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Isolation, identification and activity researching of ACC deaminase-containing rhizobacteria from lavender in Ili Kazakh Autonomous Prefecture

[Aislamiento, identificación e investigación de la actividad de rizobacterias con ACC desaminasa de lavanda en la Prefectura Autónoma Kazaja de Ili]

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Abstract: Lavender is one of the important cash crops in Ili Kazakh Autonomous Prefecture. In this study, lavender rhizobacteria were isolated and purified, and their antibacterial activity was tested by filter paper method. The antioxidant activity and growth promotion ability were determined by DPPH, ABTS and PTIO assay as well as wheat seeds and rice seeds. The isolated strains were identified by morphological methods, physiological and biochemical experiments and molecular biological methods. Five strains of ACC deaminase-containing rhizobacteria were isolated, and four of them had antibacterial activity, and scavenging ability for DPPH, ABTS and PTIO free radicals, and has growth promotion effect on wheat and rice seedlings. Through 16sRNA sequence analysis, strains were identified as *Pseudomonas rhizophila*, *Pseudomonas savastanoi*, *Klebsiella pasteurii*, *Klebsiella pneumoniae* and *Serratia plymuthica*. In conclusion, this study provides a certain theoretical basis for the utilization of rhizobacteria in plant resources.

Keywords: *Lavandula angustifolia* Mill; Rhizobacteria; ACC deaminase activity; Antibacterial activity; Antioxidant activity.

Resumen: La lavanda es uno de los cultivos comerciales importantes en la Prefectura Autónoma Kazaja de Ili. En este estudio, se aislaron y purificaron rizobacterias de lavanda, y se evaluó su actividad antibacteriana mediante el método del papel de filtro. La actividad antioxidante y la capacidad de promoción del crecimiento se determinaron mediante ensayos DPPH, ABTS y PTIO, así como con semillas de trigo y arroz. Las cepas aisladas se identificaron mediante métodos morfológicos, experimentos fisiológicos-bioquímicos y métodos de biología molecular. Se aislaron cinco cepas de rizobacterias con ACC desaminasa, de las cuales cuatro presentaron actividad antibacteriana, capacidad de eliminación de radicales libres DPPH, ABTS y PTIO, y efecto promotor del crecimiento en plántulas de trigo y arroz. Mediante análisis de secuencia 16sRNA, las cepas se identificaron como *Pseudomonas rhizophila*, *Pseudomonas savastanoi*, *Klebsiella pasteurii*, *Klebsiella pneumoniae* y *Serratia plymuthica*. En conclusión, este estudio proporciona una base teórica para el aprovechamiento de rizobacterias en recursos vegetales.

Palabras clave: *Lavandula angustifolia* Mill; Rizobacterias; Actividad ACC desaminasa; Actividad antibacteriana; Actividad antioxidante.

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INTRODUCTION

Lavandula angustifolia Mill, a plant of the genus Lavandula in the family Labiatae, has the function of clearing heat and relieving itching, and is often used in folklore to treat headaches, oral inflammation and other conditions (Bavarsad *et al.*, 2023; Betlej *et al.*, 2023; Tundis *et al.*, 2023). Lavender is mainly grown in France, Spain and Japan. China in the 1950s began to introduce lavender from France, currently in Xinjiang, Gansu, Shaanxi, Inner Mongolia and other places have a large area of planting, of which the Ili Kazakh Autonomous Prefecture (Geographic location: 40°14'-49°10' N, 80°09'-91°01' E) has a relatively high yield, has become the world's third largest producing area (Guo *et al.*, 2020). Lavender not only stimulates Ili's tourism industry, lavender-related products such as essential oils, skin lotions, face masks, etc. have greatly boosted economic benefits and solved Ili's related employment problems (Yap *et al.*, 2019; Samuelson *et al.*, 2020; Sharmeen *et al.*, 2021). But unlike the French lavender products and Japanese lavender products are sold at home and abroad, Ili lavender products consumers are mostly local residents of Ili and tourists to Ili. This is because Ili lavender in order to increase production, in the planting technology is still more dependent on pesticides and fertilizers, however, most of the residues of pesticides and fertilizers are toxic and not easy to decompose (Rahman *et al.*, 2020; Thomas *et al.*, 2020). This results in the quality of Ili lavender products being inferior to their French and Japanese counterparts, and the large-scale use of pesticides and chemical fertilizers is also harmful to the soil. As environmental issues in China are receiving more and more attention, lavender farmers in Ili are reducing their reliance on pesticides and fertilizers. Hence, there is an urgent need to explore sustainable cultivation techniques that can maintain high lavender yields and also improve lavender quality.

Plant growth promoting rhizobacteria (PGPR) are a collection of microbes that engage with plant roots to enhance plant growth and development by forming a mutually beneficial bond with the plant (Bhattacharyya & Jha, 2012; de Andrade *et al.*, 2023). These microorganisms can form a micro-ecological environment with the plant root system and contribute significantly to the well-being of the plants. 1-Aminocyclopropane-1-carboxylic acid (ACC) is a direct precursor substance for the synthesis of ethylene and is involved in the regulation of plant growth (Gamalero *et al.*, 2023). With the research on PGPR in recent years, it has been shown that some PGPR contain ACC deaminase, which can degrade ACC into ammonia and α-butyronic acid, and can provide the plant with the required nitrogen and carbon sources while at the same time lowering the ethylene content of the plant's body weight, thus promoting the plant's growth (Ali & Kim, 2018; Glick & Nascimento, 2021). It can be seen that by investigating the PGPR of lavender, it may be possible to find a cultivation technique that improves the quality of lavender through mycorrhizal fertilizers, while maintaining high yields.

Therefore, in this study, we screened rhizobacterias with ACC deaminase activity from the rhizosphere of Ili lavender, and studied its antibacterial activity, antioxidant, grown-promoting properties, and identified the rhizobacteria by 16S rRNA gene sequencing. The aim of this study is to provide a reference for the utilization of rhizobacteria in plant resources and the development of bacterial fertilizers.

MATERIALS AND METHODS

Plant samples

The healthy and intact plants of the test lavender were collected in June 2022 at the lavender base of the Institute of Agricultural Science of Ili Kazakh Autonomous Prefecture. Intact plants with disease-free appearance were randomly selected for collection, and intact plants and plant rhizospheres with rhizosphere-attached soil were collected with a shovel, brought back to the laboratory in a sealed bag, kept in a refrigerator at -20°C for backup, and processed for fresh plant samples within 24 h.

The rice seed was New Rice 46, sourced from Kan Township, Chabuchar Xibe Autonomous County, Ili Kazakh Autonomous Prefecture, Xinjiang. The wheat seed was Ningchun 16, sourced from Seven Townships, Gongliu County, Ili Kazakh Autonomous Prefecture, Xinjiang.

Isolation, purification, and screening of rhizobacteria with ACC deaminase activity

Isolation of rhizobacteria from Ili lavender rhizospheres was carried out using the soil dilution method (Zhong *et al.*, 2023). Take 1 g of rhizosphere soil and 10 mL of sterile PBS buffer in a 50 mL beaker to make a suspension, take 1mL of the suspension, add it to the beaker containing 9 mL of sterile PBS buffer, mix well to make a suspension at a 10-1 dilution, and then take 1mL of the suspension from it, add it to the beaker containing 9 mL of sterile PBS buffer, mix well to make a suspension at a 10-2

dilution. By analogy, suspensions of 10-1, 10-2, 10-3, 10-4, and 10-5 dilutions were made, and 0.1 mL of the diluted suspensions were coated into the isolation medium, and inverted and incubated at 28°C for 24 h for observation.

Based on the morphology and color of the colonies, different colonies were picked out and inoculated into the purification medium. Under aseptic conditions, cultures were incubated using the plate delineation method. The inoculated medium was inverted and incubated at 28°C for 24 h for observation. After three passages, the colony morphology was observed and the strains were preserved in glycerol at -80℃.

Single colonies were picked and inoculated in 50 mL Pseudomonas agar F (PAF) liquid medium (Coolaber, Beijing, China), and incubated in a shaker at 28℃ for 48 h. After that, 1 mL of the bacterial solution was added into 50 mL DF liquid medium (MM6150-2, Coolaber), and then incubated for 24 h again. After that, 1 mL was taken and added to 50 mL of ADF liquid medium (MM6150, Coolaber) and incubated for 24 h. A 10-fold gradient dilution of the bacterial solution was performed with ADF liquid medium. 100 μL each of strain suspensions of 10-4, 10-5 and 10-6, respectively, were coated in ADF solid medium. After colonies grew on the plate, single colonies were picked, and the streaking inoculation was repeated to finally obtain the purified strain. The obtained target strains were inoculated into 150 mL tryptic soy broth (TSB) liquid medium (Hope Bio-Technology Co., Ltd, Qingdao, Shandong, China), and placed in a shaker at 28°C for 24 h. After that, they were stored in glycerol in an ultra-low temperature refrigerator at -80°C.

ACC deaminase activity assay

A 100 mmol/L of α-butanedioic acid (K401, Sigma-Aldrich, St. Louis, MO, USA) solution was prepared in 0.1 mol/L Tris-HCl (T5941, Sigma-Aldrich) buffer (pH=8.5), after gradient dilution, added 0.2% of 2,4-dinitrophenylhydrazine (D2630, Sigma-Aldrich) solution and incubated for 30 min at 30°C. Color phenylhydrazone was developed by adding 2 mol/L NaOH (S835850, Macklin, Shanghai, China) solution, the absorbance was recorded at 540 nm using a spectrophotometer (UV-2800, Unicosh, Shanghai, China). The concentration of α-butanoic acid (mmol/L) was plotted as the abscissa, and the absorbance (A 540) was plotted as the ordinate.

The bacteria were inoculated into 15 mL of TSB liquid medium and incubated at 28℃ for 24 h. After centrifugation, the bacteria were collected and washed with 5 mL of DF medium, then suspended in 7.5 mL of ADF medium and incubated for 24 h, promoting the generation of ACC deaminase. Determination of ACC deaminase activity with reference to Anand *et al.* (Anand *et al.*, 2021), the bacteria were centrifuged and collected, washed and re-suspended in 0.1 mol/L Tris-HCl (pH=7.6) buffer. Toluene (244511, Sigma-Aldrich) was added to extract the cytosol, and 200 μL of the cell extract was mixed with 20 μL of 0.5 mol/L ACC (A3903, Sigma-Aldrich) solution and incubated at 30°C for 15 min. Added 300 μL of 0.2% 2,4-dinitrophenylhydrazine, incubated at 30°C for 30 min, and added 2 mL of 2 mol/L NaOH solution to develop the color of phenylhydrazone, and assessed the absorbance at 540 nm. The cell extracts were analyzed for their protein content using the Bradford method with bovine serum protein as a reference standard (Kielkopf *et al.*, 2020). The amount of 1 μmol α-butyronic acid formed per minute was defined as one unit of enzyme activity.

Antibacterial activity assay

Escherichia coli (Serial No. CMCCB44102), Bacillus subtilis (Serial No. CMCCB63501), and *Staphylococcus aureus* (Serial No. ATCC43300) were purchased from HuanKai Bioiogy (Zhaoqing, Guangdong, China). The above three bacterial strains were inoculated into 10 mL of nutrient broth (NB) liquid medium (022010, HuanKai Bioiogy), and incubated in a constant temperature shaker at 37℃ for 12 h. Next, the NB liquid medium was diluted with NB liquid medium to an OD600 of 0.2~0.3 (about 1×106 cfu/mL), and 100 μL of bacterial solution was pipetted and spread onto the NB solid medium, respectively. The plate was divided into 4 sections and a sterile filter paper sheet with a diameter of 6 mm was attached to each section.

The screened bacterial strains with ACC deaminase activity were seed into 150 mL of TSB liquid medium, respectively, then incubated at 28°C for 24 h. After centrifugation and collection of the bacterial cells, they were rinsed two times with sterile PBS solution. The bacteria were resuspended in sterile water, the bacteria were broken by ultrasonic crushing to prepare the bacterial liquid, and centrifuged to separate the broken bacteria, and the supernatant was taken for spare. 100 mL of the supernatant was concentrated to 1 mL using a rotary evaporator (Büchi Labortechnik, Flawil, Switzerland), and 10 μL of each was added dropwise

to the above sterile filter paper sheets after being filtered with a 0.22 μm needle to remove bacteria. At the same time, 10 μL of sterile water was put into the sterile filter paper as a blank control, and 10 μL of antibiotic (L780388, Macklin) was added to the sterile filter paper as a positive control, and the culture was inverted at 37℃ for 24 h. Each plate was repeated three times, observed the appearance of the inhibition zone around the sterile filter paper, and recorded the size of the inhibition zone.

Antioxidant activity test

The purpose of the bacterial antioxidant assay is to assess the scavenging ability of antioxidant substances in the bacterial solution against free radicals or other oxidants. The above bacterial supernatant was stored at -80°C for 24 h before being freeze-dried to produce a solid substance, which was configured with sterile water to form an antioxidant solution to be tested at a certain concentration gradient. Using the 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH) Free Radical Scavenging Capacity Assay Kit (BC4750, Solarbio, Beijing, China), 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) Free Radical Scavenging Capacity Assay Kit (BC4775, Solarbio), and Phenyl-4,4,5,5-tetramethylimidazoline-3-oxide-1-oxyl (PTIO) assay (P5084, Sigma-Aldrich) to determine the antioxidant activity of bacterial solution from different strains of bacteria (Li *et al.,* 2021; Rumpf *et al.,* 2023). In all the above three antioxidant experiments, the free radical scavenging rate of 0.1 mg/mLvitamin C (VC, A4403, Sigma-Aldrich) was recorded as 100%, and the ratio of the free radical scavenging rate of bacterial suspension of different strains to VC was used to express the relative free radical scavenging rate.

Growth-promoting effects test

In this experiment, the growth-promoting ability of ACC deaminase-active strains on rice and wheat was examined by using the bacterial solution inoculation method. The five ACC deaminase-active strains and Escherichia coli were first cultured and prepared into 108 CFU/mL suspensions, respectively. Next, full and disease-free rice and wheat seeds were randomly selected, and the seeds were surface sterilized and later rinsed three to five times with sterile water. A sterile filter paper was placed on the bottom of a sterile Petri dish, and 10 mL of sterile water, Escherichia coli suspension, and ACC deaminase-active strain suspension were used to moisten the sterile filter paper on the bottom of the dish, respectively. Thirty sterilized seeds were randomly spread in each petri dish and three sets of replicates were set up for each strain and sterile water. The germination rate of the seeds was observed by placing the petri dishes in incubator at 25°C, and the root length and fresh weight were determined after 7d.

Classification and identification of rhizobacteria

Single colonies of bacteria were picked and inoculated into 150 mL TSB liquid medium, incubated at 28℃ for 12 h to logarithmic phase, then centrifuged and collected, and the genomic DNA of the target bacteria was extracted by Ezup Column Bacterial Genomic DNA Extraction Kit (Sangon Biotech, Shanghai, China). Genomic DNA was used as a template for PCR amplification using universal primers 27F (5′-AGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) for bacterial identification. DNA molecular weight standard marker (10,000 bp), DNA molecular weight standard marker (2,000 bp), dNTP Mixture, PCR Buffer (Mg^{2+} plus), and Taq enzyme were purchased from Takara (Tokyo, Japan). The amplified PCR bands were detected using 1% agarose gel electrophoresis and the PCR products were sent to the company for sequencing. Finally, thhe sequences obtained were analyzed by comparing them with NCBI using BLAST.

Statistical methods

A minimum of 3 repetitions in each experiment, with the result being reported as mean ± standard deviation. For statistical analysis of data and image plotting, we employed SPSS 26.0 software (IBM SPSS Statistics 26) and Origin Pro 2022 software. Student's t-test evaluated the distinctions between the two groups, and analysis of variance (ANOVA) was performed to make comparisons between sub-multiple groups. Using MEGA-X-10.1.7, the phylogenetic tree was constructed by Neighbor-Joining method. *p<0.05 denotes significant difference.

RESULTS

Biological morphology and physiological and biochemical characterization of rhizobacteria with ACC deaminase activity

Five bacterial strains containing ACC deaminase activity were isolated and purified from the rhizosphere of Ili lavender, and the biomorphological

and morphological characteristics of the strains and some of their culture traits are shown in Table No. 1. All five bacterial strains obtained from the screening showed Gram-negative reaction; two were short rod-shaped and three were rod-shaped; five strains were free of spores; two were polar flagellum, one periplasmic flagellum, and two were flagellum-free; three had no capsules and two had capsules.

Table No. 1
Morphology and culture characteristics of ACC deaminase-containing rhizobacteria from Ili lavender

Rhizobacteria number	YSX-12	YSX-23	YSX-36	YSX-47	YSX-61
Gram stain	-	-	-	-	-
Morphology	short rod	short rod	short rod	short rod	rod-shaped
Spore	none	none	none	none	none
Flagellum	polar	polar	none	none	periplasmic
Capsule	none	none	have	have	none
Culture characteristics	Clusters fibrous, uneven, serrated, level, translucent, yellow-tinged	Clusters round, smooth, even edges, translucent, snowy	Growths spherical, smooth-surfaced, with even borders, pad-like, non-transparent, creamy white	Growths spherical, smooth, with undulating borders, convex, non-transparent, creamy white	Clusters round, rough, irregular edges, convex, translucent, ivory white

"-" indicates a negative Gram stain

The morphological characteristics and culture traits of each strain containing ACC deaminase activity are shown in Table No. 2. All five Ili Lavander rhizobacteria strains containing ACC deaminase could produce contact and oxidase enzymes, and all were also able to utilize glucose for fermentation, were positive in the nitrate reduction reaction and did not produce H2S. On the basis of all the physical and chemical traits of these strains, YSX-12 and YSX-23 were initially identified as Pseudomonas strains. YSX-36 and YSX-47 were preliminarily identified as *Klebsiella* strains, and YSX-61 was preliminarily identified as a *Serratia* strain.

Table No. 2
Results of physiological and biochemical experiments of Rhizobacteria containing ACC deaminase in Ili Lavender

Test program	Rhizobacteria				
	YSX-12	YSX-23	YSX-36	YSX-47	YSX-61
Oxidase reaction	+	+	+	+	-
Catalase reaction	+	+	+	+	+
Glucose fermentation reaction	+	+	+	+	+
V.P test	+	+	+	-	+
M.R test	-	-	-	+	+
Cellulose hydrolysis	-	-	+	-	-
Citrate utilization	-	-	+	+	+
Gelatin liquefaction	-	-	-	-	+
Tween 20 reaction	-	-	-	-	-
Tween 80 reaction	-	-	-	+	-
Casein hydrolysis	-	-	-	+	+
Nitrate reduction	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-
H_2S reaction	-	-	-	-	-

"+" indicates positive; "-" indicates negative

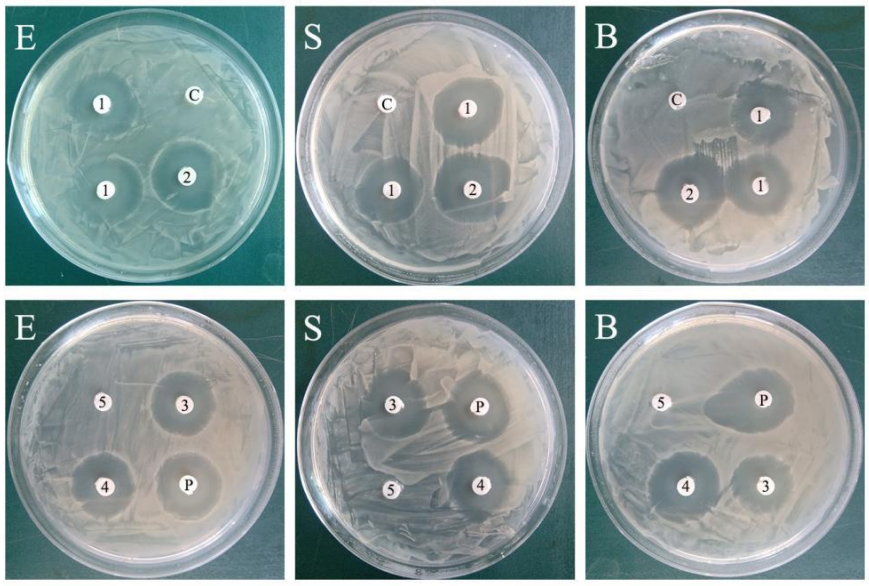
ACC deaminase activity of rhizobacteria

The ACC deaminase activity of the five rhizobacteria strains was assayed by determining the absorbance of α-butyronic acid. The ACC deaminase activity of strains YSX-12, YSX-23, YSX-36, YSX-47, and YSX-61 was 0.546 U/mg, 0.487 U/mg, 0.277 U/mg, 0.312 U/mg, and 0.081 U/mg, respectively. YSX-12 had the highest ACC deaminase activity, while YSX-61 had the lowest ACC deaminase activity.

Antibacterial activity of rhizobacteria

The results of the inhibition experiments are shown in Figure No. 1, Four of the five ACC deaminase-active strains, YSX-12, YSX-23, YSX-36, and YSX-47, showed different degrees of inhibition against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. The diameters of inhibition zone were between 17 mm and 23 mm. However, strain YSX-61 did not show any inhibitory effects on the three bacteria tested.

Figure No. 1
Antibacterial activity of ACC deaminase-containing rhizobacteria from Ili lavender against the tested bacteria

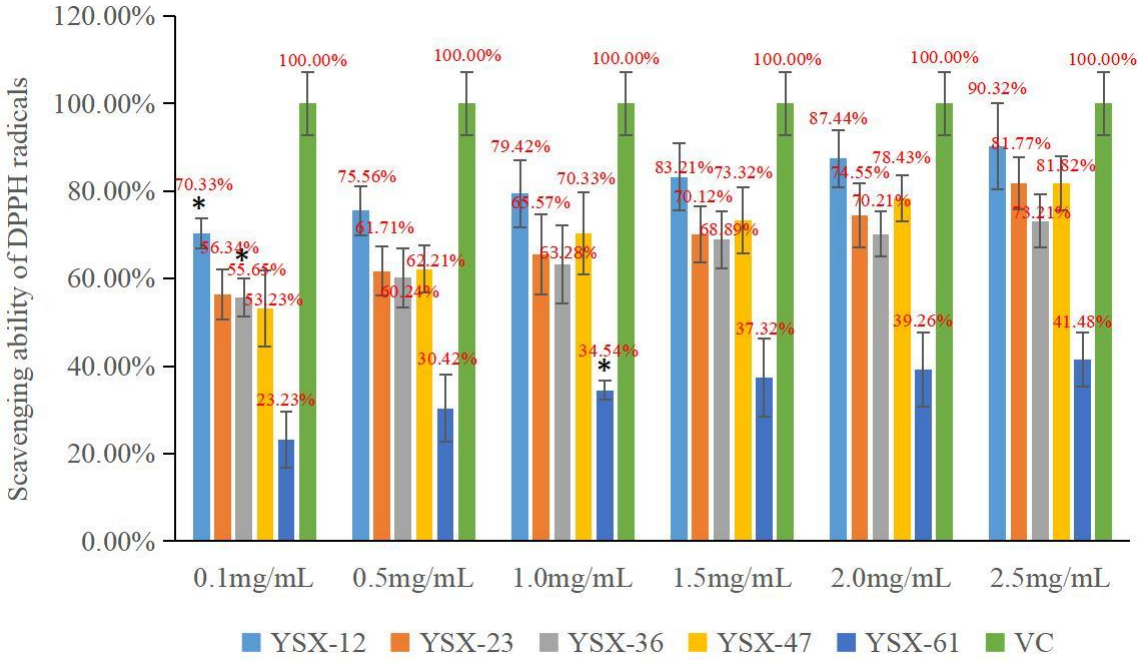


E: *Escherichia coli*; S: *Staphylococcus aureus*; B: *Bacillus subtilis*; C: sterile water; P: antibiotic. 1: YSX-12; 2: YSX-23; 3: YSX-36; 4: YSX-47; 5: YSX-61

Antioxidant activity of rhizobacteria

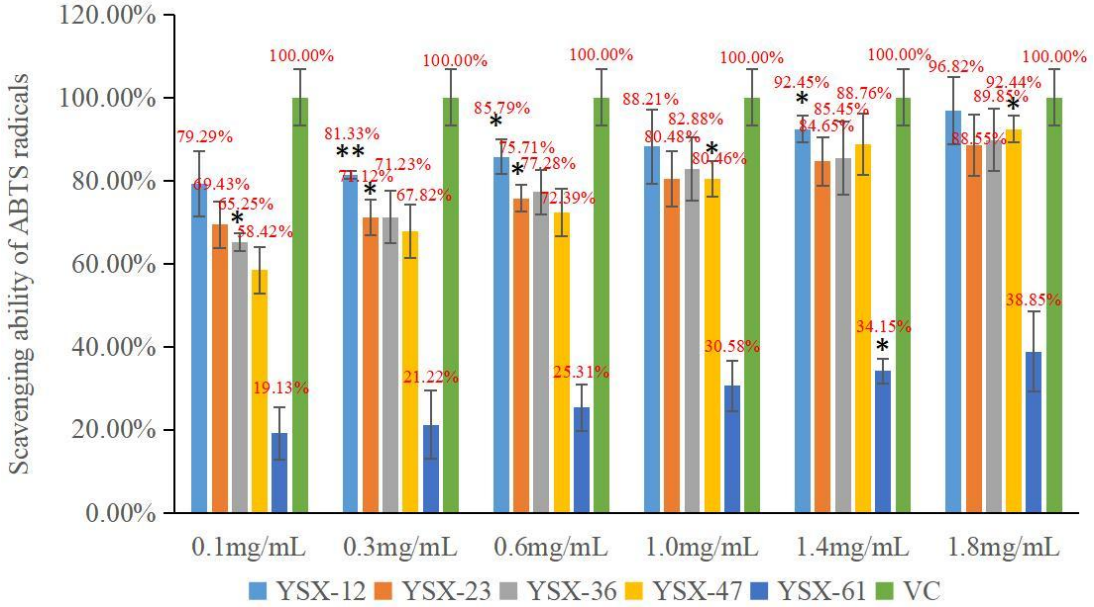
The findings of antioxidant experiments were shown in Figure No. 2, Figure No. 3 and Figure No. 4. Among the five strains with ACC deaminase activity, four strains (YSX-12, YSX-23, YSX-36, and YSX-47) showed different degrees of antioxidant effects on DPPH radicals, ABTS radicals, and PTIO radicals, and the higher the concentration, the stronger the antioxidant capacity was. Notably, the strain with the strongest antioxidant effect was YSX-12. However, strain YSX-61 had a low scavenging capacity for DPPH, ABTS, and PTIO radicals.

Figure No. 2
The scavenging ability of strains to DPPH free radicals



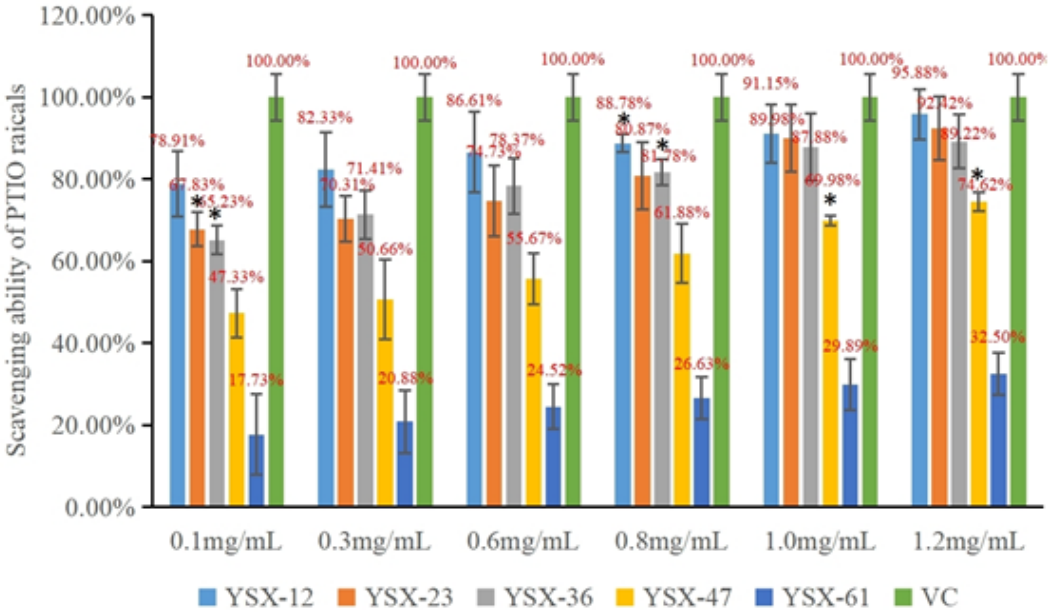
VC indicates vitamin C. * indicates $p<0.05$

Figure No. 3
The scavenging ability of strains to ABTS free radicals



VC indicates vitamin C. * indicates $p<0.05$; ** indicates $p<0.01$

Figure No. 4
The scavenging ability of strains to PTIO free radicals



VC indicates vitamin C. * indicates $p<0.05$

Growth-promoting effects of rhizobacteria

In rice seedling and wheat seedling promotion experiments, the YSX-12, YSX-23, YSX-36 and YSX-47 all showed different degrees of growth promoting ability on the root development of rice seedlings and wheat seedlings. Meanwhile, during the germination process of rice seeds and wheat seeds, none of the five bacterial strains with ACC deaminase activity exhibited a particularly pronounced growth-promoting effect on germination rates affecting rice seedlings.

No notable variation was observed in the germination rate of rice seeds between the sterile water treatment group and the group treated with the ACC deaminase-active strain, indicating that the ACC deaminase-active strain did not affect the germination of rice seeds. The promotion of rice seedling growth by five ACC deaminase-active strains is shown in Table No. 3. Strains YSX-12, YSX-23, YSX-36, and YSX-47 promoted the growth of rice seedlings to varying degrees. Among them, the growth-promoting effect of YSX-23 was the most obvious. The average root length of rice seedlings treated with strain YSX-23 increased by 62% and the average root fresh weight increased by 37% after 7 d of growth compared to the control group (Figure No. 5).

Table No. 3
Growth-promoting effect of rhizobacteria with ACC deaminase activity on root length and fresh weight of rice seedlings

Process	Root length		Root fresh weight	
	Average value/cm	Relative elongation rate/%	Average value/mg	Relative fresh weight rate/%
YSX-12	4.8±0.26**	150	17.38±1.72*	128
YSX-23	5.2±0.41*	162	18.61±1.88*	137
YSX-36	4.0±0.68*	125	16.49±1.71	121
YSX-47	3.9±0.25	121	17.03±1.85	125
YSX-61	3.4±0.77*	106	14.81±1.83	109
Escherichia coli	3.1±0.45	97	12.26±1.63	91
sterile water	3.2±0.31	100	13.55±1.31	100

*** indicates $p<0.05$; ** indicates $p<0.01$**

Figure No. 5
Growth-promoting Effect of Strain YSX-23 on Root System of Rice Seedlings

The five ACC deaminase-active strains also had no notable impact on the germination rate of wheat seeds by comparison with the *Escherichia coli*-treated group and the sterile water-treated group. However, the results of the growth promotion test showed that four of the five ACC deaminase-active strains (YSX-12, YSX-23, YSX-36, and YSX-47) exerted different degrees of growth promotion effects on the root development of wheat seedlings (Table No. 4). The root length of wheat in the strain YSX-23 inoculation treatment group was 5.8 cm and the root fresh weight was 19.81 g, which increased by 65% and 30%, respectively, which indicated that strain YSX-23 had a strong growth-promoting effect (Figure No. 6).

Table No. 4
Growth-promoting effect of rhizobacteria with ACC deaminase activity on root length and root fresh weight of wheat seedlings

Process	Root length		Root fresh weight	
	Average value/cm	Relative elongation rate/%	Average value/mg	Relative fresh weight rate/%
YSX-12	5.1±1.20*	145	18.62±1.31*	122
YSX-23	5.8±0.81**	165	19.81±1.71*	130
YSX-36	4.1±0.28*	117	17.73±1.66	116
YSX-47	4.0±0.44	114	17.26±1.42*	113
YSX-61	3.6±0.78	103	16.09±1.39	105
Escherichia coli	3.4±0.51	97	14.23±1.77	94
sterile water	3.5±0.32	100	15.21±1.26	100

*** indicates $p<0.05$; ** indicates $p<0.01$**

Identification and analysis of rhizobacteria with ACC deaminase activity

Using MEGA-X-10.1.7, the phylogenetic tree was constructed by Neighbor-Joining method. Strain YSX-12 showed 99% similarity to *Pseudomonas rhizophila* S211, strain YSX-23 showed 99% similarity to *Pseudomonas savastanoi* pv. *phaseolicola* 1448A (sequence number CP000058.1), strain YSX-36 has 100% similarity to strain *Klebsiella pasteurii* SPARK1448C2 (sequence number MN104666.1), strain YSX-47 showed 100% similarity to strain *Klebsiella pneumoniae* INF102 (sequence number CP110762.1), strain YSX-61 showed 99% similarity to strain *Serratia plymuthica* NCTC12961 (sequence number LS483469.1) (Figure No. 7). Phylogenetic tree analysis by integrating morphological, physiological and biochemical characters and molecular characterization, strain YSX-12 was identified as *Pseudomonas rhizophila*, strain YSX-23 was identified as *Pseudomonas savastanoi*, strain YSX-36 was identified as *Klebsiella pasteurii*, strain YSX-47 was identified as *Klebsiella pneumoniae*, strain YSX-61 was identified as *Serratia plymuthica*.

Figure No. 6
Growth-promoting effect of strain YSX-23 on root system of wheat seedlings

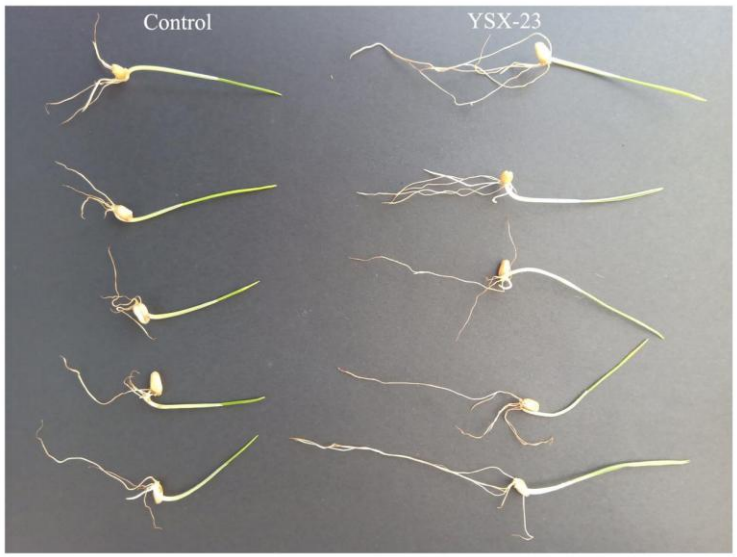
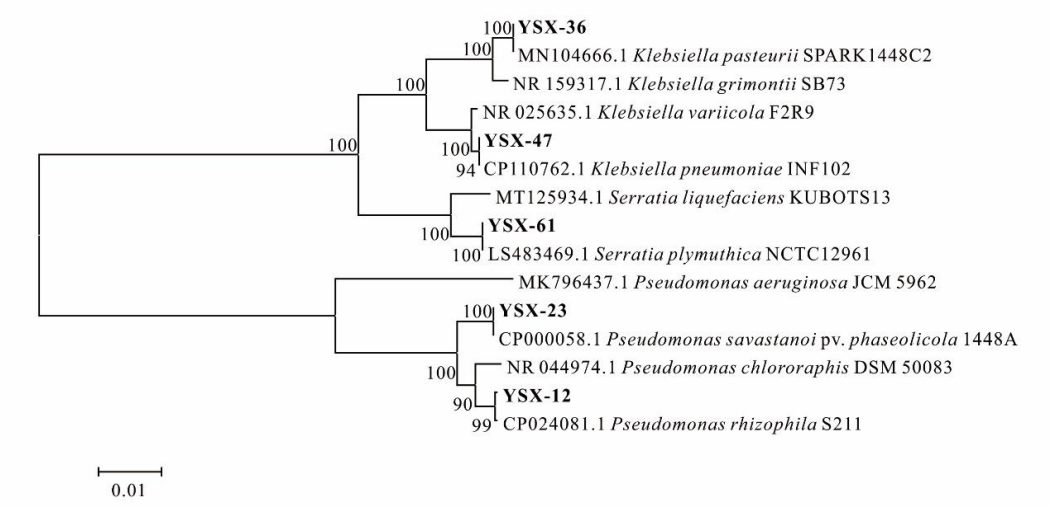


Figure No. 7
Phylogenetic tree of ACC deaminase-containing rhizobacteria and reference strains based on 16S rRNA sequences



DISCUSSION

Lavender is one of the important cash crops in Ili Kazakh Autonomous Prefecture. However, during the cultivation of Ili lavender, the heavy use of pesticides and chemical fertilizers has caused soil nitrogen and phosphorus overload and soil acidification, resulting in a decline in the quality of lavender and difficulties in increasing yields, and other crops face the same problems during cultivation as well (Liu *et al.*, 2021). Plants and microorganisms in the same surroundings have a strong connection, with microorganisms being crucial for plant development. Therefore, more and more researchers and scholars have started to look for new biological resources from rhizosphere microorganisms to improve crop yield and quality through research on mycorrhizal fertilizers (Haque *et al.*, 2022; Kumawat *et al.*, 2022).

Currently, Singh et al. isolated 12 bacterial strains with ACC deaminase activity from *Zizania latifolia* roots, and the bacterial strain with the highest enzyme activity was identified as *Enterobacter cloacae*, and this strain has the ability to solubilize phosphate, enhancing wheat growth in challenging environments (Singh *et al.*, 2022). Gupta *et al.* (2021), screened 25 strains of PGPR with ACC deaminase, the most active in ACC deaminasei were dentified as *Bacillus marisflavi* and *Bacillus cereus*. Their measured ACC deaminase activities of 0.395 mmol/mg/h and 0.368 mmol/mg/h, respectively, promoted *Pisum sativum* growth under salt stress conditions (Gupta *et al.*, 2021). Five strains containing ACC deaminase activity were isolated by soil-fold dilution, plate delineation methods and screening media in this research. These strains were YSX-12, YSX-23, YSX-36, YSX-47 and YSX-61 with ACC deaminase activities of 0.546 U/mg, 0.487 U/mg, 0.277 U/mg, 0.312 U/mg, 0.081 U/mg, respectively. Four of these strains exhibited greater ACC deaminase activity compared to the strain with ACC deaminase activity found in alfalfa rhizosphere soil by Kong *et al.* (2015), (0.217 U/mg).

Numerous studies have shown that strains with higher ACC deaminase activity have stronger nitrogen fixation, stress tolerance, bacterial inhibition, and growth-promoting ability (Orozco-Mosqueda *et al.*, 2020; Brunetti *et al.*, 2021; Sagar *et al.*, 2021). Compared to YSX-61, which has lower ACC deaminase activity, YSX-12, YSX-23, YSX-36, and YSX-47, which have higher enzyme activities, showed significant antibacterial activities against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. It has been shown that PGPR affects the antioxidant system of the plant by eliminating the excessive accumulation of radicals, thus protecting the metabolism of the plant (Najafi Zilaie *et al.*, 2022; Abdelkrim *et al.*, 2023). These four strains were also more capable of scavenging DPPH radicals, ABTS radicals and PTIO radicals; and more pronounced in promoting the growth of wheat seedlings and rice seedlings. At present, there are many species of ACC deaminase-containing rhizosphere promoting bacteria reported at home and

abroad, most of which belong to the genera *Pseudomonas*, *Bacillus*, *Enterobacter*, *Stenotrophomonas*, *Arthrobacter*), *Pantoea*, *Microbacterium*, *Vibrio* and *Klebsiella* (Glick, 2004; Cheng *et al.*, 2007; Zarei *et al.*, 2020). In this research, five Ili lavender rhizobacteria strains containing ACC deaminase activity were identified by morphology and molecular biology as *Pseudomonas rhizophila*, *Pseudomonas savastanoi*, *Klebsiella pasteurii*, *Klebsiella pneumoniae*, *Serratia plymuthica*. Two of the strains were identified as the genus *Pseudomonas*, two were the genus *Klebsiella*, and the remaining strain was the genus *Serratia*. Among the studies on strains with ACC deaminase activity, there are fewer reports on *Serratia*. This may be caused by the inconsistency in the distribution of bacterial populations with ACC deaminase activity in the rhizobacteria of different plants or in different soil environments and thus awaits further study and confirmation.

In summary, a variety of elements can impact the isolation and screening of ACC deaminase-containing strains and their enzyme activity levels. For example, differences in the plants from which the strains were isolated, differences in the soils in which the plants were grown, and the humidity, temperature, and pH of the laboratory environment can affect the isolation of ACC deaminase-active strains and their ACC deaminase activity, antibacterial, antioxidant, and growth-promoting abilities. This is generally consistent with the findings of our studies (Chandra *et al.*, 2020; Chen *et al.*, 2021; Nadeem *et al.*, 2021).

CONCLUSION

In this research, five ACC deaminase-containing active strains were isolated from the rhizosphere soil of Ili lavender, and four of them (*Pseudomonas rhizophila*, *Pseudomonas savastanoi*, *Klebsiella pasteurii* and *Klebsiella pneumoniae*) possessed bacteriostatic activity, antioxidant and growth-promoting abilities. In subsequent experiments, the active components of the strain products can be chemically analyzed and explored by chromatography. Research on the anthelmintic effects of the strain products can also be carried out, to provide a certain theoretical basis for the rational and comprehensive utilization of microbial resources.

CONSENT TO PUBLISH

The manuscript has neither been previously published nor is under consideration by any other journal. The authors have all approved the content of the paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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CONFLICTS OF INTEREST

The authors declare that they have no financial conflicts of interest.

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