{h}^{-1}$ . Strain YB1701 inhibited 16 different plant pathogenic fungi by at least 50%, with more than 80% inhibition observed against *Exserohilum turcicum* and *Sclerotinia sclerotiorum*. Additionally, treatment with strain YB1701 significantly promoted the growth of rice seedlings, increasing shoot height, root length, and shoot and root dry weight by 64.54%, 20.39%, and 71.94%, respectively. Root dry weight alone increased by 2.36%. These findings suggest that *B. amyloliquefaciens* strain YB1701 has potential applications in agriculture and other fields as a novel biocontrol agent and growth promoter. This strain could enrich microbial species resources and provide a basis for the utilization of bacterial resources in various applications.

**Keywords:** PGPR, ACC deaminase activity, IAA content, antagonistic ability, rice seedling growth promotion

## Introduction

PGPR refers to a group of beneficial bacteria that enhance plant growth by improving the absorption and utilization of mineral nutrients. They also help inhibit harmful organisms that closely associate with the rhizosphere [1]. PGPR and their host plants have

\*e-mail: malianju@163.com

\*\*e-mail: lxmls132@163.com

developed a unique, mutually beneficial symbiotic relationship and dynamic balance [2]. PGPR is rich in resources, producing a wide variety of secondary metabolites and active substances [3]. Currently, more than 20 genera of PGPR have been isolated from plant rhizospheres, including *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Azotobacter*, *Klebsiella*, and *Burkholderia* [4]. The proportion of beneficial bacteria that can directly or indirectly promote plant growth or enhance stress adaptation is approximately 2% to 5% [5]. The volatile organic compounds secreted by PGPR have been found to promote root growth and enhance the synthesis of monoterpenoids in peppermint [6]. *B. amyloliquefaciens* has efficient phosphorus and potassium solubilization capabilities, and secretes siderophores and IAA, which have a significant role in promoting plant growth and development through colonization in plants, improving photosynthesis ability, and antioxidant enzyme activity. The symbiosis between PGPR and host plants not only promotes plant growth but also provides a certain level of biocontrol. PGPR inhibits pathogens and prevents plant diseases by antagonizing pathogens or producing some secondary metabolites. Literature has shown that *B. subtilis* exhibits the strongest antagonistic effect in vitro, with their antimicrobial activity primarily attributed to polypeptides [7]. *B. amyloliquefaciens*, closely related to *B. subtilis*, produces a range of metabolites that inhibit fungal and bacterial activity during its growth [8]. The strain *B. amyloliquefaciens* NCPSJ7, isolated from the rhizosphere soil of ginger and applied as a wettable powder, demonstrated biocontrol efficiency comparable to that of procymidone at a concentration of  $8.1 \times 10^7$  cfu·mL<sup>-1</sup> [9].

*Suaeda salsa* (L.) Pall. is a salt-tolerant plant that thrives in saline and alkaline environments such as beaches, river valleys, roadsides, and fields. Belonging to the genus *Suaeda* in the Amaranthaceae family, it is widely distributed across Inner Mongolia, Liaoning, and other provinces in China. Microorganisms with strong stress resistance can survive under extreme conditions such as low or high temperatures, high alkalinity, high salinity, and hypoxia. Exploring their characteristics can help develop microbial resources that are resilient, adaptable, environmentally friendly, and secure. This approach can also enhance yield and improve crop quality [10]. It is noteworthy that the rhizosphere of *Suaeda salsa* is rich in microorganisms that aid in nutrient absorption and promote plant growth. From the Red Beach in Panjin, 80 strains of saline-alkaliphilic bacteria have been isolated and purified, all of which have demonstrated capabilities in biocontrol and growth promotion [11].

Rice (*Oryza sativa* L.) is a crucial global food crop, feeding over half of the world's population. Various factors impact rice production and quality, but the utilization rate of microbial fertilizers in rice cultivation remains low. Research indicates that PGPR can enhance rice germination, seedling growth, and, to some extent, both the quantity and quality of the crop [12].

Additionally, PGPR is known to promote overall plant growth, stimulate resistance mechanisms, and improve plant vigor [13]. PGPR has indeed become a major focus in soil microbiology research as well as in ecology and microbial fertilizer studies. Despite this interest, there is a limited number of bacterial strains specifically identified as promoting rice growth, and there are relatively few reports detailing the properties of rice growth-promoting bacteria. More research in this area could uncover new bacterial strains and mechanisms that enhance rice cultivation.

Microbial fertilizers play a crucial role in agricultural production. They are regarded as an efficient and environmentally friendly alternative to chemical pesticides and fertilizers, helping to reduce their usage [14]. Microbial fertilizer is an efficient, low-cost, and pollution-free biofertilizer produced through the artificial cultivation and quantitative production of one or more beneficial microorganisms found in the environment. Bacteria, in particular, form the foundation for microbial fertilizer production [15]. To investigate the biocontrol and growth-promoting effects of *Suaeda salsa* PGPR, it is important to develop new biocontrol and growth promotion resources and apply them across agriculture, industry, and other fields [16]. Therefore, this study investigated the optimal pH and growth curve of *B. amyloliquefaciens* YB1701, isolated from the rhizosphere of *Suaeda salsa*. It also evaluated the strain's ACC deaminase activity, IAA content, and antagonistic effects against various pathogenic fungi. Additionally, the study examined the growth-promoting effects of strain YB1701 on rice with the aim of developing an effective microbial fertilizer to enhance rice growth. The basic activity of strain YB1701 was assessed to provide a theoretical foundation for future field experiments and the production of microbial agents.

## Materials and Methods

### Isolation of PGPR

*Suaeda salsa* was collected from the Red Beach. The entire plant, along with the soil in its rhizosphere, was uprooted and carefully handled to prevent root damage by covering it with a plastic bag. The plant was then placed in a sealed bag and transported to the lab. A fresh sample of the rhizosphere-containing soil (10 g) collected was suspended in 90 mL of sterile saline with glass beads. The mixture was oscillated at 120 rpm at room temperature for 30 minutes to create a soil suspension. Following this, the soil suspension was serially diluted ( $10^{-3}$  to  $10^{-5}$ ) with sterile saline. From each dilution, 100  $\mu$ L of the soil suspension was spread on agar plates and incubated at 37°C for 24 h. Single colonies were then selected for isolation and purification.

### Identification of PGPR

The purified strain was inoculated on agar plates and incubated at 37°C for 1-2 d. Regular observations were made to record characteristics such as colony size, color, transparency, surface morphology, edge morphology, and growth rate. Additionally, the morphology of strain YB1701 was examined using the direct preparation method under an optical microscope. This included single staining, Gram staining, and endospore staining to determine its structural and morphological features.

The biochemical characteristics of the strain were investigated by a carbohydrate fermentation test, H<sub>2</sub>S test, VP test, MR test, gelatin liquefaction test, citrate test, litmus milk test, peptone test, and glucose gas production test. The biochemical tests of the strain were determined by referring to Bergey's manual [17].

Identification of the strain was performed by 16S rDNA sequencing. The total genomic DNA was extracted using the Bacterial DNA Isolation Kit according to the manufacturer's instructions. The total genomic DNA was extracted by the colony method and used as the template for gene amplification with 16S rDNA universal primers 27F: (5'AGAGTTTGATCCTGGCTCAG-3') and 1492r: (5'-TACGGTTACCTTGTTACGACTT3'), was amplified by PCR. The reaction system was as follows: 2 × PremixTaq (5.0 U/μL) 25 μL, the two primers were (10 μM) 1 μL, respectively, the amplification template was 2 μL, and the system was supplemented with ddH<sub>2</sub>O to 50 μL. Reaction procedure: 95°C for 1 min, 35 cycles of 95°C for 1 min, 55°C for 1 min, 72°C for 2 min, and finally extension at 72°C for 10 min, and storage at 4°C. The PCR product was amplified and purified and then tested for sequencing. The DNA sequencing was completed by Sangon Biotech (Shanghai, China). After the sequencing was completed, the returned sequences were compared by the EzBioCloud (<https://www.ezbiocloud.net/>) database, and the sequences were analyzed for homology and similarity. The sequences with high homology in the gene bank were selected to construct a phylogenetic tree with MEGA 11.0 software [18].

### Determination of Starch-Degrading Ability

Strain YB1701, during its logarithmic growth stage, was inoculated on agar medium plates containing starch in a three-point pattern. After 2 d of incubation, iodine tincture was added to the plates. After 10 min, the presence of a clear zone around the colonies was observed to indicate starch hydrolysis.

### Analysis of Growth Characteristics

Beef extract peptone liquid medium was used as the base medium, with its pH adjusted to range from 4.0 to 11.0. Three replicates were prepared for each pH level. Strain YB1701 was inoculated into each liquid medium at a 1.0% (v/v) concentration. The cultures were

incubated at 37°C with shaking at 180 rpm for 24 h. The optical density at 600 nm (OD<sub>600</sub>) was then measured using an ultraviolet-visible (UV-Vis) spectrophotometer to assess bacterial growth.

Strain YB1701 (1.0% V/V) was inoculated in beef extract peptone liquid medium and incubated at pH 7.5, 37°C, 180 rpm. Samples were taken at 2 h intervals, and the OD<sub>600</sub> was measured to assess bacterial growth. Three parallel replicates were set for each time point. Bacterial growth curves were plotted with OD<sub>600</sub> values as the vertical axis and time as the horizontal axis.

### Determination of IAA Content

Determination of IAA content of strain YB1701 by colorimetric method [19]. The strains were inoculated in a DF medium for overnight and then transferred to a DF medium containing 0.1% L-tryptophan. The strain YB1701 was cultured at 28°C for 7 days and then centrifuged at 8000 rpm for 10 min. One mL suspension was mixed into a 2 mL FeCl<sub>3</sub>-H<sub>2</sub>SO<sub>4</sub> solution and left in darkness for 45 min. The absorbance of the sample at 450 nm was measured to calculate the IAA content according to the standard curve.

### Determination of ACC Deaminase Activity

The strain YB1701 was added to 5 mL beef extract peptone liquid medium and cultured at 28°C for 24 h. 0.5 mL of strain YB1701 solution was added to 60 mL of Beef Extract Peptone Liquid Medium for 36 h. Bacteria were collected for use by centrifugation at 4°C and 8000 rpm for 10 min, and the supernatant was discarded. ACC deaminase activity was determined according to the method of Saravanakumar and Samiyappan with slight modification to assess the ability of the strain to utilize ACC as nitrogen [20].

### Determination of Inhibitory Rate Against Plant Pathogenic Fungi

The three-point confrontation method was used to determine the inhibitory rate of the antagonistic pathogen fungi of the strain YB1701. Plant pathogenic fungi were inoculated at three points on the potato dextrose agar plate, and filter paper of the fermentation liquid of strain YB1701 was inoculated at the middle position. An agar plate containing only the pathogenic fungi, without the strain YB1701, was used as the control. Each group was repeated three times and cultured in an incubator at 28°C for 4-5 d. The diameter of each treatment colony was measured, the average value was obtained, and the antibacterial rate (%) was calculated.

### Growth Promotion Experiment for Rice Seedlings

Rice seeds of similar size and full grain were selected, soaked at 28°C for 24 h, and then germination was promoted at 30°C for 24 h. The germinated seeds

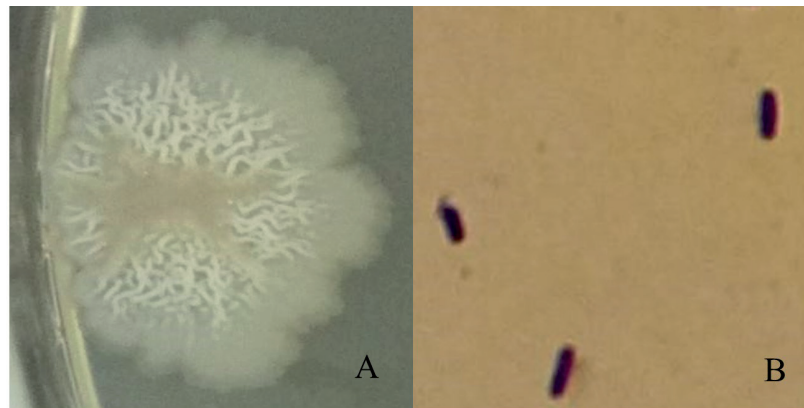


Fig. 1. Morphological observation of strain YB1701, the colony morphology of strain YB1701 (A) and the 100 x microscopic morphology of strain YB1701 (B).

Table 1. Physiological and biochemical characteristics of strain YB1701

Physiological and biochemical characteristics	Results	Physiological and biochemical characteristics	Results
D-glucose	+	Litmus milk test	+
D-Sucrose	+	Peptone test	+
D-Maltose	+	Glucose gas production	+
D-Lactose	-	D-Mannitol	+
MR test	+	H <sub>2</sub> S test	-
Gelatin liquefaction test	+	VP test	-
Citrate test	-		

Note: ("+" means positive, "-" means negative).

were seeded on 750 mL plastic cups filled with Hoagland nutrient solution and covered with gauze mesh, and each group was repeated three times. It was cultured in a light incubator with diurnal and nocturnal temperatures of 28°C/22°C, photoperiod of 12 h/12 h, air humidity of 80%, and light intensity of 300  $\mu\text{M}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . When the seedlings reached two leaves, they were treated with 3% beef extract peptone medium as control and 3% strain YB1701 fermentation solution as treatment. Shoot height, root length, and shoot and root dry weight were measured on the 10th, 12th, 14th, and 16th days after treatment.

### Statistical Analysis

The obtained data were checked and analyzed using SPSS 21.0 software.

## Results

### Isolation and Identification of Strain YB1701

The colony of strain YB1701 is milky white and opaque, with a wrinkled surface and irregular edges, making it easy to pick (Fig. 1A). The bacteria are short

rod-shaped, Gram-positive, and form spores. They measure approximately 1.6-3.1  $\mu\text{m}$  in length and 0.9-1.1  $\mu\text{m}$  in width (Fig. 1B).

D-glucose, D-sucrose, D-maltose, D-mannitol, MR Test, gelatin liquefaction test, litmus milk test, peptone test, and glucose gas production test were positive. However, the D-lactose, H<sub>2</sub>S test, VP test, and citrate test were negative (Table 1).

The 16S rDNA gene sequence was determined and compared in the nucleic acid database after amplification to determine the taxonomic status of the strain and download the corresponding sequence. The results showed that the 16S rDNA sequence of strain YB1701 was the most similar to that of a *B. amyloliquefaciens* strain in the genus *Bacillus*. MEGA11.0 was used to construct a phylogenetic tree for the 16S rDNA sequence of strain YB1701 and the sequences of related strains (Fig. 2). Strain YB1701 belongs to the same branch as other strains. The similarity between strain YB1701 and *B. amyloliquefaciens* was high, indicating that they had higher homology and a closer developmental relationship. According to the morphological observation and biochemical results, the strain YB1701 was classified as *Firmicutes*, *Bacillus* classes, *Bacillus* orders, *Bacillus* families, *Bacillus* genera, and *B. amyloliquefaciens*.



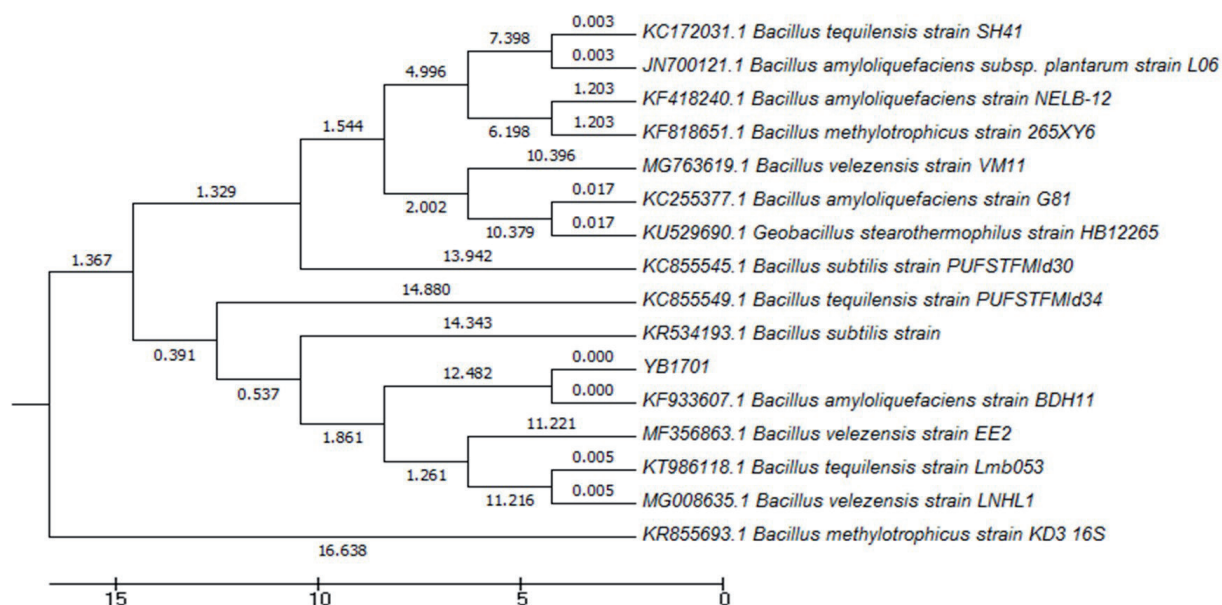


Fig. 2. Phylogenetic tree of obtained strains and their closest sequences in GenBank. (the number before strain name or species name is the entry number)

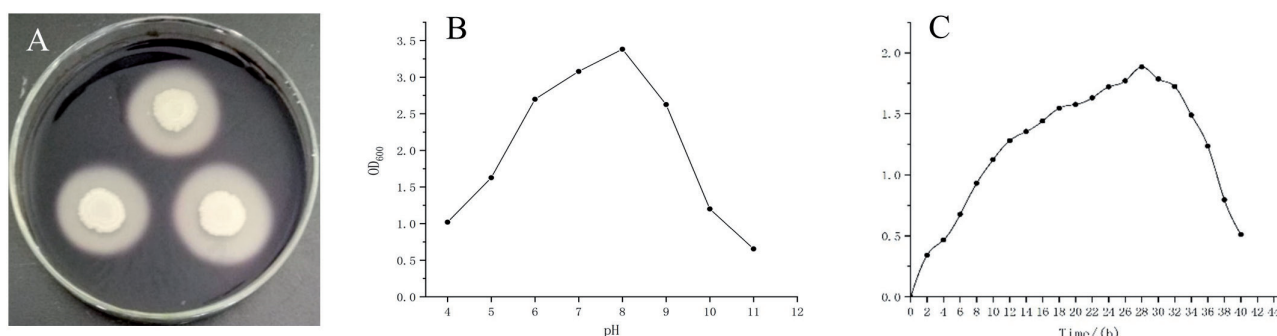


Fig. 3. Ability to degrade starch (A), optimum pH (B) and growth curve (C) of strain YB1701

### Starch-Degrading Capacity

The presence of an obvious transparent zone around the colonies on the medium indicated that strain YB1701 possessed strong starch decomposition ability (Fig. 3A).

### Growth Characteristics Analysis

Strain YB1701 exhibited gradual growth increases when the pH was between 4.0 and 8.0, with the maximum growth observed at pH 8.0. Growth began to decline when the pH exceeded 8.0, although the strain was still able to grow at pH 11.0, indicating its capability to survive in alkaline environments. The optimal pH range for strain YB1701 was found to be between 7.0 and 8.0 (Fig. 3B).

Before 4 h, the  $OD_{600}$  value of strain YB1701 remained unchanged, and the  $OD_{600}$  value of strain YB1701 increased with the increase of time within 4-16 h, and the  $OD_{600}$  value reached the maximum at 28 h.

After 32 h, strain YB1701 began to enter the decline period (Fig. 3C).

### IAA Production Capacity

The IAA content of strain YB1701 was measured at  $137.58 \mu\text{g}\cdot\text{mL}^{-1}$ , indicating that the strain was capable of producing IAA.

### ACC Deaminase Production Activity

The ACC deaminase activity of strain YB1701 was measured at  $3.03 \mu\text{mol}\cdot\text{mg}^{-1}\cdot\text{h}^{-1}$ . This indicated that strain YB1701 possessed ACC deaminase activity, which could enhance the plant's resistance to drought, high temperatures, diseases, insect infestations, and heavy metal pollution.

Table 2. Inhibition rate of strain YB1701 on pathogenic fungi.

Pathogenic fungus	Inhibition rate (%)	Pathogenic fungus	Inhibition rate (%)
<i>Acremonium strictum</i>	74.8	<i>Fusarium graminearum</i>	61.5
<i>Exserohilum turcicum</i>	85.3	<i>Fusarium culmorum</i>	65.7
<i>Bipolaris sorghicola</i>	66.9	<i>Fruentum subglutinans</i>	71
<i>Bipolaris zeicola</i>	73.6	<i>Botrytis cinerea Pers.</i>	72.7
<i>Pyricularia grisea</i>	79.3	<i>Pythium aphanidermatum</i>	72.1
<i>Phytophthora capsici Leonian</i>	56.9	<i>Sclerotinia sclerotiorum</i>	82.5
<i>Trichothecium roseum</i>	78.8	<i>Pythium graminicola Subra</i>	59.6
<i>Fusarium oxysporum f. sp. melonis</i>	65.7	<i>Curvularia lunata</i>	64.4

### Inhibitory Rate Effect on Plant Pathogenic Fungi

The inhibition rate of strain YB1701 to 16 plant pathogenic fungi tested was  $\geq 50\%$ . In particular, the inhibition rate for the tested *Exserohilum turcicum* and *Sclerotinia sclerotiorum* was  $\geq 80\%$  (Table 2; Fig. 4).

### Seedling Growth and Biomass Accumulation of Rice Seedlings

The hydroponic experiments revealed that strain YB1701 significantly improved rice seedling growth, biomass, and main agronomic traits compared to the control (Fig. 5A; Fig. 5B).

Strain YB1701 demonstrated notable effects on rice seedling growth. The shoot height significantly ( $P < 0.05$ ) increased by 41.36% and 64.54% on the 10th and 16th days after treatment, respectively, compared to the control (Fig. 5C); strain YB1701 also promoted root length, which markedly ( $P < 0.05$ ) increased by 17.44% and 20.39% on the 14th and 16th days, respectively, compared to the control, with significant differences observed ( $P < 0.05$ ) (Fig. 5D). The shoot dry weight in the treatment group increased by 13.95% and 71.94% on the 10th and 16th days, respectively, compared to the control (Fig. 5E). The difference in root dry weight between the treatment and control groups decreased over time, and there was no significant difference on the 16th day. However, root dry weight increased after the 16th day (Fig. 5F). These results suggest that strain YB1701 effectively enhances shoot and root growth and contributes to increased biomass in rice seedlings.

### Discussion

It has been reported that over 80% of plant root microorganisms can synthesize IAA. IAA is a crucial hormone for plant growth, playing an essential role in various developmental stages. It is involved in processes such as cell elongation, cell division, tissue differentiation, apical dominance, lateral root, and root

hair formation, as well as regulating taproot length. IAA promotes root development and seedling growth, contributing significantly to overall plant growth [21]. Interestingly, PGPR can secrete IAA to promote the growth and development of host plants. For example, IAA-producing PGPR was isolated from the rhizosphere soil of volcanic plants in Mexico [22]. The strain Dabac TI-8, isolated from rhizosphere soil samples in Antarctica, produced IAA at lower temperatures at concentrations sufficient to affect plant growth [23]. Twenty-six PGPR were isolated from the rhizosphere soil of wild Musa in a coal mining area, and 7 had good growth-promoting ability [24]. Idris validated the growth-promoting effects of the PGPR strain *B. amyloliquefaciens* FZB42, which was isolated from plant rhizosphere soil and has been commercialized. The effects on plant growth were found to be equivalent to IAA concentrations of  $10^{-6}$  to  $10^{-7}$  mol·L<sup>-1</sup> IAA [25]. Twenty-four PGPR were isolated from the rhizosphere and roots of surviving snowbushes, all capable of producing IAA. Among them, 17 strains produced IAA at concentrations exceeding 10 µg·mL<sup>-1</sup> [26]. In the present study, strain YB1701 was found to secrete 137.58 µg·mL<sup>-1</sup> of IAA, suggesting that it has the potential to regulate plant growth and development through IAA secretion. ACC deaminase, an enzyme unique to many PGPRs, can break down the precursor of ethylene synthesis, thereby reducing ethylene levels in plants. Studies have shown that ACC deaminase activity must exceed 20 nmol·mg<sup>-1</sup>·h<sup>-1</sup> to have a noticeable growth-promoting effect on plants. This reduction in ethylene helps alleviate stress conditions and enhances plant growth [27]. Ten PGPR strains with ACC deaminase activity were isolated from the rhizosphere of the semi-desert weeds [28]. Three new strains were isolated from the rhizosphere of crops affected by salinization along coastal India, with ACC deaminase activities ranging from  $1.87 \pm 0.27$  to  $2.88 \pm 0.71$  µmol·mg<sup>-1</sup>·h<sup>-1</sup> [29]. The isolation of a strain from the rhizosphere of *Avena fatua* L., which can utilize ACC as its sole nitrogen source, exhibited ACC deaminase activity  $0.576 \pm 0.055$  µmol·mg<sup>-1</sup>·h<sup>-1</sup> [30]. ACC deaminase activity was

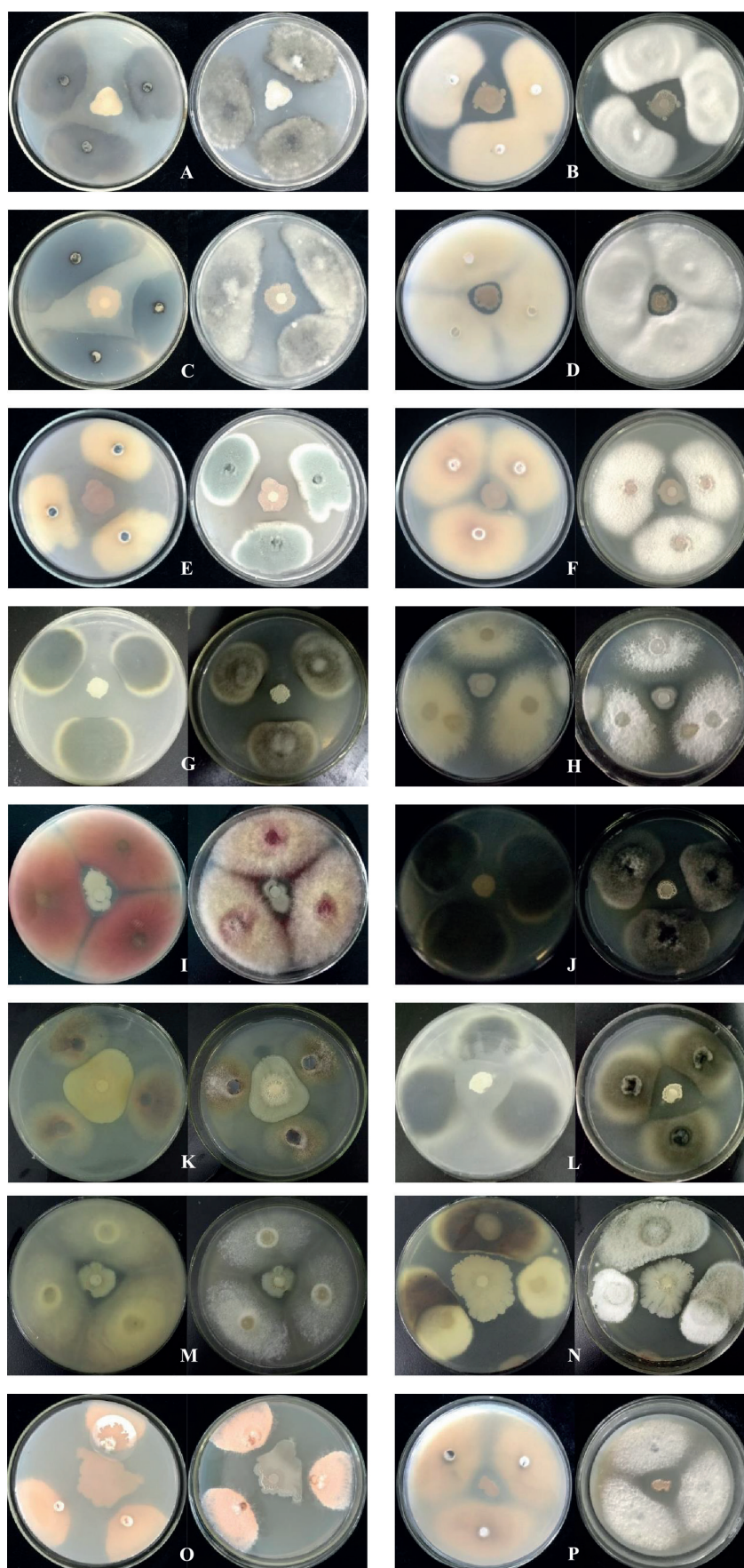


Fig. 4. The plate faceoff method was used to determine the pairs of strain YB1701 against *Pyricularia grisea* (A), *Frumentum subglutinans* (B), *Bipolaris zeicola* (C), *Phytophthora capsici* Leonian (D), *Botrytis cinerea* Pers. (E), *Pythium aphanidermatum* (F), *Bipolaris sorghicola* (G), *Fusarium culmorum* (H), *Fusarium graminearum* (I), *Curvularia lunata* (J), *Sclerotinia sclerotiorum* (K), *Exserohilum turcicum* (L), *Pythium gramincola* Subra (M), *Acremonium strictum* (N), *Trichothecium roseum* (O), *Fusarium oxysporum* f. sp. *Melonis* (P).



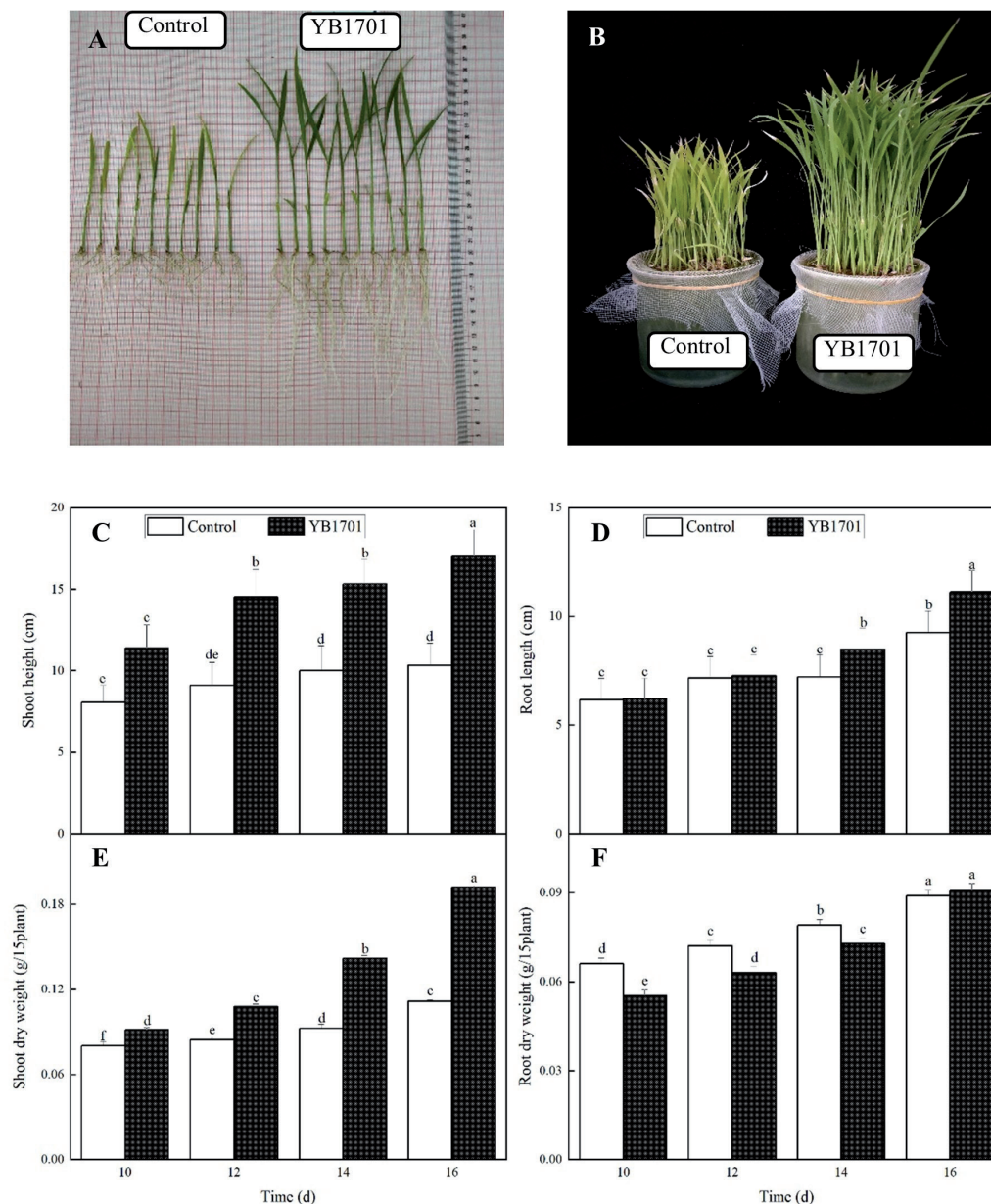


Fig. 5. Effect of strain YB1701 treatment on growth parameters of rice seedlings on the 16th day after treatment (A, B). Effect of strain YB1701 treatment on growth parameters of rice seedlings at 10, 12, 14 and 16 days. Shoot height (C), root length (D), shoot dry weight (E) and root dry weight (F) are means  $\pm$  SD of independent experiments. Different letters indicate significant differences at  $P < 0.05$ .

detected in all PGPR strains isolated from pineapple rhizosphere, particularly *Brevundimonas* sp. CHTJ 5H consumed 88% ACC over 24 h, with the highest deaminase activity of  $13.37 \mu\text{mol}\cdot\text{mg}^{-1}\cdot\text{h}^{-1}$  [31]. In a study involving the isolation of 96 PGPR strains from the rhizosphere of pearl millet, 28 strains exhibited ACC deaminase activity. Among them, *B. amyloliquefaciens* MMR04 showed the highest ACC deaminase activity, reaching  $2.19 \mu\text{mol}\cdot\text{mg}^{-1}\cdot\text{h}^{-1}$  [32]. In the current study, the ACC deaminase activity of strain YB1701 reached  $3.03 \mu\text{mol}\cdot\text{mg}^{-1}\cdot\text{h}^{-1}$ , suggesting a strong potential for promoting plant growth. PGPR like YB1701 colonizes the roots of plants and produces beneficial metabolites that enhance nutrient uptake and improve nutrient use efficiency. Inoculation of the PGPR 5B1 in *Arabidopsis*

*Thaliana* altered root growth and branching patterns, thereby promoting root development [33]. PGPR *Burkholderia* was screened from acid sulfate soil and an experimental farm of the nuclear agriculture research institute and inoculated to rice, which promoted rice growth. All measured plant growth parameters were improved over the untreated control, showing that the application of these two bacteria could reduce the primary fertilizer in rice production by 50% [34]. Field application of 7 selected PGPR strains led to a significant increase in the productivity of *Crocus sativus* L. (saffron). This included improvements in various growth parameters such as the number of germinated bulbs, plant height, stigma length, and the fresh and dry weight of the stigma, which is the most valuable



part of the saffron plant [35]. The strain SM33, isolated from the rhizosphere of *Minthostachys verticillata*, demonstrated a positive effect on the seedlings of *M. verticillata* [36]. The growth-promoting effects of PGPR strains *B. amyloliquefaciens* BS006 and *Pseudomonas* PS006 on banana growth parameters, such as plant height, leaf number, leaf area, root and shoot dry weight, and fresh weight, were found to outperform those of 100% chemical fertilizer [37]. In the current study, strain YB1701 significantly promoted the growth of rice seedlings, increasing shoot height, root length, and shoot and root dry weight by 64.54%, 20.39%, 71.94%, and 2.36%, respectively, which further proved the growth-promoting effect of strain YB1701 on rice seedlings (Fig. 5).

In addition, PGPR has indeed garnered significant attention for its biocontrol capabilities. These bacteria can suppress plant diseases through various mechanisms, such as producing antimicrobial compounds, outcompeting pathogens for resources, and inducing systemic resistance in plants. Five strains of PGPR were isolated from the rhizosphere of forest plants. These strains exhibited strong antagonistic effects against *Colletotrichum truncatum* in chili peppers, with spore germination inhibition rates approaching 100% [38]. Three strains of PGPR isolated from the rhizosphere of *Arabidopsis thaliana* demonstrated over 65% inhibition of *Phytophthora capsici*, *Phytophthora citricola*, *Phytophthora palmivora*, and *Phytophthora cinnamomi* [39]. A *Bacillus* was isolated from the grain rhizosphere and screened for microorganisms resistant to plant pathogenicity, foodborne pathogenicity, and spoilage in vitro [40]. *B. amyloliquefaciens* exhibits inherent high resistance to stress and holds significant potential for biological control and the sustainable development of agriculture. *B. amyloliquefaciens* is a bacterium with broad-spectrum antifungal activity, capable of producing a large number of secondary metabolites and various antifungal substances. *B. amyloliquefaciens* PPCB004 had antifungal activity against 7 different postharvest fungal pathogens [41]. *B. amyloliquefaciens* Bc2 was isolated from the rhizosphere soil of strawberries, which had good antipathogenic activity against strawberry anthracnose [42]. Zhao isolated a strain of *B. amyloliquefaciens* and used it to produce the antifungal lipopeptide Iturillin A, which exhibited strong activity against *Fusarium oxysporum* [43]. Lu isolated a strain of *B. amyloliquefaciens* TB2, which had a good inhibitory effects on *Aspergillus flavus* and *Fusarium oxysporum* [44]. The lipopeptides surfactin, iturin, and fengycin were isolated from *B. amyloliquefaciens* with inhibitory against *C. difficile* growth and viability [45]. *B. amyloliquefaciens* SC-B15, isolated from the rhizosphere soil of chestnut cultivation, demonstrated broad-spectrum antifungal activity with an inhibition rate exceeding 64% against various mycotoxins and fungi [46]. *B. amyloliquefaciens* ZK-9 isolated from wheat rhizosphere soil exhibited inhibitory effects on various pathogenic fungi, including 82.14% inhibition

against *Fusarium* crown rot and 71.76% inhibition against *Fusarium* head blight [47]. In the present study, strain YB1701 exhibited a significant inhibitory effect on 16 test pathogens. Notably, the inhibition rates against *Exserohilum turcicum* and *Sclerotinia sclerotiorum* were  $\geq 80\%$ , demonstrating that strain YB1701 has broad-spectrum resistance and inhibition capabilities against a range of pathogenic fungi (Table 2; Fig. 4).

The proportion of microbial pesticides among existing pesticide varieties has significantly increased compared to the past. Additionally, various biocontrol *Bacillus* species have been processed and widely utilized as microbial pesticides. The use of biological control agents like *B. amyloliquefaciens* addresses some of the shortcomings associated with air pollution, environmental contamination, and harm to human health. It serves as an effective alternative to chemical control methods and synthetic fertilizers [48, 49]. In the future, strain YB1701 is expected to be developed into a microbial fertilizer for agricultural production. Since different PGPRs exhibit varying growth-promoting effects on different plant species, their selection should be tailored to the specific plant species and growth environment. Researchers and experts must continue to explore and refine their approaches to achieve more efficient and safer agricultural production methods [50].

## Conclusions

In this study, strain YB1701 was isolated from the rhizosphere soil of *Suaeda salsa*. It exhibits starch decomposition abilities, produces IAA and ACC deaminase significantly inhibits a variety of pathogens, and can significantly promote the growth of rice seedlings. Therefore, strain YB1701 has the potential for pathogen resistance and growth promotion, offering opportunities for developing new biocontrol and growth-promoting resources. It could be applied in agriculture, industry, and other fields, thereby enriching microbial strain resources and providing a foundation for their utilization.

## Acknowledgements

This work was supported by the Special fund for basic scientific research business expenses of undergraduate universities in Liaoning Province (LJ202410166008), Undergraduate innovation and entrepreneurship training program of Liaoning Province (S202410166053), PhD Initiation Fund of Shenyang Normal University (BS202318).

## Conflicts of Interest

The authors declare no conflict of interest.

## References

- FENG L.C., LI Q., ZHOU D.Q., JIA M.Y., LIU Z.Z., HOU Z.Q., REN Q.J., JI S.D., SANG S.F., LU S.P., YU J.P.B. *Subtilis* CNBG-PGPR-1 induces methionine to regulate ethylene pathway and ROS scavenging for improving salt tolerance of tomato. *Plant Journal*, **117** (1), 193, **2024**.
- NGALIMAT M.S., HATA E.M., ZULPERI D., ISMAIL S.I., ISMAIL M.R., MOHD ZAINUDIN N.A.I., SAIDI N.B., YUSOF M.T. Plant growth-promoting bacteria as an emerging tool to manage bacterial rice pathogens. *Microorganisms*, **9** (4), 682, **2021**.
- SUN L., CHENG L.F.Y., MA Y.H., LEI P., WANG R., GU Y.A., LI S., ZHANG F.H., XU H. Exopolysaccharides from *Pantoea alhagi* NX-11 specifically improve its root colonization and rice salt resistance. *International Journal of Biological Macromolecules*, **209** (Pt A), 396, **2022**.
- BHAT B.A., TARIQ L., NISSAR S., ISLAM S.T., ISLAM S.U., MANGRAL Z., ILYAS N., SAYYED R.Z., MUTHUSAMY G., KIM W., DAR T.U.H. The role of plant-associated rhizobacteria in plant growth, biocontrol and abiotic stress management. *Journal of Applied Microbiology*, **133** (5), 2717, **2022**.
- ZHOU Y.F., BAI Y.S., YUE T., LI Q.W., HUANG Y.N., JIANG W., HE C., WANG J.B. Research progress on the growth-promoting characteristics of plant growth-promoting rhizobacteria. *Microbiology China*, **50** (02), 644, **2023**.
- SANTORO M.V., ZYGADLO J., GIORDANO W., ET A.L. Volatile organic compounds from rhizobacteria increase biosynthesis of essential oils and growth parameters in peppermint (*Mentha piperita*). *Plant Physiology and Biochemistry*, **49** (10), 117, **2011**.
- QIAO H.T., ZHANG B., CHEN X.N., SU L.J., JIAO C., CHEN S., FAN J.F., LIU H.J. Short peptides secreted by *Bacillus subtilis* inhibit the growth of mold on fresh-cut pumpkin (*Cucurbita pepo*). *Journal of the Science of Food and Agriculture*, **100** (3), 936, **2019**.
- FAHAD S., HUSSAIN S., BANO A., SAUD S., HASSAN S., SHAN D., KHAN F.A., KHAN F., CHEN Y.T., WU C., TABASSUM M.A., CHUN M.X., AFZAL M., JAN A., JAN M.T., HUANG J.L. Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. *Environmental Science and Pollution Research*, **22**, 4907-4921, **2015**.
- WANG J.H., ZHANG X.W., ZHANG Y.H., QIN S.X., QIU J.Y., LI X.H., ZHANG Z.Y., CHEN X.Y., CHEN L.L., ZHOU Q.X. Formulation of the microbial agent *Bacillus amyloliquefaciens* NCPSJ7 and its application against *Botrytis cinerea* in tomato. *European Journal of Plant Pathology*, **169** (3), 611, **2024**.
- ADNAN M., SHAH Z., SHARIF M., RAHMAN H. Liming induces carbon dioxide (CO<sub>2</sub>) emission in PSB inoculated alkaline soil supplemented with different phosphorus sources. *Environmental Science and Pollution Research*, **25**, 9501, **2018**.
- AWLACHEW Z.T., MENGISTIE G.Y. Growth promotion of rice (*Oryza sativa* L.) seedlings using plant growth-promoting rhizobacteria (PGPR) isolated from northwest Ethiopia. *Advances in Agriculture*, **8**, **2022**.
- CHINACHANTA K., SHUTSRIRUNG A., HERRMANN L., LESUEUR D. Isolation and characterization of KDML105 aromatic rice rhizobacteria producing indole-3-acetic acid: impact of organic and conventional paddy rice practices. *Letters in Applied Microbiology*, **74**, 354, **2022**.
- ZHOU Y.Y., HAO L.P., JI C., ZHOU Q.S., SONG X., LIU Y., LI H.Y., LI C.H., GAO Q.X., LI J.T., ZHANG P.C., LIU X.L. The effect of salt-tolerant antagonistic bacteria cz-6 on the rhizosphere microbial community of winter jujube (*Ziziphus jujuba* Mill. "Dongzao") in saline-alkali land. *BioMed Research International*, **13**, 5171086, **2021**.
- SAMET M., GHAZALA I., KARRAY F., ABID C., CHIAB N., NOURI-ELLOUZ O., SAYADI S., GARGOURI-BOUZID R. Isolation of bacterial strains from compost teas and screening of their PGPR properties. *Environmental Science and Pollution Research*, **29** (50), 75365, **2022**.
- LIU H., LI S.S., QIANG R.W., LU E.J., LI C.L., ZHANG J.J., GAO Q. Response of soil microbial community structure to phosphate fertilizer reduction and combinations of microbial fertilizer. *Frontiers in Environmental Science*, **10**, **2022**.
- SUN T., LIU Y.Y.N., WU S., ZHANG J.Z., QU B., XU J.G. Effects of background fertilization followed by co-application of two kinds of bacteria on soil nutrient content and rice yield in Northeast China. *International Journal of Agricultural and Biological Engineering*, **13** (2), 154, **2020**.
- BUCHANAN R.E., GIBBONS N.E. *Bergey's manual of determinative bacteriology*, 8rd ed.; Science Press: Beijing, China, 751, **1984**.
- RAMALOKO W.T., OSEI SEKYERE J. Phylogenomics, epigenomics, virulome, and mobilome of gram-negative bacteria co-resistant to carbapenems and polymyxins: a one-health systematic review and meta-analyses. *Environmental Microbiology*, **24** (3), 1518, **2022**.
- TIAN W., LI L., XIAO X., WU H., WANG Y., HU Z., BEGUM N., ZOU Y.P., LOU L.Q., CHANG M., CAI Q.S. Identification of a plant endophytic growth-promoting bacteria capable of inhibiting cadmium uptake in rice. *Journal of Applied Microbiology*, **132**, 520, **2022**.
- LU L., CHANG M., HAN X., WANG Q., WANG J., YANG H., GUAN Q., DAI S. Beneficial effects of endophytic *Pantoea ananatis* with ability to promote rice growth under saline stress. *Journal of Applied Microbiology*, **131**, 1919, **2021**.
- MUKHTAR S., ZAREEN M., KHALIQ Z., MEHNAZ S., MALIK K.A. Phylogenetic analysis of halophyte-associated rhizobacteria and effect of halotolerant and halophilic phosphate-solubilizing biofertilizers on maize growth under salinity stress conditions. *Journal of Applied Microbiology*, **128**, 556, **2020**.
- RINCÓN-MOLINA C.I., MARTÍNEZ-ROMERO E., RUÍZ-VALDIVIEZO V.M., VELÁZQUEZ E., RUIZ-LAU N., ROGEL-HERNÁNDEZ M.A., VILLALOBOS-MALDONADO J.J., RINCÓN-ROSALES R. Plant growth-promoting potential of bacteria associated to pioneer plants from an active volcanic site of Chiapas (Mexico). *Applied Soil Ecology*, **146**, 103390, **2020**.
- BERRÍOS G., CABRERA G., GIDEKEL M., GUTIÉRREZ-MORAGA A. Characterization of a novel antarctic plant growth-promoting bacterial strain and its interaction with antarctic hair grass (*Deschampsia antarctica* Desv). *Polar Biology*, **36**, 349, **2012**.
- TATUNG M., DEB C.R. Screening and characterization of heavy metal tolerant rhizobacteria from wild Musa rhizosphere from coal mining area of Changki, Nagaland, India and assessment of their growth promoting potential under Cd/Cu contaminated conditions. *South African*

- Journal of Botany, **165**, 217, **2024**.
25. IDRIS E.E., BOCHOW H., ROSS H., BORRIS R. Use of *Bacillus subtilis* as biocontrol agent. VI. Phytohormone-like action of culture filtrates prepared from plant growth-promoting *Bacillus amyloliquefaciens* FZB 24, FZB 42, FZB 45 and *Bacillus subtilis* FZB 37. Journal of Plant Diseases and Protection, **111**, 583, **2004**.
  26. GANESH J., HEWITT K., DEVKOTA A.R., WILSON T., KAUNDAL A. IAA-producing plant growth promoting rhizobacteria from *Ceanothus velutinus* enhance cutting propagation efficiency and Arabidopsis biomass. Frontiers in Plant Science, **15**, 1374877, **2024**.
  27. PENROSE D.M., GLICK B.R. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol Plant, **118** (1), 10, **2003**.
  28. ORTEGA-ORTEGA Y., SARMIENTO-LÓPEZ L.G., BAYLÓN-PALOMINO A., VÁZQUEZ-LEE J., MALDONADO-BONILLA L.D., FLORES-OLIVAS A., VALENZUELA-SOTO J.H. *Enterobacter* sp. DBA51 produces ACC deaminase and promotes the growth of tomato (*Solanum lycopersicum* L.) and tobacco (*Nicotiana tabacum* L.) plants under greenhouse condition. Current Research in Microbial Sciences, **6**, 100207, **2023**.
  29. KRISHNAN R., LANG E., MIDHA S., PATIL P.B., RAMESHKUMAR N. Isolation and characterization of a novel 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing plant growth promoting marine *Gammaproteobacteria* from crops grown in brackish environments. Proposal for *Pokkaliibacter* plantistimulans gen. nov., sp. nov., *Balneatrichaceae* fam. nov. in the order *Oceanospirillales* and an emended description of the genus *Balneatrix*, Systematic and Applied Microbiology, **41** (6), 570, **2018**.
  30. GUO C.H., FANG F., LIU J.L. Isolation of acc deaminase-containing plant growth-promoting rhizobacteria from petroleum contaminated soil. Advanced Materials Research, **356-360**, 244, **2011**.
  31. RATNANINGSIH H.R., NOVIANA Z., DEWI T.K., LOEKITO S., WIYONO S., GAFUR A., ANTONIUS S. IAA and ACC deaminase producing-bacteria isolated from the rhizosphere of pineapple plants grown under different abiotic and biotic stresses. Heliyon, **9** (6), e16306, **2023**.
  32. MURALI M., SINGH S.B., GOWTHAM H.G., SHILPA N., PRASAD M., AIYAZ M., AMRUTHESH K.N. Induction of drought tolerance in *Pennisetum glaucum* by ACC deaminase producing PGPR-*Bacillus amyloliquefaciens* through Antioxidant defense system. Microbiology Research, **253**, 126891, **2021**.
  33. JIMÉNEZ-VÁZQUEZ K.R., GARCÍA-CÁRDENAS E., BARRERA-ORTIZ S., ORTIZ-CASTRO R., RUIZ-HERRERA L.F., RAMOS-ACOSTA B.P., CORIA-ARELLANO J.L., SÁENZ-MATA J., LÓPEZ-BUCIO J. The plant beneficial rhizobacterium *Achromobacter* sp. 5B1 influences root development through auxin signaling and redistribution. Plant Journal, **103** (5), 1639, **2020**.
  34. KHAN M.M.A., HAQUE E., PAUL N.C., KHALEQUE M.A., AL-GARNI S.M.S., RAHMAN M., ISLAM M.T. Enhancement of growth and grain yield of rice in nutrient deficient soils by rice probiotic bacteria. Rice Science, **24** (5), 264, **2017**.
  35. THAKUR R., SONI R., DHAR H., RANA A., SHARMA A., KAUSHAL K., SHAH M.A., RESHI Z.A., MATHEW S., GULATI A. Enhancing saffron (*Crocus sativus* L.) growth in the Kashmir valley with resilient and widely effective Plant Growth-Promoting Rhizobacteria (PGPR) under field conditions. Industrial Crops and Products, **222** (1), 119475, **2024**.
  36. MENEGUZZI R.D.V., FERNANDEZ M., CAPPELLARI L.D.R., GIORDANO W., BANCHIO E. Isolation and characterization of plant growth-promoting bacteria from the rhizosphere of medicinal and aromatic plant *Minthostachys verticillata*. Plants-Basel, **13** (15), 2062, **2024**.
  37. GAMEZ R., CARDINALE M., MONTES M., RAMIREZ S., SCHNELL S., RODRIGUEZ F. Screening, plant growth promotion and root colonization pattern of two rhizobacteria (*Pseudomonas fluorescens* Ps006 and *Bacillus amyloliquefaciens* Bs006) on banana cv. Williams (*Musa acuminata* Colla). Microbiology Research, **220**, 12, **2019**.
  38. SANDANI H.B.P., RANATHUNGE N.P., LAKSHMAN P.L.N., WEERAKOON W.M.W. Biocontrol potential of five *Burkholderia* and *Pseudomonas* strains against colletotrichum truncatum infecting chilli pepper. Biocontrol Science and Technology, **29** (8), 727, **2019**.
  39. SYED-AB-RAHMAN S.F., CARVALHAIS L.C., CHUA E., XIAO Y.W., WASS T.J., SCHENK P.M. Identification of soil bacterial isolates suppressing different *Phytophthora* spp. and promoting plant growth. Frontiers in Plant Science, **9**, 1502, **2018**.
  40. FÖLDES T., BÁNHÉGYI I., HERPAI Z., VARGA L., SZIGETI J. Isolation of *Bacillus* strains from the rhizosphere of cereals and in vitro screening for antagonism against phytopathogenic, food-borne pathogenic and spoilage micro-organisms. Journal of Applied Microbiology, **89** (5), 840, **2000**.
  41. ARREBOLA E., JACOBS R., KORSTEN L. Iturin A is the principal inhibitor in the biocontrol activity of *Bacillus amyloliquefaciens* PPCB004 against postharvest fungal pathogens. Journal of Applied Microbiology, **108** (2), 386, **2010**.
  42. ES-SOUFI R., TAHIRI H., AZAROUAL L., OUALKADI A.E., MARTIN P., BADOC A., LAMARTI A. Biocontrol potential of *Bacillus amyloliquefaciens* Bc2 and *Trichoderma harzianum* TR against strawberry anthracnose under laboratory and field conditions. Agricultural Sciences, **11**, 260, **2020**.
  43. ZHAO X., ZHOU Z.J., HAN Y. Antifungal effects of lipopeptide produced by *Bacillus amyloliquefaciens* BH072. Advances in Bioscience and Biotechnology, **8**, 295, **2017**.
  44. LU H.D., YANG P.P., ZHONG M.Y., BILAL M., XU H., ZHANG Q.H., XU J.N., LIANG N.G., LIU S., ZHAO L., ZHAO Y.P., GENG C.X. Isolation of a potential probiotic strain *Bacillus amyloliquefaciens* LPB-18 and identification of antimicrobial compounds responsible for inhibition of food-borne pathogens. Food Science & Nutrition, **11**, 2186, **2023**.
  45. LV J., DA R., CHENG Y., TUO X.H., WEI J., JIANG K.C., MONISAYO A.O., HAN B. Mechanism of antibacterial activity of *Bacillus amyloliquefaciens* C-1 lipopeptide toward anaerobic clostridium difficile, BioMed Research International, **12**, 3104613, **2020**.
  46. ZHAO Z.T., LIU D.M., RUAN L., WANG T.L., LIANG Z.H. Antifungal mechanism of *Bacillus amyloliquefaciens* SC-B15 and its application in cereal mildewproof and grape preservation. Food Bioscience, **56**, 103287, **2023**.
  47. YI Y.J., LUAN P.Y., FAN M.H., WU X.Q., SUN Z.K., SHANG Z.J., YANG Y.Z., LI C.W. Antifungal efficacy of *Bacillus amyloliquefaciens* ZK-9 against Fusarium graminearum and analysis of the potential mechanism



- of its lipopeptides. *International Journal of Food Microbiology*, **422**, 110821, **2024**.
48. ADNAN M., FAHAD S., ZAMIN M., SHAH S., MIAN I.A., DANISH S., ZAFAR-UL-HYE M., BATTAGLIA M.L., NAZ R.M.M., SAEED B., SAUD S., AHMAD I., YUE Z., BRTNICKY M., HOLATKO J., DATTA R. Coupling phosphate-solubilizing bacteria with phosphorus supplements improve maize phosphorus acquisition and growth under lime induced salinity stress. *Plants-Basel*, **9** (7), 900, **2020**.
  49. ALAM F., KHAN A., FAHAD S., NAWAZ S., AHMED N., ALI M.A., ADNAN M., DAWAR K., SAUD S., HASSAN S., RAZA M.A.S., NAVEED K., ARIF M., DATTA R., DANISH S. Phosphate solubilizing bacteria optimize wheat yield in mineral phosphorus applied alkaline soil, *Journal of the Saudi Society of Agricultural Sciences*, **21** (5), 339, **2022**.
  50. SARKAR D., SANKAR A., DEVIKA O.S., SINGH S., SHIKHA, PARIHAR M., RAKSHIT A., SAYYED R. Z., GAFUR A., ANSARI M.J., DANISH S., FAHAD S., DATTA R. Optimizing nutrient use efficiency, productivity, energetics, and economics of red cabbage following mineral fertilization and biopriming with compatible rhizosphere microbes. *Scientific Reports*, **11**, 15680, **2021**.