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# *Alcaligenes faecalis* Juj3 alleviates *Plasmodiophora brassicae* stress to cabbage via promoting growth and inducing resistance

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Clubroot is a devastating disease threatening global cruciferous vegetable production caused by *Plasmodiophora brassicae* (*Pb*). We have evaluated the positive effects of the *Alcaligenes faecalis* Juj3 on cabbage growth promotion and *Pb* stress alleviation through pot and field experiments. The Juj3 strain was isolated from a healthy cabbage rhizosphere with growth-promoting characteristics and was identified as *A. faecalis* based on morphological traits and phylogeny. Seed germination assays revealed that Juj3 inoculation enhances cabbage bud shoot and root growth. In pot experiments, inoculation with Juj3 fermentation powder at cabbage sowing dates significantly improved the seedling biomass. Combining seed treatments with root irrigation after transplanting considerably reduced the clubroot disease index and resulted in appreciable biocontrol efficacy (83.7%). Gene expression analyses of cabbage after Juj3 inoculation showed that *PR2* and *EIN3* expression were significantly up-regulated. Physiologically, Juj3 inoculation enhanced cabbage chlorophyll content and root activity in a normal environment. Irrespective of whether plants were under normal environment or *Pb* stresses, Juj3 improved photosynthesis. Field trial analyses revealed that Juj3 exhibits satisfactory biocontrol efficacy in cabbage (51.4%) and Chinese cabbage (37.7%). Moreover, Juj3 could also enhance cabbage and Chinese cabbage biomass to improve the yield quality. These findings pave the way for future use of *A. faecalis* as biocontrol agents for clubroot and reveal the great potential of the rhizobacterium for plant growth-promoting applications in agriculture and horticulture.

## KEYWORDS

PGPR, *Plasmodiophora brassicae*, biocontrol efficacy, photosynthesis, induced disease resistance

## Introduction

Brassica crops provide vital sources for vegetable, cooking oil, and condiment uses for human consumption (Karamat et al., 2021). In recent years, soilborne diseases have become increasingly severe as the cultivated areas of cruciferous vegetables have increased. Clubroot, as one of the most economically severe diseases of cruciferous vegetables, caused a substantial proportion of these losses (Strelkov and Hwang, 2014) and is elicited by the obligate biotrophic parasite, *Plasmodiophora brassicae* (*Pb*) (Zhang et al., 2019). The pathogen induces abnormal cell division and enlargement of host root cells to produce spindle-like, spherical, knobby, or club-shaped root galls (Kageyama and Asano, 2009). *Pb* infections result in carbohydrate sinks from the viable gall cells, leading to wilting or plant death (Ludwig-Müller and Schuller, 2008; Ludwig-Müller et al., 2009). Hence, *Pb* substantially alters the morphology, development, and physiology of diseased plants (Malinowski et al., 2012) resulting in nutrients and severe yield losses in cruciferous vegetables. To ensure sustainable vegetable yields in cruciferous agrosystems during ever-increasing *Pb* stresses, integrated strategies involving host resistance, crop rotation, fungicides (Peng et al., 2014), and biocontrol agents (BCAs) (Zhu et al., 2019) are necessary for clubroot management. Among the various methods, the use of plant growth-promoting rhizobacteria (PGPR) has attracted the attention of many researchers.

The rhizosphere provides the frontline defense for plant roots against attack by soilborne pathogens (Cook et al., 1995). Their interactions have improved plant growth, yield, and stress tolerance of crops, and can be included in biofertilizers and biopesticide applications, which makes them a very effective organic alternative (Qessaoui et al., 2019; Singh et al., 2020). Several rhizosphere bacteria, including *Pseudomonas* spp., *Bacillus* spp., and *Alternaria* spp., are being used to successfully control soilborne diseases that have emerged in vegetable production (Agrillo et al., 2019; Zhang et al., 2020; Huang et al., 2021). PGPRs support plant growth under biotic stress by at least one of the direct and indirect mechanisms. For example, PGPRs increase the availability of nutrients to growing plants under nutritional imbalance conditions by influencing solubility or uptake of nutrients to improve plant vigor and increase root development (Etesami and Maheshwari, 2018). They can indirectly repress pathogens by stimulating the plant's defense system. Induced systemic resistance (ISR) is typically activated upon colonization of plant roots by beneficial microorganisms, and it is associated with priming for enhanced defense rather than direct activation of defense (Pozo et al., 2008). This priming leads to an enhanced expression of host defense-related genes upon subsequent attack by a pathogen.

*Alcaligenes faecalis* is a gram-negative bacterium that is widespread in soils. As a PGPR, *A. faecalis* has been

reported to have an active role in promoting the growth and development of plants (Li, 2011). Several investigations have shown that *A. faecalis* has antifungal activity against *Aspergillus niger*, *A. flavus*, *Paecilomyces variotii*, *Candida albicans*, *Alternaria alternata*, *Cercospora arachicola*, *Rhizoctonia solani*, and *Fusarium oxysporum* (Honda et al., 1999; Santoa et al., 2011; Sayyed and Patel, 2011). In recent years, *A. faecalis* has been found to biodegrade organic pollutants such as anionic surfactants (Fedeila et al., 2018), which is of great significance to research in the field of environmental protection. However, to our knowledge, there is no evidence as to whether *A. faecalis* can induce tolerance against *Pb* stress in cruciferous vegetables. In this study, we have isolated microorganisms from a healthy cabbage rhizosphere and have selected a bacterium, *A. faecalis* Juj3, with growth-promoting characteristics. To characterize the roles of *A. faecalis* on cabbage responses to *Pb* stresses, we assessed the effects of inoculating cabbage with Juj3 on (i) plant growth and development, (ii) disease resistance responses, and (iii) the infestation levels of *Pb* in greenhouse and field trials.

## Materials and methods

### Isolation of rhizospheric bacteria

Rhizobacteria soil samples of healthy cabbage were collected from a field infected with *Pb* at Baoji, Shaanxi, China (107°18'35" E, 34°2'51" N). Isolation of bacteria from cabbage rhizospheres was performed as previously stated (Cordovez et al., 2018). Briefly, the floating soil of the cabbage root was shaken off, and a sterile brush was used to gently brush the soil within 4 mm near the root as rhizosphere soil. Subsequently, the soil sample was air-dried for 3 days and passed through a 200-mesh sieve. Ten g rhizosphere soil was placed in 100 mL sterile distilled water (SDW) and shaken for 30 min at 180 r/min. The supernatant was serially diluted in SDW ( $10^{-1}$  to  $10^{-7}$ ) and plated on Luria-Bertani (LB) medium (10 g NaCl, 10 g tryptone, 5 g yeast extract, and 15 g agar per liter). After incubation at 30°C for 1–2 d, different colonies on the agar plates were observed. The morphologically distinct colonies of isolates were selected and streaked on LB plates. Purified bacterial cultures were preserved in 50% v/v glycerol stock at –80°C for further experiments.

### Inoculum production for the isolates

The isolated bacteria were cultivated on LB plates for 24 h at 30°C. A single colony was selected and incubated in conical flasks containing liquid LB (250 mL conical flasks, 100 mL each) at 30°C with shaking at 180 rpm for 48 h. The fermentation broth (FB) was then harvested and prepared for root irrigation test. After centrifuging at 8,000 rpm for 15 min, the supernatant

was filtered through a 0.22  $\mu\text{m}$  cellulose nitrate filter as cell-free suspensions used for metabolite detection. The cells were collected by centrifugation and resuspended in SDW as bacterial suspension (BS) used for seed treatment.

For formulations preparation, solid fermentations were carried out following the method described by Guo et al. (2020) with a slight modification. One kilogram powder could be generated with solid fermentation material composed of 600 g soybean meal, 600 g bran, 100 g sucrose, 100 g yeast extract, 1 g  $\text{MgSO}_4$ , 0.2 g  $\text{MnSO}_4$ , and 1,800 mL water. After all, the ingredients were mixed for about 60 min, and the solid fermentation medium was packed in 250 mL conical flasks (80 g per flask) and autoclaved for sterilization. The FB was inoculated in the sterile solid fermentation medium in a volume ratio of 1/10 and incubated at 30°C for 72 h. Finally, the semi-processed fermentation medium was dried in a 40°C oven for 96 h, crushed by a grinder, and the powder was collected from across a 200-mesh sieve used for the greenhouse trial.

## Pathogen inoculum preparation

Clubroot galls were collected from diseased cabbages in a heavily infested field, washed, dried, and stored at  $-20^\circ\text{C}$  until used. The crude resting spores were extracted based on the method of Zhu et al. (2019) with slight modifications. Briefly, about 10 g of stored galls were immersion in 50 mL SDW at 25°C for 2 h to soften the tissue and then were ground in a mortar. The resulting suspension was filtered through 8 layers of gauze. The obtained *Pb* suspension was estimated for resting spores concentration with a hemacytometer and diluted with SDW to  $1 \times 10^7$  spores/mL as crude inoculum. For *Pb* inoculation, a soil-free planting mix was infested with a *Pb* suspension ( $1 \times 10^7$  resting spores/mL) to result in an infestation level of about  $2 \times 10^6$  spores per gram of growth medium. The growth medium was then sealed for more than 48 h at 23°C before planting.

## Screening of bio-control strains with plant growth-promoting traits

### Siderophore production

The detection of siderophore production was performed according to a modified method by Bonaldi et al. (2015). A mixture of 10 mL of Chrome-Azurol S (CAS) reagent and 90 mL of sterilized LB agar medium was used for siderophore detection in CAS agar plates. After incubation, the formation of a clear zone around the colonies was reported as positive. The experiments were conducted in triplicate.

### IAA-like compounds production

Indole-3-acetic acid (IAA) production was determined via Salkowski's colorimetric method (Sachdev et al., 2009). Briefly, 1 mL of cell-free suspensions was added to 2 mL of Salkowski reagent. After 30 min of incubation, the appearance of the color pink indicated a positive production of IAA. The experiments were conducted in triplicate.

### Growth in nitrogen-free medium

The strains were evaluated for their nitrogen fixation ability on Burk's modified N-free medium (pH 7.00) (Franche et al., 2008). In short, the appearance of bacterial growth on the medium was taken as a positive test for the nitrogen fixation experiment. The experiments were conducted in triplicate.

### Phosphate solubilization

The phosphate-solubilizing activities of the strains were evaluated on the organophosphorus medium (Yang and Yang, 2020). Following bacterial culture at 30°C for 5 d, the occurrence of a transparent phosphorus solubilizing circle on the medium was interpreted as the bacteria having the capability of dissolving organic phosphorus. The experiments were conducted in triplicate.

## *In vitro* plant growth promotion assays

For *in vitro* assays, the Haoyuan (Shanghai Xinglv Vegetable Seedling Research Institute) cabbage variety that is susceptible to clubroot disease was used in this study. Cabbage seeds were surface-sterilized by soaking in 70% ethanol for 2 min, immersed in 10% sodium hypochlorite for 5 min, and rinsed four times with  $\text{ddH}_2\text{O}$  to remove residues. At least 27 sterile seeds were soaked in bacterial suspensions at concentrations of  $1 \times 10^6$  colony-forming units (CFU)/mL or SDW for 2 h to make Juj3 colonize on the cabbage seeds, followed by rinsing three times in  $\text{ddH}_2\text{O}$ . Seeds were then germinated on 90 mm diameter Petri dishes containing two water-soaked filter papers in a climate cabinet at 23°C (16 h: 8 h, light: dark). The germination rates of the seeds were tested on the second day, and three replicates were used per treatment.

To observe the effects of the isolates on cabbage growth at the seedling stage, a root elongation test was conducted in a growth pouch. Surface-sterilized cabbage seeds were inoculated with the isolates described above. Twenty-five seeds per treatment were transferred into sterile growth pouches that had been saturated with sterile Hoagland's nutrient solution. Three replicates were used for each treatment, and the seedlings were kept in the dark at 23°C for 1–2 days until they germinated. They were then cultured at 23°C for 5 days under

a 16-h photoperiod, and their plant heights and root lengths were measured.

## Identification of Juj3

Morphological and molecular characteristics of Juj3 were used for taxonomic identification after streaking on LB plates and incubation at 30°C for 48 h and observations by scanning electron microscope (Hitachi model S-3400N, Japan). To characterize isolates phylogenetically, 16S rRNA genes were amplified by Polymerase Chain Reaction (PCR) with the primers 27F (5'-ATGCCATTCGTGCGGAGGTTG-3') and 1492R (5'-CGTCTCTGCTGTCATCACTTCGTAT-3') (Zhang et al., 2021). Genomic DNA extractions from the Juj3 strain were performed by use of the Bacterial Genomic DNA Extraction kit (Tiangen Biotech co. LTD). The 25- $\mu$ L reaction mixtures contained 2  $\mu$ L of DNA template, 1  $\mu$ L (0.4  $\mu$ M) of each primer, and 12.5  $\mu$ L Taq Mix (Vazyme). PCR conditions for 16S rRNA gene amplification were as follows: 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1.5 min. A final extension step was carried out at 72°C for 10 min. The amplified 16S rRNA fragments were sequenced (Qingke, Xian, China) and submitted to the EzTaxon-e server (<http://www.ezbiocloud.net/>) for comparative analyses. Phylogenetic trees were constructed with MEGA 7.0 software using the Maximum Likelihood method with 1,000 bootstrap replications (Kumar et al., 2016b).

## Effects of Juj3 on photosynthesis and root viability of cabbage seedlings

### Experimental design

Photosynthesis and root viability assays were carried out under normal and stress conditions to determine whether Juj3 could enhance plant health and growth. For the *Pb* stress test, sterile cabbage seeds were sowed in the plant plug trays (15  $\times$  7-well) filled with a growth medium containing *Pb* ( $2 \times 10^6$  spores/g). Fermentations with different root irrigation concentrations of Juj3 ( $1.00 \times 10^6$ ,  $1.00 \times 10^5$ , and  $1.00 \times 10^4$  CFU/mL) with 25 mL per pot were performed at 7 days and 14 days after seeding. Six hundred times dilutions of cyazofamid (20%) with 25 mL per pot were served as positive controls, and blank controls received 25 mL of water only. The non-stress experiments were conducted in plant plug trays filled with V9 soilless planting mix in which sterile cabbage seeds were inoculated with Juj3, cyazofamid, and water, respectively, as described above. All treated plants were placed in a growth cabinet at 23°C with a 16-h photoperiod. Each treatment consisted of five plants in a trial, and the trial was replicated at three different times. The physiological indexes such as

photosynthesis, chlorophyll content, and root viability were determined after 30 days of sowing.

### Photosynthesis measurement

Leaf photosynthetic characteristics (i.e., the net photosynthetic rate, stomatal conductance, intercellular CO<sub>2</sub> concentration, and transpiration rate) were measured on top leaves with a CIRAS-3 portable photosynthesis system (PP SYSTEMS CIRAS-3) on sunny days. The chlorophyll content was determined as described previously (Zhang et al., 2016; Zhou et al., 2021). In brief, 0.10 g of fresh cabbage leaf tissue was harvested from each treatment, homogenized in 80% acetone, and centrifuged at 8000 r/min for 5 min. Then, the supernatant was obtained, and absorbance was measured through the UV-Vis spectrophotometer at 645 nm and 663 nm.

### Analysis of root activity

Root activities were measured by the triphenyl tetrazolium chloride (TTC) method. After weighing the roots, they were immersed in 10 mL of an equally mixed solution of 1% TTC and phosphate buffer and kept in the dark at 37°C for 1 h. Subsequently, 2 mL of 1 M H<sub>2</sub>SO<sub>4</sub> was added to end the reaction with the root. Samples, carefully wiped and cut into segments, were then dried and ground with 3–4 mL ethyl acetate and a small amount of quartz sand to extract triphenyl tetrazolium formazan (TTF). Transfer the red extract into the test tube with a small amount of ethyl acetate, washing the residue two to three times, and then transferring the residue into the test tube. Finally, add ethyl acetate to make the total amount of 10 mL, and the absorbance of the extract at 485 nm was recorded. Root activity was expressed as TTC reduction intensity, and the formulae are described below.

$$\text{Root activity} = \frac{\text{Amount of TTC reduction } (\mu\text{g})}{\text{Fresh root weight (g)} \times \text{time (h)}}$$

### Relative expression of cabbage defense-related genes

Surface-sterilized cabbage seeds were inoculated with the Juj3 as described in Section *In vitro* plant growth promotion assays, and seeds soaked in SDW were used as controls. After being fully air-dried, the seeds were then sowed in the plant plug trays (15  $\times$  7-well) filled with the growth medium containing *Pb* ( $2 \times 10^6$  spores/g). All treated plants were placed in a growth cabinet at 23°C with a 16-h photoperiod. Each treatment consisted of five plants in a trial, and the trial was replicated at three different times. Fourteen days after sowing, roots from three cabbages (a replicate) as well as the first true leaves of each plant were collected. All

harvested samples were immediately put into liquid nitrogen. Total RNA from cabbages were extracted using Trizol and converted to cDNA using Hipure Plant RNA Mini Kit (Magen) protocols.

To examine the defense gene expression in cabbage, a quantitative real-time PCR (qRT-PCR)-based assay was developed for several putative genes, such as pathogenesis-related protein 1 (*PR1*), *PR2*, *PR3*, transcription factor ethylene insensitive 3 (*EIN3*), phenylalanine ammonia-lyase (*PAL*), and NIMI-interacting 2 (*NIMI2*). The gene *Actin* was chosen as the internal control gene. Sequences for gene-specific primer pairs were designed based on sequences available in the GenBank database, and primer sequences were designed using Primer Premier 5.0. Details of these genes and their primer sequences are given in [Supplementary Table S3](#). The qRT-PCR was performed using UltraSYBR Mixture (CW BIO, China), and the amplification was performed according to the manufacturer's instructions. The relative fold change of target gene expression in Juj3-treated plants was calculated and normalized against its expression levels in water-treated plants, using the  $\Delta\Delta C_t$  method as previously described method ([Kenneth and Thomas, 2001](#)).

## Greenhouse experiments

### Effects of Juj3 on cabbage seedling growth

For seedling growth assays, surface-sterilized cabbage seeds were sown in substrates mixed with different doses of Juj3 solid fermentation powder. The numbers of viable bacteria in the Juj3 bacteria power were counted. The Juj3 bacterial powder and sterile substrates were mixed uniformly at a mass ratio of 1:9, 1:19, 1:39, 1:79, and 1:159 to provide final bacteria concentrations in the soil of  $1.00 \times 10^7$ ,  $5.00 \times 10^6$ ,  $2.50 \times 10^6$ ,  $1.25 \times 10^6$ , and  $6.25 \times 10^5$  CFU/g, respectively. Seeds were germinated in 72-cell plant plug trays filled with the above growth medium and were sown in sterile substrates as blank controls. Two seeds were sown per hole, and each treatment was repeated three times. The fresh weight, dry weight, root weight, shoot weight, stem diameter, and plant heights were measured 35 days after sowing.

### Biocontrol assay

To evaluate the biocontrol efficacies of the Juj3 strain against clubroot under greenhouse conditions, seeds were processed by combining Juj3 solid formulations and fermentation broth. Based on the experimental results of the Section Effects of Juj3 on cabbage seedling growth protocols, a Juj3 formulation of  $2.50 \times 10^6$  CFU/g was mixed evenly with the soil-less mix. Seeds were sown in plant plug trays filled with the mixture and grown under the same conditions as above. Then after 35 days of culture, single cabbage seedlings were transplanted

into nutrient bowls filled with the *Pb* mix. For root irrigations, the initial Juj3 fermentation broth was diluted with SDW to produce 2-, 4-, and 8-fold diluted suspensions. Root irrigations were performed at transplanting time, 7 days, and 14 days after transplanting with 25 mL of solution for each pot. Positive controls consisted of 600-fold dilutions of 20% cyazofamid, and SDW was used as a blank control. Each treatment involved 30 transplanted seedlings, and the experiment was repeated three times. The treated plants were kept in a growth chamber at 23°C (16-h photoperiod, 90% relative humidity for 40 days). A disease severity index (DSI) was then calculated by the [Klewer \(2009\)](#) formula:

$$DSI = \frac{\sum (n \times 0 + n \times 1 + n \times 2 + n \times 3 + n \times 4)}{N \times 4} \times 100$$

where *n* is the number of plants in a class; *N* is the total number of plants in an experimental unit; and 0, 1, 2, 3, and 4 are the symptom severity classes. The control efficiency formula was:

$$\text{Control efficacy} = \frac{(CDI - TDI)}{CDI} \times 100\%$$

where CDI refers to the DSI of the control group, and TDI denotes the DSI of the treatment group.

## Field trials

### Experimental design

To evaluate the biocontrol effects of Juj3 on clubroot disease under field conditions, we chose the Haoyuan cabbage and Chinese cabbage (Qingza No. 3, Qingdao Xingye Seeds Co., Ltd.) as the target vegetables. The experimental test plot was located in Tangkou Village, Taibai County, Shaanxi province (107°18'35" E, 34°2'51" N). Two fields infested with *Pb* were used for testing from March 2018 to October 2018. The preceding crop was Chinese cabbage. Before winter, subsoiling and basal fertilizers of N, P, and K 100 kg/667 m<sup>2</sup> were applied to the experimental plots.

Seeds were sown in 72-cell plant plug trays filled with substrate, with 2 seeds per hole to ensure germination. After sowing, the seedling trays were placed on pre-leveled seedling beds in the experimental field, and the soils were kept moist throughout the seedling stage. Both Cabbage and Chinese cabbage field trials included seven treatments with three replications consisting of a planting density of 35 × 50 cm in a completely randomized block design. There were 5 concentration gradients of fermentation broth in biocontrol treatment, and 2-fold serial dilutions (2-, 4-, 8-, 16-, and 32-fold) were made in SDW. The 600-fold dilutions of 20% cyazofamid were used as a positive control, and SDW acted as a blank control. Root irrigation was carried out at transplanting time, 7 days, and 14 days after transplanting, and the amount of root irrigation was 100 mL/plant per time. The area of each



plot was 0.0012 hm<sup>2</sup>, and there were 42 plots in this trial. An isolation zone of 1 m was set up to prevent water and gas flow. A protection line was set around the test site, and the field was routinely managed.

## Measurements

A five-point sampling method was used, and at least 10 plants were obtained to calculate the cabbage and Chinese cabbage disease indexes in each plot. The total number of inspected plants and the number of diseased plants at each grade were recorded. Infected cruciferous vegetables were scored according to the following: 0, no galls; 1, a few small clubs on the lateral roots; 3, most lateral roots swelling and obvious swelling of expanding primary roots; 5, enlarged napiform taproot, no wilting and yellowing symptoms; 7, all taproots are swollen but not rotted, plant wilting or yellowing and growth cessation; 9, swollen and rotten roots, and nearly dead plants. Field disease severity indexes (FDSI) were calculated as follows, and the field control efficacy was counted as described above in Section Biocontrol assay.

$$\text{FDSI} = \frac{\sum (n \times 0 + n \times 1 + n \times 3 + n \times 5 + n \times 7 + n \times 9)}{N \times 9} \times 100$$

To assess the effects of Juj3 on the cabbage's growth and quality, ten plants per plot were used for the experiments. The relevant growth-promotion parameters, including cabbage height, commodity fresh weight, and stem diameter, were evaluated at the yield stage. Concurrently, the height and commodity fresh weight of Chinese cabbage were investigated. A group of treatments with the best control effect of Juj3 was selected to determine cabbage leaf quality. The nitrate, total amino acid, vitamin C and reducing sugar contents were measured using salicylate spectrophotometric, ninhydrin colorimetry, ultraviolet spectrophotometry, and 3, 5-dinitrosalicylic acid (DNS) method (Miller, 1959; Stevens et al., 2006; Wei et al., 2018), respectively.

## Data analysis

All data were presented as mean values  $\pm$  standard deviation (SD), except for data from field experiments, and the values are presented as  $\pm$  standard error mean (SEM). Data were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range tests using SPSS11.0 (SPSS Inc., Chicago, IL, USA). Different lowercase letters indicate a significant difference at the 0.05 level. Data were analyzed by the two-tailed *t*-test using the Student's *t*-test, as appropriate.

## Results

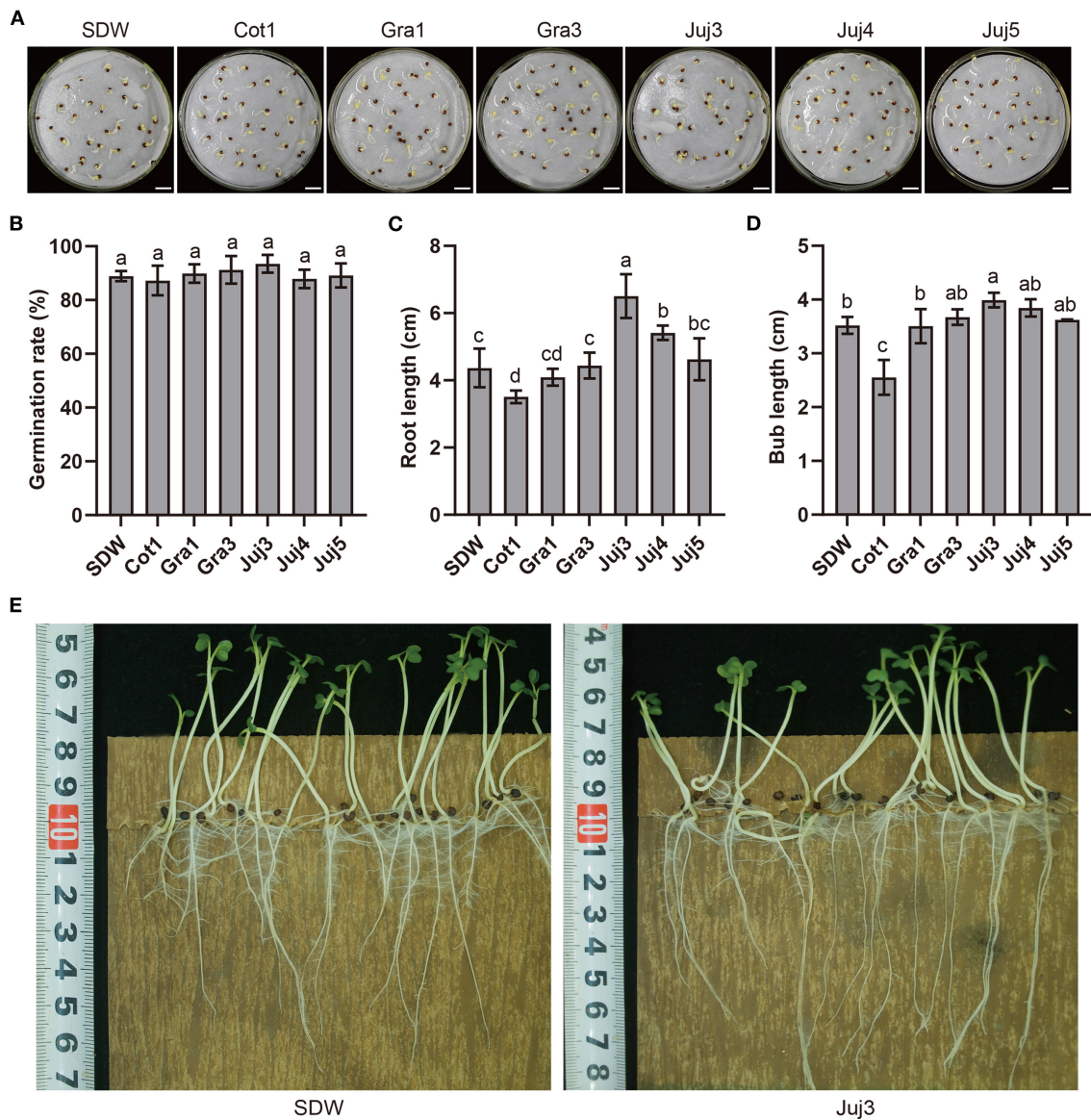
### PGP attributes of the isolates

Eighteen morphologically different bacteria strains (numbered Juj1 to Juj6, Cot1 to Cot4, Gra1 to Gra5, and Cab1 to Cab3) were isolated from rhizosphere soils collected in Taibai County, Shaanxi Province, China (data not shown). The isolates were analyzed for their plant growth-promoting (PGP) attributes. Isolates Juj3, Juj4, Juj5, Cot1, Gra1, and Gra3 were promising for PGP attributes (Supplementary Table S1), which showed nitrogen fixation abilities (Supplementary Figure S1) and siderophore production (Supplementary Figure S2). The Juj3 strain was positive for phosphorous solubilization ability (Supplementary Figure S3). None of the six isolates affected cabbage seed germination (Figures 1A,B). Among these isolates, Juj3 was rated the best because it could enhance the length of cabbage seedlings and facilitate plant growth. As shown in Figure 1B, although there were no significant differences from the control, the germination rates of cabbage seeds treated with Juj3 were 5.2% higher than the control group. The significant effects of Juj3 on the root length and bud length of the seeds at early time points (5 dpi) increased by 49.0% (Figure 1C) and 13.4% (Figure 1D) respectively compared with the control (Figure 1E).

### Identification of Juj3

The rhizosphere bacterium Juj3 was identified as *Alcaligenes faecalis* based on phenotypic characterization coupled with phylogeny analyses of 16S rRNA gene sequences. Morphologically, the Juj3 colonies after 48 h incubation on LB medium were creamy white in appearance, had a smooth surface, with smooth, regular margins, and were opaque, moist, and did not produce pigment (Figure 2A). Under the scanning electron microscope, the Juj3 cells were rod-shaped with peritrichous flagella and consisted of cell sizes between 0.6 and 1.0  $\mu$ m that developed into long and elliptical spores (Figure 2B).

To confirm the Juj3 phylogenetic relationship with *A. faecalis*, we used the original 16S rRNA sequences available in the NCBI SRA database with the accession number ON028636 to conduct alignments in the EzTaxon-e server. Analysis of the 16S rRNA sequences demonstrated that Juj3 matches *A. faecalis* subsp. *Phenolicus* DSM 16503 at 99.3% in the EzTaxon-e server, and they cluster into a single group (Figure 2C). These results clearly show that strain Juj3 is *A. faecalis*.



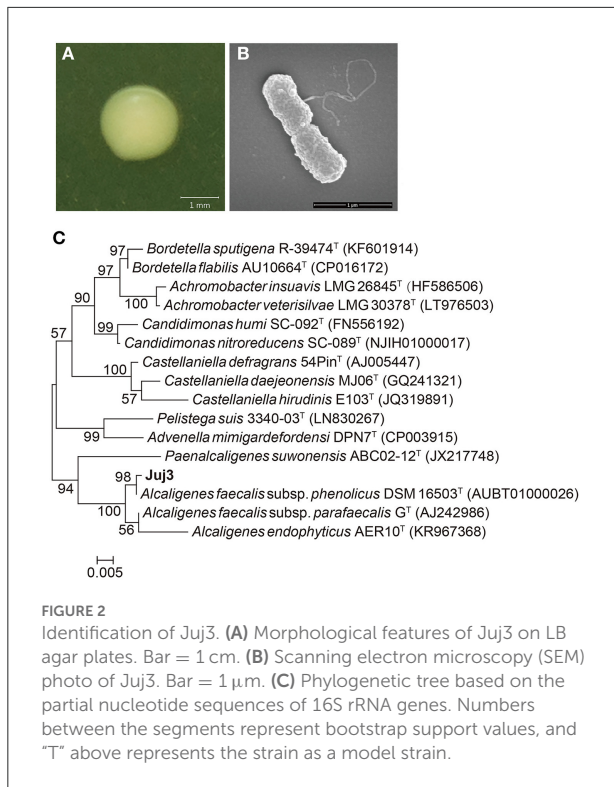
**FIGURE 1** Cabbage seeds growth-promoting effects of the isolates. **(A)** Effect of six isolates on cabbage seed germination. Bar = 1 cm. **(B)** Seed germination rates after isolate treatments. Germination rates were determined 2 days after sowing. Values in the chart were mean ± SD. Different lowercase letters indicate significant differences at the 0.05 percent levels by the Duncan’s new multiple range test. **(C,D)** Average root and bud lengths (cm) of cabbage treated with the six isolates. **(E)** Effects of Juj3 on cabbage growth at the 5-day-old seedling stage.

### Photosynthetic rate, chlorophyll content, and root activity analysis

To further investigate the physiological impacts of Juj3 on cabbages, we determined the photosynthetic rates, chlorophyll content of leaves, and root activity of Juj3 treatments during normal and *Pb*-stress conditions.

### Photosynthetic rate

Photosynthesis is the basis of plant growth and development, and the rate of photosynthesis directly determines the growth rate of plants. As shown in **Figure 3**, photosynthesis of *Pb*-infected cabbage plants was significantly lower than healthy plants. Stomatal conductance was also markedly ( $P < 0.01$ ) reduced after *Pb* infection and the CK treatment values under *Pb* stress were 57.5% lower than that of healthy plants (CK-normal)



(Figure 3B). Regardless of whether cabbages were grown under normal conditions, all photosynthesis-related parameters except for intercellular CO<sub>2</sub> concentrations were maintained at relatively high levels or were significantly improved after the addition of Juj3 at concentrations of  $1.00 \times 10^6$  CFU/mL (T1) (Figure 3D). Under *Pb* stress after the addition of Juj3, the leaf assimilation rates, stomatal conductance, and transportation rates increased by 45.2% (Figure 3A), 0.2% (Figure 3B), and 7.8% (Figure 3C), respectively, each of which were significantly higher than that of the CK group. Similar trends (~1.3-fold greater than CK treatments) were observed under normal conditions when the concentration of Juj3 was  $1.00 \times 10^6$  CFU/mL (Figure 3A).

## Chlorophyll content

To explore whether changes in photosynthetic pigment content resulted in photosynthesis alterations, we examined three different photosynthetic pigment content traits: chlorophyll a, chlorophyll b, and total chlorophyll (Table 1). In summary, the chlorophyll content of all treatment groups after *Pb* infection was higher than under the normal environment, and the major changes were for chlorophyll b. In the case of *Pb*-stress, the chlorophyll content of the T1 treatment ( $1.00 \times 10^6$  CFU/mL) group decreased significantly, compared with the control group, and there was no significant difference with cyazofamid (Cy) treatment. Under normal conditions, each concentration of Juj3 increased the chlorophyll a and

total chlorophyll contents to different degrees. When the concentration was  $1.00 \times 10^6$  (T1) and  $1.00 \times 10^5$  CFU/mL (T2), the chlorophyll a content was enhanced significantly compared with the control. The T1 treatment increased total chlorophyll contents, and there was no significant difference in comparison to the CK. The T2 treatment increased the total chlorophyll content by 23.3% compared to the control.

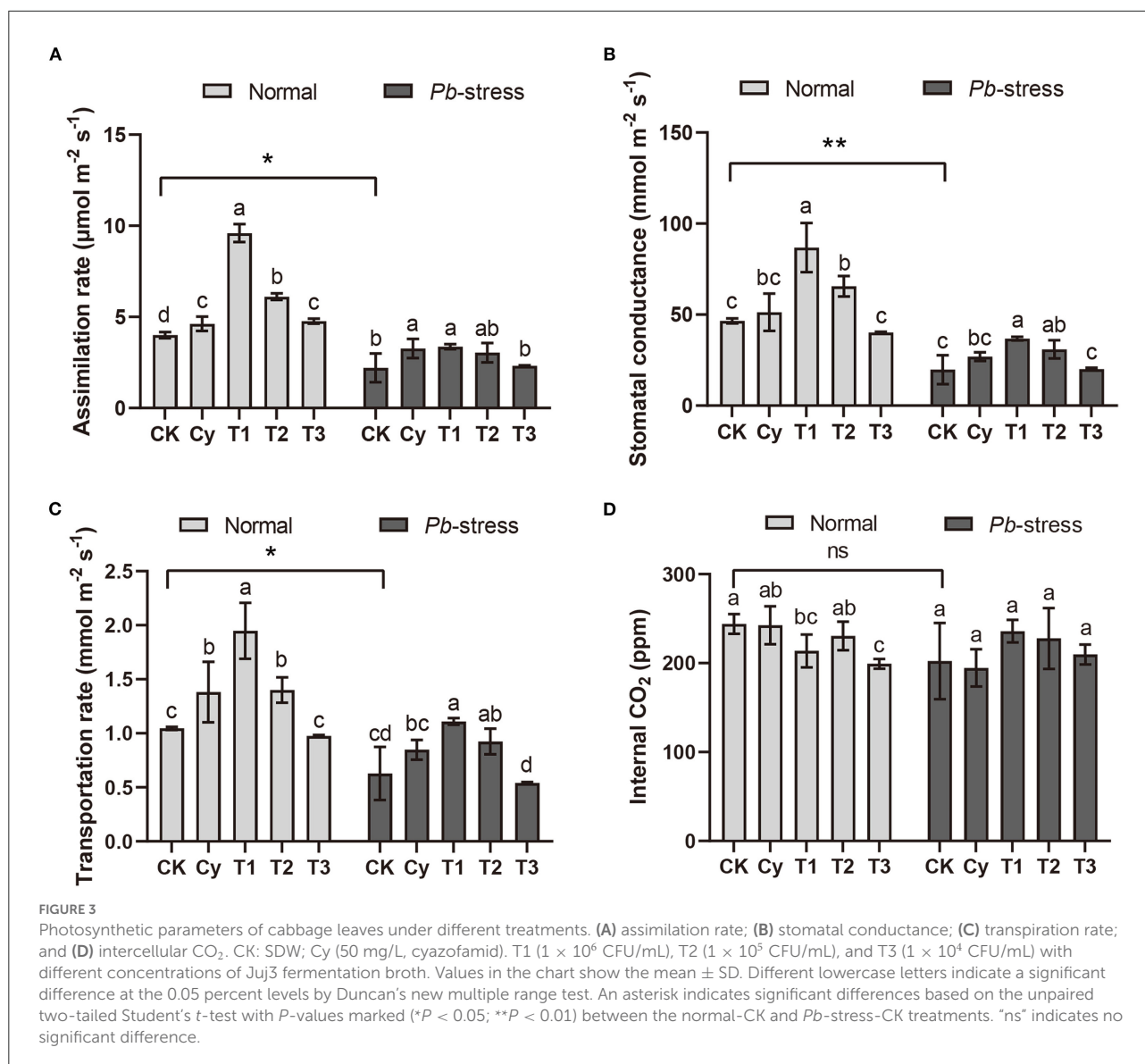
## Root activity

As a good root metabolic reactive agent, TTC receives electrons directly from the electron transport link, and transport reductions are directly related to the mitochondrial respiration rate (Comas et al., 2000; Ruf and Brunner, 2003). In our experiments, there was a significant increase in the root TTC activity under *Pb* stress (Table 1). For CK treatments, the TTC reductive intensity in roots with *Pb*-infected was 4.0-fold higher than in healthy cabbage roots and the root activities of CK treatments were higher than in other *Pb* stress treatments. The T1 ( $1.00 \times 10^6$  CFU/mL) and T2 ( $1.00 \times 10^5$  CFU/mL) treatments were significantly different CK treatments and were very similar to the Cy treatments. The TTC reductive intensity in all three concentrations of Juj3 was higher than with CK, and the most significant difference was from the T3 treatment.

## Beneficial rhizobacteria trigger changes in the expression of defense-related genes

We evaluated if the application of strain Juj3 could activate the expression of defense-related genes in cabbage. When cabbage plants were treated with Juj3 plus *Pb*, gene *EIN3* (transcription factor ethylene insensitive 3) activity generally and its expression increased 3.4- and 1.1-fold in the roots and leaves relative to *Pb* inoculated (Figure 4). The gene expression related to PR2 protein biosynthesis ( $\beta$ -1,3-glucanase) was also induced by inoculation with Juj3 plus *Pb*. Even though the gene expression of *PR2* was increased 2.8-fold compared with the controls, the expression of this gene did not change significantly in leaves. The genes *PR1* and *NIM12*, associated with salicylic acid (SA) signaling pathways, showed the same expression tendency. That is, gene expression was not significantly different from control in the root but significantly decreased in leaves. The gene *PR3*, encoding a class I chitinase, was down-regulated in leaves of treated plants, although the level of gene *PR3* expression in roots was not significantly different from the control. Only expression of gene *PAL* (involved in the phenylpropanoid pathways) decreased substantially in roots and leaves.





## Growth-promoting effects of Juj3 on cabbage in planta and plot experiments

Growth-promoting effects of Juj3 on cabbage at the seedling stage were assessed after 35 days of seeding in the pot experiments. As shown in Figure 5A, cabbage treated with Juj3 have better developmental processes. However, seedling emergence of cabbage was inhibited by  $1.00 \times 10^7$  CFU/g concentrations of Juj3 (data not shown). According to the bar graph (Figures 5B–G), among 6 parameters, the cabbage dry weight, stem diameter, and height significantly increased during Juj3 treatment. Concentrations of  $2.50 \times 10^6$  and  $1.25 \times 10^6$  CFU/g significantly increased the numerical data for all the test

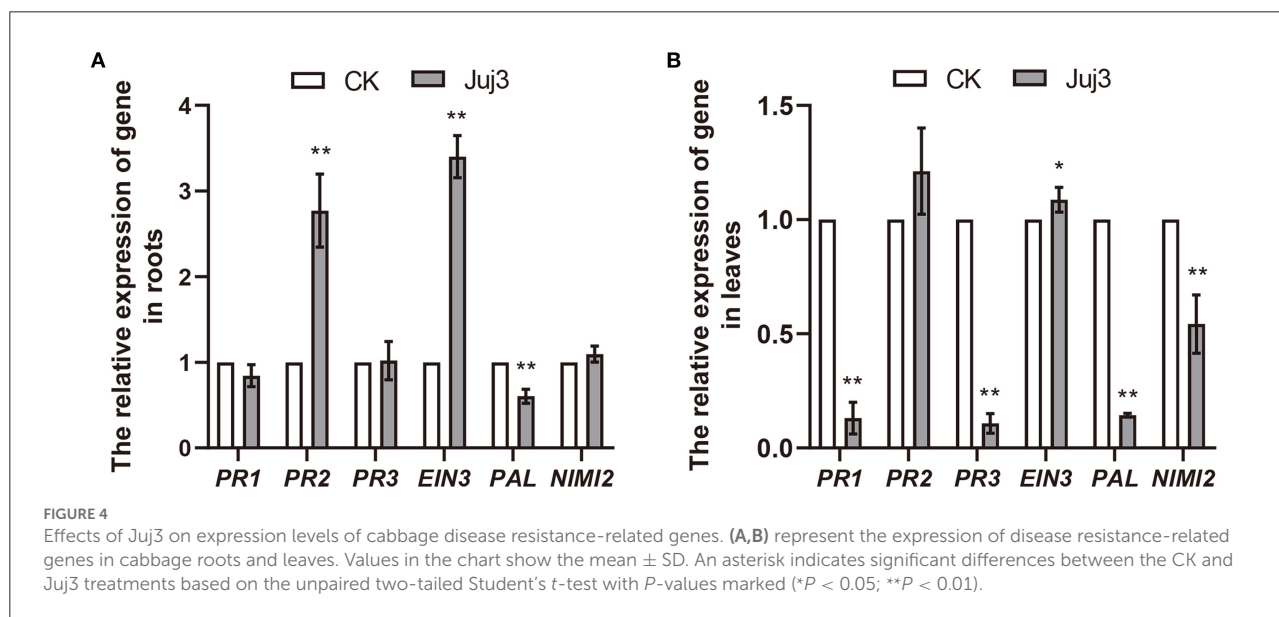
parameters compared to the SDW control and exhibited the best growth-promoting effects among the treatments.

In field trials, the cabbage and Chinese cabbage biomasses were measured at the harvest stage. The fresh weights of plants treated with Juj3 and combined with 8-fold (8n) and 16-fold (16n) dilutions of fermentation broth had significant improvements in fresh weight in both cabbage (Figure 6A) and Chinese cabbage (Figure 6B) when compared to the controls (CK). As shown in Supplementary Table S2, 2-fold (2n), 4-fold (4n), and 8-fold (8n) dilutions of fermentation broth significantly increased the cabbage and Chinese cabbage height and cabbage stem diameter. Additionally, the results of quality parameter measurements demonstrated that Juj3 fermentation

TABLE 1 Effects of Juj3 and cyazofamid on chlorophyll content and root activity in cabbage seedlings.

Set	Chlorophyll a (mg/g)		Chlorophyll b (mg/g)		Total Chlorophyll (mg/g)		Root activity ( $\text{g g}^{-1} \text{h}^{-1}$ )	
	Normal	<i>Pb</i> -stress	Normal	<i>Pb</i> -stress	Normal	<i>Pb</i> -stress	Normal	<i>Pb</i> -stress
CK	1.68 ± 0.10 b	2.32 ± 0.09 a	0.71 ± 0.02 a	1.04 ± 0.10 a	2.48 ± 0.06 b	3.52 ± 0.18 a	0.95 ± 0.28 b	4.72 ± 1.77 a
Cy	1.67 ± 0.14 b	1.89 ± 0.08 b	0.74 ± 0.11 a	0.87 ± 0.09 ab	2.50 ± 0.07 b	2.87 ± 0.13 b	1.24 ± 0.25 ab	1.46 ± 0.12 b
T1	2.00 ± 0.12 a	1.89 ± 0.09 b	0.66 ± 0.20 a	0.81 ± 0.05 b	2.76 ± 0.17 ab	2.83 ± 0.17 b	0.99 ± 0.18 b	1.49 ± 0.44 b
T2	2.06 ± 0.17 a	2.15 ± 0.10 a	0.88 ± 0.09 a	0.95 ± 0.06 ab	3.06 ± 0.20 a	3.27 ± 0.19 a	1.20 ± 0.15 ab	2.23 ± 0.16 b
T3	1.84 ± 0.08 ab	2.29 ± 0.09 a	0.81 ± 0.08 a	1.02 ± 0.05 a	2.68 ± 0.20 b	3.51 ± 0.14 a	1.61 ± 0.29 a	3.09 ± 0.61 ab

CK: SDW; Cy (50 mg/L, cyazofamid). T1 ( $1 \times 10^6$  CFU/mL), T2 ( $1 \times 10^5$  CFU/mL), and T3 ( $1 \times 10^4$  CFU/mL) indicate the treatment of different concentration fermentation broth of Juj3. Values in the table show the mean ± SD. Different lowercase letters indicate a significant difference between different at the 0.05 percent levels by Duncan's new multiple range test.



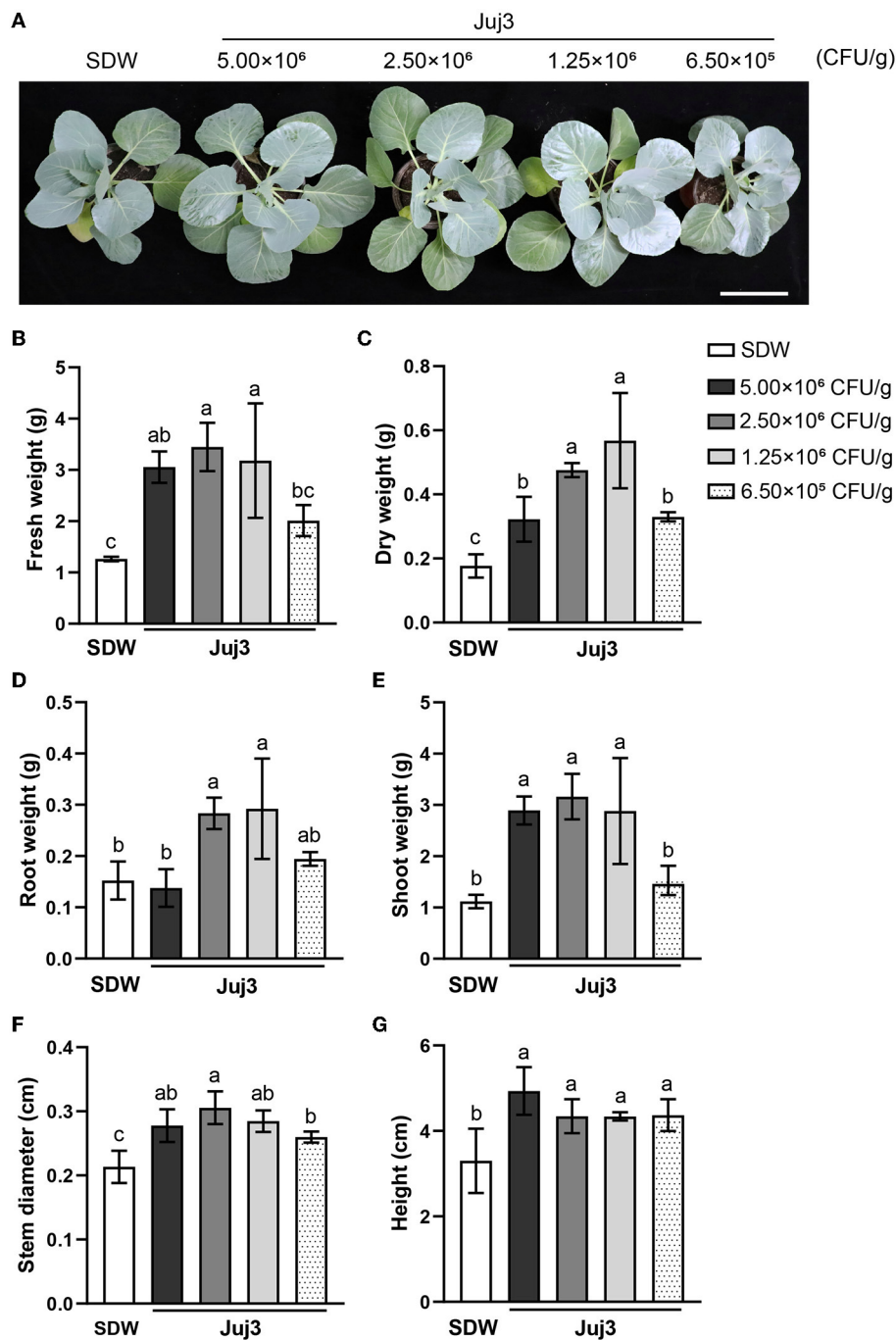
broth resulted in a significant improvement in cabbage quality. Juj3 reduced the nitrate content in cabbage leaves to <19.3% of the control-treated cabbages and conspicuously elevated the reducing sugar contents to 26.0 % over the SDW treatment (Table 2). Unexpectedly, the vitamin C content in cabbages treated with Juj3 was 175.0 mg/100 g, ~1.17-fold higher than that of in-control plants.

## Control effects of Juj3 on cabbage clubroot in pot and field experiments

In the pot experiments, all treatments inoculated with Juj3 ( $2.50 \times 10^6$  CFU/g) in the sowing period exhibited significantly reduced disease indices when compared with the blank control (CK) (Figures 7A,B). Even roots irrigated with SDW at transplanting had significantly lower disease indexes (51.7) than the control (65.6). Among all treatments, the 2-fold

dilution treatment (2n) resulted in the best cabbage clubroot biocontrol effects of 83.7%, which were significantly higher than the 73.7% cyazofamid treatment (Figure 7C).

In the field experiments, the clubroot disease severity was assessed according to the disease index at the harvest stage (Table 3). Viewed as a whole, the disease indices of the cabbage plots were generally lower than those of the Chinese cabbage plots. However, all five tested Juj3 concentrations had significant effects on disease severity compared with the control treatments, with the cabbage DSIs ranging from 18.0 to 30.4 and the Chinese cabbage ranges from 30.4 to 38.2. Among all of the Juj3 concentrations, in contrast, the 8-fold dilution treatment (8n) exhibited high control efficiency for clubroot in both cabbage and Chinese cabbage fields. The 8n treatment provided the highest control efficiency in the cabbage fields (51.4%) and was significantly higher than the cyazofamid group (32.7%). Taken together, these results indicate that Juj3 is a very effective clubroot biological control agent.



**FIGURE 5** Dosage effects of various concentrations of Juj3 solid fermentation powder (SFP) on cabbage seedling growth. (A) Cabbage growth at 35 days after sowing. Soil treatments were carried out with different Juj3 SFP doses before sowing. SDW: sterile distilled water. Bar = 10 cm. Effect of Juj3 on the (B) fresh weight, (C) dry weight, (D) root weight, (E) shoot weight, (F) stem diameter, and (G) height of cabbage seedlings. Values in the chart are the mean ± SD. Different lowercase letters indicate significant differences at the 0.05 percent levels by Duncan's new multiple range test.

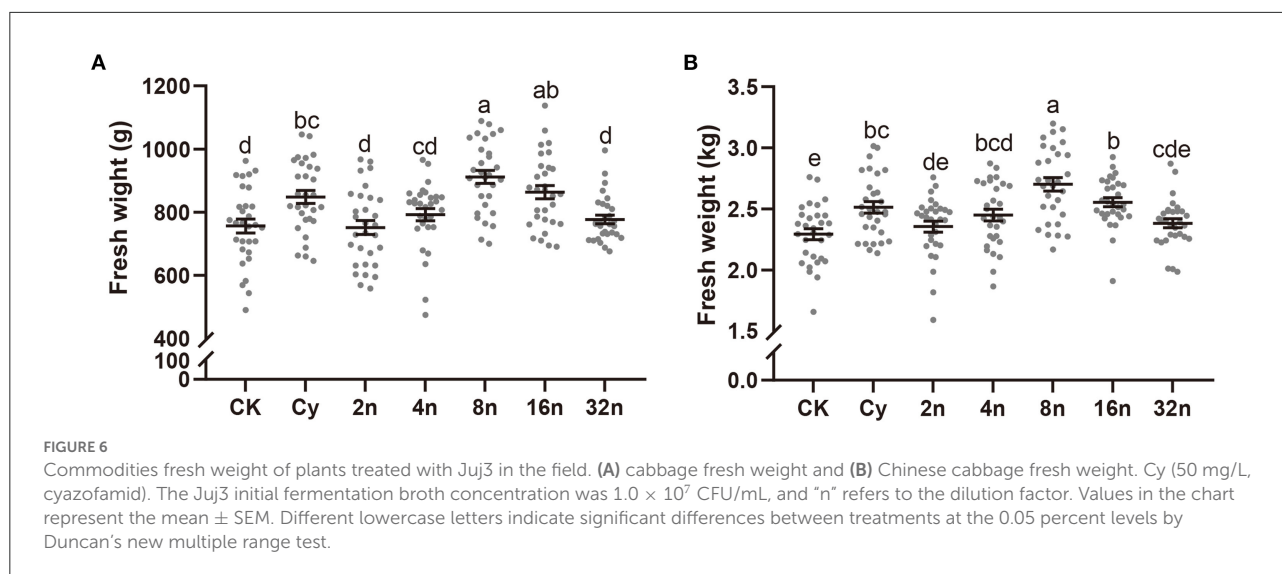


TABLE 2 Effects of Juj3 on cabbage leaf quality.

Treatment	Amino acid content (mg/g)	Reducing sugar content (mg/g)	Vitamin C content (mg/100 g)	Nitrate content (mg/g)
CK	37.83 $\pm$ 7.20 b	38.84 $\pm$ 0.83 c	80.78 $\pm$ 8.24 b	8.95 $\pm$ 0.24 a
Cy	66.32 $\pm$ 1.80 a	45.44 $\pm$ 0.99 b	218.77 $\pm$ 38.96 a	9.21 $\pm$ 0.15 a
Juj3	44.20 $\pm$ 1.71 b	48.93 $\pm$ 0.97 a	175.01 $\pm$ 20.75 a	7.22 $\pm$ 0.71 b

CK: SDW; Cy (50 mg/L, cyazofamid); the concentration of Juj3 fermentation broth was  $1.25 \times 10^6$  CFU/mL. Values in the table are mean  $\pm$  SD. Different lowercase letters indicate a significant difference between different at the 0.05 percent levels by Duncan's new multiple range test.

## Discussion

Plant growth-promoting rhizobacteria (PGPR) provide attractive sustainable ways for agriculture to cope up with biotic and abiotic stresses (Alexander et al., 2021). These agents improve plant growth and development directly and indirectly. Direct effects include more effective nitrogen fixation, phosphate solubilization, siderophore, and phytohormone production and indirect effects are as biocontrol actions or activation of ISR in host plants (Vessey, 2003; Van Loon, 2007). Although *Alcaligenes faecalis* has been reported to enhance rice growth, it can also inhibit the growth of various pathogenic fungi (Li, 2011; Santoa et al., 2011). However, the roles that *A. faecalis* might play in the resistance of cabbage to *Pb* stresses have not been previously investigated. In this study, we isolated the *A. faecalis* Juj3 strain from healthy cabbage rhizospheres in severely infested disease fields and found that Juj3 can enhance cabbage growth and development and induce defense-related gene expression under *Pb* stresses, as well as decrease the clubroot disease index.

PGPR enhances the growth of crops either by the production of growth substances produced by them or by improving plant uptake of environmental nutrients (Khan et al., 2019). The results suggest that PGPR helps promote plant growth

by an improving combination of physiological attributes such as asymbiotic  $N_2$  fixation, production of phytohormones such as IAA, siderophore production, solubilization of inorganic phosphate, zinc, potassium, and various trace elements, as well as by inhibiting deleterious fungal pathogen infections (Whipps, 2001; Kumar et al., 2020). Previous studies have demonstrated that *A. faecalis* affects PGPR due to the production of IAA, ACC-deaminase, the fungicidal siderophore bavistin, and phosphate solubilization and fixation of atmospheric nitrogen (Sayed and Chincholkar, 2009; Kumar et al., 2016a). In the current study of Juj3, we tested multiple PGP traits during the initial screening process. Among the 18 rhizobacterial isolates evaluated, *A. faecalis* Juj3 had the most efficient nitrogen fixation, phosphate solubilization, and siderophore production properties. Moreover, Juj3 was shown to possess positive plant growth promotion activities that enhanced the growth of cabbage seedlings. Therefore, we selected the Juj3 as a PGPR to determine its biological role in the cabbage rhizosphere.

Bacterial inoculation to enhance the productivity of different crops has been practiced since the discovery of beneficial bacterial effects on plants. Applications of seed treatments contribute mainly to the survival efficiency of the bacteria in the soil and on seeds. Previous researchers have reported that seed treatments with PGPR improved germination and seedling



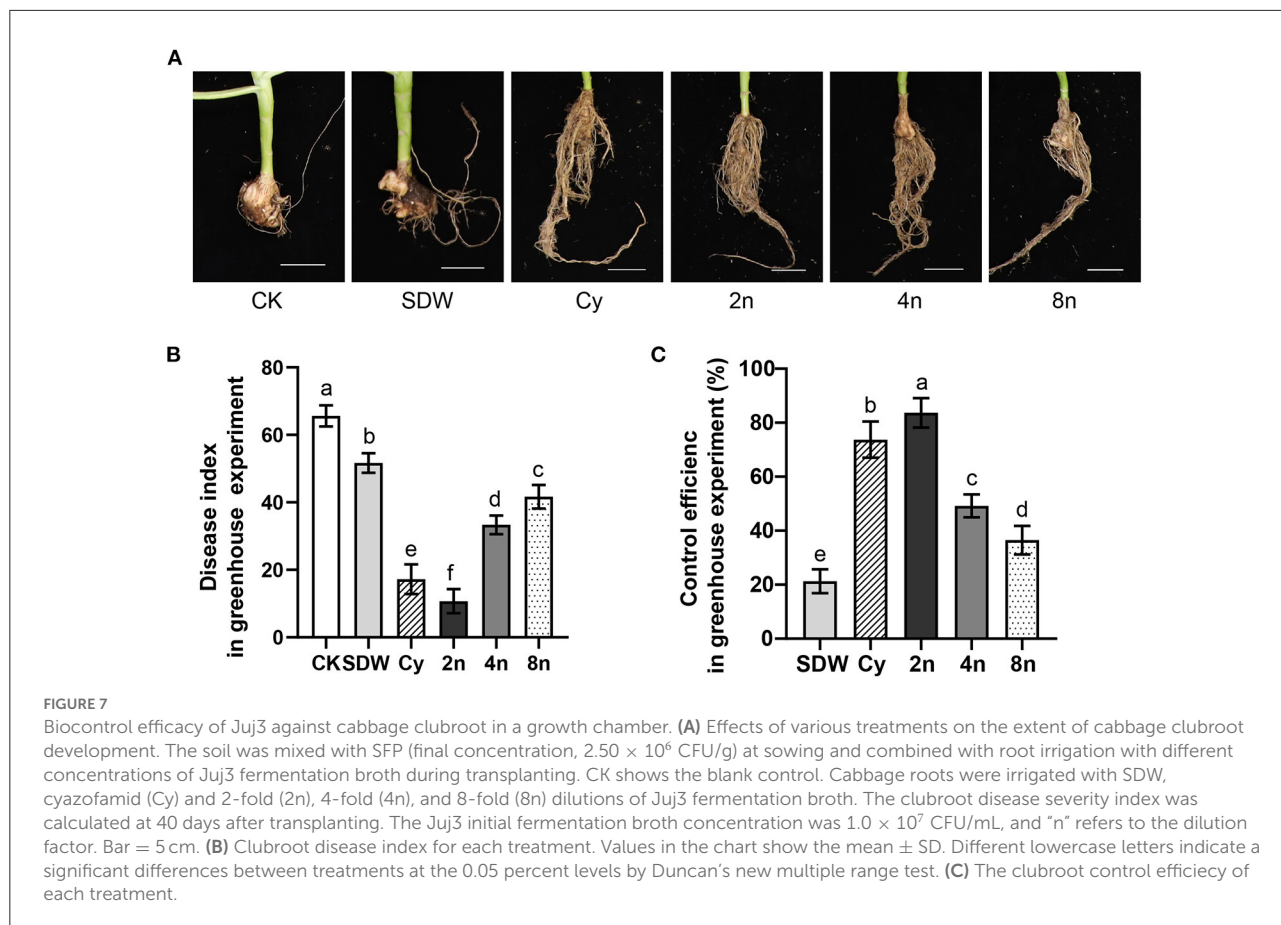


TABLE 3 Clubroot field control effects of Juj3.

Treatment	Disease index in field trials		Control efficiency (%)	
	Cabbage	Chinese cabbage	Cabbage	Chinese cabbage
CK	37.13 $\pm$ 2.22 a	48.75 $\pm$ 0.93 a	-	-
Cy	24.97 $\pm$ 1.22 c	27.04 $\pm$ 1.89 d	32.72 $\pm$ 3.30 bc	44.52 $\pm$ 4.10 a
2n	30.37 $\pm$ 4.19 b	38.15 $\pm$ 0.52 b	18.18 $\pm$ 11.29 c	21.71 $\pm$ 2.40 d
4n	21.21 $\pm$ 2.30 cd	32.66 $\pm$ 0.95 bc	42.86 $\pm$ 6.22 ab	32.99 $\pm$ 2.13 bc
8n	18.02 $\pm$ 1.85 d	30.44 $\pm$ 1.10 c	51.44 $\pm$ 4.98 a	37.56 $\pm$ 2.26 b
16n	25.93 $\pm$ 1.05 bc	32.22 $\pm$ 0.91 bc	30.16 $\pm$ 2.82 bc	33.91 $\pm$ 1.40 bc
32n	22.78 $\pm$ 2.36 cd	34.44 $\pm$ 1.81 c	38.64 $\pm$ 6.34 ab	29.37 $\pm$ 2.99 c

CK: SDW; Cy (50 mg/L, cyazofamid); the concentration of the original fermentation broth with Juj3 was  $1.00 \times 10^7$  CFU/mL, "n" represents the dilution factor. Values in the table are mean  $\pm$  SD. Different lowercase letters indicate a significant difference between different at the 0.05 percent levels by Duncan's new multiple range test.

vigor (Liu et al., 2019), plant emergence rates, biomass, and significantly increased plant health (Kusstascher et al., 2020). Our results are compatible with these conclusions. We found that bio-inoculation with Juj3 fermentation powder before sowing improves the growth of cabbage and decreases the clubroot disease index at the seedling stage. In our field trials, the optimal root irrigation concentration for Juj3 fermentation broth was determined; it could promote cabbage and Chinese cabbage growth, improve the quality of cabbage leaves, and

provide a positive effect on clubroot control. The results herein establish the foundation for Juj3 application in agricultural production practices.

Improved photosynthesis can increase plant growth and stress resistance (Bendaly et al., 2016). Therefore, studying the effects of PGPR Juj3 on plant photosynthetic parameters under *Pb* stress is of great significance for improving biotic tolerance. It is well-known that most pathogen invasions lead to a decline in the photosynthesis rate of the host

(Walters et al., 2008; Kasuga et al., 2021). The net photosynthesis rate and related parameters of susceptible tomato leaves are affected by inoculation with *Fusarium oxysporum* f. sp. *lycopersici* or *Verticillium albo-atrum* (Lorenzini et al., 1997). The photosynthesis capacity of maize leaves is also inhibited by infection with *Colletotrichum musae* and *Fusarium moniliforme*, and these inhibitions are accompanied by sharp decreases in chlorophyll content (Pinto et al., 2000). Similarly, we found that *Pb* stress can reduce cabbage photosynthesis. It has been reported that PGPR improves the stomatal aperture to result in increased water uptake via roots and enhanced stomatal CO<sub>2</sub> conductance compared to non-PGPR inoculated plants (Vejan et al., 2016). Other PGPR, such as *Bacillus siamensis* and *Pseudomonas aeruginosa*, have been reported to improve leaf photosynthesis and chlorophyll maintenance under stressful environmental conditions (Sarma and Saikia, 2013; Awan et al., 2020). Our study suggests that the Juj3 strain can effectively alleviate stress conditions by enhancing cabbage assimilation rates, transportation rates, and stomatal conductance.

Root activity reflects the ability of roots to synthesize, assimilate and transform essential substances such as amino acids and hormones. Several early studies found that *Pb* infection stimulates meristematic activity in the hypocotyl (Malinowski et al., 2012). In growing root galls, the pathogen alters host metabolism, especially plant hormones such as auxins and cytokinins that are important for cell division and elongation induction (Siemens et al., 2006; Ludwig-Müller, 2008). Overexpression of these hormones enhances abnormal lateral root growth to produce pathological galls. Our experiments identified a similar phenomenon in that *Pb* stress significantly increased the TTC content in cabbage roots. Devos et al. (2005) studied Chinese cabbage and found that the zeatin content increased earlier in infected plants during clubroot formation. Additionally, zeatin has been previously reported to promote chloroplast development and increase the chlorophyll content (Criado et al., 2009; Zulfqar et al., 2020). This is probably the reason why the chlorophyll in *Pb*-stressed cabbage was higher than that of control plants in our study. Interestingly, inoculation with Juj3 improved root vigor and chlorophyll content of cabbage in normal settings; however, the two physiological indicators of cabbage treated with Juj3 were lower than control treatments under *Pb* stress. Thus, our results indicate that Juj3 can help cabbage reduce the *Pb* stress effects and maintain regular metabolic activity. But the specific mechanism mediating these effects remains to be determined.

Apart from their ability to promote plant growth, PGPRs have also been recognized to improve the health of plants by enhancing their defense system by the mechanism of ISR (Beneduzi et al., 2012; van de Mortel et al., 2012; Li and Chen, 2019). For instance, three rhizobacteria, *Burkholderia territorii* strain A63, *B. metallica* strain A53, and *Pseudomonas geniculata* strain 95, were found to effectively activate plant defense and significantly reduce symptom development in leaves challenged with *Xanthomonas citri* subsp. *citri* (Riera et al.,

2018). Treatment of plant roots with the cyclic lipopeptides secreted from the strain *Bacillus* UCMB5113 resulted in disease suppression when challenged with *Alternaria*, indicating primed systemic defense as ISR (Asari et al., 2017). Additionally, the application PGPRs to the seeds or seedlings of certain plants have resulted in increased efficiency of ISR against several pathogens (Ramamoorthy et al., 2001). Regardless of the method of its application (foliar spraying or soil drenching), the 100-fold-diluted fermentation broth of *Streptomyces* sp. JCK-6131 suppressed the development of tomato bacterial wilt and cucumber *Fusarium* wilt by ~50%, suggesting that strain JCK-6131 could enhance plant defense resistance (Le et al., 2021). Our study demonstrated that strain Juj3 reduces the clubroot disease index and provides a higher biocontrol efficacy in the greenhouse than in the field. Many studies have also found that PGPR elicits greater greenhouse than field effectiveness (Boregowda et al., 2019). The complex field environment likely contributes to this reduced effectiveness.

Emerging evidence has shown that phytohormones, especially SA and jasmonic acid (JA), and ethylene (ET) signaling pathways regulate plant defense against various pathogens (Zhao et al., 2009). The gene *PR2* induction in plants provides them resistance against biotrophic fungi and is regulated by SA to activate systemic acquired resistance (SAR) (Ali et al., 2017). Researchers have indicated that an exogenous application of SA to broccoli resulted in up-regulation of *PR1* and *PR2* genes and a moderate reduction in clubroot severity (Lovelock et al., 2012). *Heteroconium chaetospora* (Hc) plus *Pb* up-regulated the expression of *PR2* relative to *Pb* inoculation alone (Lahlali et al., 2014). The strong expression of *PR2* in this study, coupled with the evidence above, suggests the possible involvement of *PR2* in Juj3-mediated plant defense against clubroot. PAL is involved in the biosynthesis of SA through the PAL pathway, and it is a critical enzyme catalyzing the first step of phenylpropanoid biosynthesis controlled by a multi-gene family (Zhao et al., 2009). A previous study reported that the genes *BnCCR* and *BnOPCL*, encoding phenylpropanoid pathways enzymes, were up-regulated in strain *Clonostachys rosea* treated plants. In another work, strain *Hc* treatments generally increased PAL activity, but the expression of the *BnCCR* and *BnOPCL* genes was not up-regulated (Lahlali et al., 2014). In the current study, gene *PAL* was not up-regulated by Juj3 plus *Pb* when compared to *Pb* inoculation alone. In the process, whether Juj3 affected the PAL synthetic pathway needs further to be investigated. The systemic resistance response induced in plants by beneficial rhizobacteria is in many cases regulated by the phytohormones JA and ET (Zamioudis and Pieterse, 2012). EIN3s encode key transcriptional factors of ET signaling and they function downstream of ET receptors (Jin et al., 2022). The strong expression of *EIN3* in the current study also contrasts with the general lack of ET-related gene up-regulation in mycorrhiza-mediated ISR, where elevated ET levels may have a negative impact on mycorrhizal development (Martin-Rodriguez et al., 2011; Fracetto et al., 2013).

Galindo-Gonzalez et al. (2020) showed that JA-mediated responses seemed to be mostly inhibited following *Pb* inoculation, especially in the resistant genotype. *PR3* is known to impart resistance in response to necrotrophs and is regulated by JA to activate ISR (Chandrashekar et al., 2018). In our research, compared to the cabbages inoculated with the *Pb* alone, cabbages inoculated with Juj3 plus *Pb* showed a much lower *PR3* gene expression level in leaves. Juj3 may likely participate in disease resistance by down-regulating the expression of *PR3*.

In conclusion, our work demonstrates that *A. faecalis* Juj3 has a positive role in resistance to clubroot and is a promising biocontrol agent for promotion of the growth and quality of brassica plants.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

## Author contributions

YW and RJ designed the study and wrote the manuscript. RJ, JC, LH, and XL performed the experiments. RJ, JC, KX, and YW analyzed the data. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2022.942409/full#supplementary-material>

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