



Antifungal effect and potential mechanism of endophytic *Pseudomonas* sp. P11 against *Botryosphaeria dothidea* in apple

Ying Zhang^{a,b,1}, Jinhua Dou^{a,b,1}, Zijun Yan^{a,b,1}, Yuqing Zhu^{a,b}, Xiaodong Zheng^{a,b}, Qiang Zhao^{a,b,*}

^a College of Horticulture, Qingdao Agricultural University, Qingdao, Shandong 266109, China

^b Engineering Laboratory of Genetic Improvement of Horticultural Crops of Shandong Province, Qingdao Agricultural University, Qingdao, Shandong 266109, China

ARTICLE INFO

Keywords:

Malus domestica
Apple ring rot
Endophytic bacteria
Biocontrol
Antagonistic action

ABSTRACT

Botryosphaeria dothidea (*B. dothidea*) is a devastating fungal pathogen responsible for apple ring rot, a disease that causes substantial economic losses in apple production worldwide. Endophytes, which colonize plant tissues without causing harm, can establish mutualistic symbioses that enhance plant growth or stress resistance. However, the functional role of endophytes in relation to apple disease resistance remains relatively limited. In this study, an endophytic bacterium *Pseudomonas* sp. P11 was isolated from the fruit and branch tissues of apple. In vitro antagonism assays revealed that strain P11 exhibits strong antifungal activity, inhibiting the growth of *B. dothidea* by 67.08%. Further investigation revealed that strain P11 also effectively suppressed *B. dothidea* infection in apple fruit, and no significant differences were observed between apple fruit inoculated with strain P11 alone and control (sterile water). Notably, fruit co-inoculated with strain P11 and *B. dothidea* had higher levels of soluble solids, soluble sugars, titratable acids, and vitamin C compared to fruit inoculated with *B. dothidea* alone, indicating that strain P11 contributed to a certain degree of improvement in the quality of apple fruit. Scanning electron microscopy (SEM) and propidium iodide (PI) staining showed that strain P11 suppressed hyphal growth and destroyed the integrity of the cell membrane of *B. dothidea* during infection on apple fruit. Taken together, our findings identify *Pseudomonas* sp. P11 as a promising biocontrol agent for the suppression of apple ring rot, highlighting its potential for application in sustainable apple production.

1. Introduction

Apples are widely cultivated fruit known for their high nutritional and economic value (Yuan et al., 2025). However, apple production is severely constrained by apple ring rot, a devastating disease caused by the fungal pathogen *Botryosphaeria dothidea* (Dong et al., 2021). The disease is one of the primary cause of fruit rot, which can affect yield and lead to a reduction in production. In severe cases, the incidence of the disease can reach as high as 80% in unbagged orchards, and the occurrence of rotary striation on branches can be as high as 77.6% (Tang et al., 2012). Additionally, this disease can persist during the storage period of the fruit, severely impacting both the yield and quality of apples (Huang et al. 2021). Currently, agricultural practices and chemical controls are the main methods employed to manage apple ring rot. While synthetic chemical fungicides (e.g., Bordeaux mixture, Tuzet, and carbendazim) effectively suppress *B. dothidea*, they also present

several issues, including threats to human health, environmental pollution, and the emergence of fungicide-resistant pathogens (Wei et al., 2024). Consequently, the exploration of biological agents has emerged as a primary strategy for the prevention and control apple ring rot (Yuan et al., 2025).

Biological control methods utilizing endophytes have garnered significant attention as a result of their environmental friendliness and non-toxicity, these methods have been widely applied in plant pathogen control and the development of new pesticides (Liu et al., 2009; Yuan et al., 2022). Plant endophytes are microorganisms that establish symbiosis relationships with host plants without inducing damage, they exert beneficial effects by secreting plant hormones to promote growth, producing bioactive antifungal metabolites to inhibit pathogenic fungi, and enhancing plant stress tolerance (Wei et al., 2024). Various endophytes, such as *Pseudomonas* spp., *Bacillus* spp. and *Streptomyces* spp., have been studied extensively for their roles in promoting growth and

* Corresponding author at: College of Horticulture, Qingdao Agricultural University, Qingdao, Shandong 266109, China.

E-mail address: zhaoqiang000666@163.com (Q. Zhao).

¹ These authors contributed equally to this work.

development, and preventing diseases. For instance, *Bacillus cereus* strain B8W8 could be an effective biological agent to control the main fungal pathogens of apples and citrus fruits during post-harvest period (Khadiri et al., 2023). Similarly, a total of 49 *Pseudomonas* strains were isolated from willows and rhizosphere soil, among which seven novel *Pseudomonas* strains were found to be resistant to *P. versicolora* (Wang et al., 2024). The strain *Bacillus velezensis* HE-23, originally isolated from root tissues of *Aucklandia lappa*, has been shown to impair cell membrane integrity in *Botrytis cinerea* hyphae and significantly reduce gray mold incidence in postharvest tomato fruit (Wei et al., 2024). An endophytic bacterium *Serratia plymuthica*, isolated from Chinese leek (*Allium tuberosum*), significantly suppressed the mycelial growth and severely damaging the hyphal morphology of *B. dothidea*, achieving 84.64% inhibition against apple ring rot in postharvest fruit assays (Sun et al., 2022). However, the current biocontrol agents for apple ring rot are predominantly derived from non-apple hosts or synthetic approaches, with limited reports on the exploitation of apple-derived endophytes against *B. dothidea* (Mu et al., 2020).

As one of the primary genera of plant endophytes, *Pseudomonas* has garnered significant attention for its roles in promoting growth, enhancing disease resistance, and elucidating the mechanism involved in host plants (Gao et al., 2021). The ecological versatility of *Pseudomonas* spp., including both beneficial endophytes and pathogens, makes them a model system for understanding plant-microbe interactions. For instance, *Pseudomonas syringae* pathovars are well-studied plant pathogens that cause substantial economic losses, yet their interactions with plants have profoundly illuminated the mechanisms of plant immunity and microbial pathogenicity (Carreras et al., 2024; Hong et al., 2025). Conversely, numerous non-pathogenic *Pseudomonas* strains have been shown to function as potent plant growth-promoting rhizobacteria (PGPR) and biocontrol agents, thereby contributing to sustainable agriculture by reducing reliance on chemical fertilizers and pesticides. *Pseudomonas* spp. are well-known for their capacity to produce phytohormones, such as auxins, gibberellins, and cytokinins, which have been demonstrated to promote root development and enhance overall plant growth (Beneduzi et al., 2012). Moreover, these endophytes enhance nutrient uptake by solubilizing phosphorus and facilitating nitrogen fixation, thereby improving plant health and productivity (Jiang et al., 2019; Singh et al., 2017). Such traits make them attractive candidates for biofertilizers, especially in nutrient-deficient soils where traditional fertilizers may not be as effective. In addition to promoting growth, *Pseudomonas* spp. play a crucial role in defending plants against various pathogens. They can induce systemic resistance in plants, whereby local infection triggers a broader activation of defence responses throughout the plant (Parsa et al., 2016; Zheng et al., 2023). For instance, *Pseudomonas fluorescens* has been shown to trigger defense mechanisms in tomato and pepper, providing enhanced resistance to fungal pathogens such as *Botrytis cinerea* and *Fusarium oxysporum* (López-Orona et al., 2019; Monaco et al., 2009). The production of secondary metabolites, including phenazines, 2,4-diacetylphloroglucinol, and hydrogen cyanide, further contributes to their antifungal activity, demonstrating the versatile strategies employed by these microbes to protect their hosts (Mazzola, 2004).

This study aims to isolate and identify endophytic microorganisms from apple tissues that exhibit significant antagonistic activity against *B. dothidea*, thereby providing a novel strategy for controlling apple ring rot.

2. Materials and methods

2.1. Plant materials and pathogen

Apple samples were collected from the Qingdao Academy of Agricultural Sciences (Qingdao, China). The apple fruit (180 days after full bloom) with uniform size, no physical damage, and apparently healthy appearance were selected for the experiments. The selected fruits had a

mean flesh firmness of 6.95 ± 0.15 kg/cm², a starch pattern index of 7.0 ± 0.1 (based on a 1–8 starch staining index), and a soluble solids content of $11.7 \pm 0.2\%$ (mean $\pm$ SD, $n = 15$). The surfaces of the fruit were disinfected using 1.5% sodium hypochlorite solution for 5 min, rinsed three times with sterile water, and then dried with sterile filter paper to remove excess moisture.

The fungus *B. dothidea* were cultured on the potato dextrose agar (PDA) media at 28 °C.

2.2. Isolation of endophytic *Pseudomonas* spp. P11

Apple fruit and branches were firstly rinsed under running tap water to remove superficial debris, followed by rinsing with sterile water. The samples were then surface-sterilized by sequential immersion in 75% (v/v) ethanol for 3 min and three subsequent rinses with sterile water. After blotting dry with sterile filter paper, the samples were treated with a 5% (v/v) sodium hypochlorite solution for 5 min and rinsed another three times with sterile water. The sterilized samples were thoroughly ground and the homogenate was allowed to stand for 30 min. Following serial gradient dilution, 100 μ L of the resulting suspension was spread onto LB agar plates and incubated at 28 °C for 48 h. *Pseudomonas* spp. strain P11 was subsequently selected for further analyses.

2.3. In vitro antifungal activity assays

The antagonistic activity of strain P11 against apple fungal pathogens was evaluated using confrontation culture assays. A 5-mm-diameter fungal mycelial disc was punched from the margin of an actively growing fungal colony and inoculated onto potato dextrose agar (PDA) plates. Subsequently, 10 μ L of the strain P11 bacterial suspension was inoculated at two sites 2.5 cm away from the fungal disc (one on each side). PDA plates inoculated only with the fungal disc (without P11) served as the control. All plates were incubated at 28 °C for 5 d. Each treatment included five biological replicates, and the experiment was repeated three times independently. The diameter of the inhibition zone formed by strain P11 against the fungal pathogens was measured using a vernier caliper, and the inhibition rate was calculated as needed.

2.4. Identification of strain P11

Gram staining of strain P11 was performed following a standard protocol involving initial staining, mordant treatment, decolorization, and counter staining, as described previously (Reynolds et al., 2009). Subsequent to Gram staining, physiological and biochemical identification assays were conducted, including the methyl red test, contact enzyme test, sugar-alcohol fermentation test, Voges-Proskauer test, starch hydrolysis test, gelatin liquefaction test, and salt tolerance test. The physiological and biochemical characterization of this endophytic bacterium followed the methodology outlined by Dong et al. (2021). The 16S rDNA and gyrB genes were amplified using specific primers (16S rDNA: 27 F: 5'-AGAGTTTGATCCTGGCTCAG-3'; 1492 R: 5'-CCCGTCAATTCATTTGAGTTT-3'). PCR products were sequenced by Shenggong Bioengineering (Shanghai) Co., Ltd., and the resulting sequences were submitted to the NCBI database for BLAST homology analysis. Subsequently, highly homologous sequences were retrieved, and a phylogenetic tree was constructed using the neighbor-joining method with MEGA 11 software.

2.5. Effect of strain P11 on growth of *B. dothidea* in vitro

2.5.1. Varying concentrations of the cell suspension

The antifungal effect of biocontrol strain P11 against *B. dothidea* was assessed using dual Petri dish method (Yuan et al., 2023). Briefly, a single colony of strain P11 was inoculated into 20 mL of LB medium overnight at 28 °C with shaking at 180 rpm. Aliquots (100 μ L) of strain P11 cell suspension at different concentrations were then

evenly spread onto LB agar plates. LB agar plates without strain P11 served as the control (CK). Each LB plate was inverted and paired with a PDA plate inoculated with a 5-mm-diameter mycelial plug of *B. dothidea*. The paired plates were co-cultured at 28 °C for 4 d. Five replicate plates were used per treatment in a single experiment, and the experiment was repeated three times independently. After incubation, the colony diameter of *B. dothidea* on each PDA plate was measured, and the inhibition rate (%) was calculated using the following formula: Inhibition rate (%) = [(colony diameter of CK – colony diameter of treatment) / (colony diameter of CK – 0.5)] × 100.

2.5.2. Different forms of the cell suspension

A 5-mm-in-diameter *B. dothidea* mycelial disc was inoculated onto PDA plates. Subsequently, 15 µL aliquots of different forms of the strain P11 cell suspension (culture filtrate, washed cell suspension, unwashed cell suspension, and autoclaved culture) were inoculated at two sites, each 2.5 cm away from the fungal disc (one on each side). PDA plates inoculated with 15 µL of sterile water were used as the control. All plates were incubated at 28 °C for 5 d. Five replicate plates were used per treatment in a single experiment, and the experiment was repeated three times independently. After incubation, the colony diameter of *B. dothidea* was measured, and the inhibition rate (%) was calculated using the same formula as above: Inhibition rate (%) = [(colony diameter of CK – colony diameter of treatment) / (colony diameter of CK – 0.5)] × 100.

2.5.3. Effects of different temperatures on the growth and antifungal activity of strain P11

Strain P11 suspension was inoculated into LB liquid medium and cultured at 28 °C with shaking at 180 rpm for 12 h. The culture was subsequently aliquoted into 2 mL centrifuge tubes and incubated at different temperatures (0 °C, 20 °C, 40 °C, 60 °C, 80 °C, and 100 °C) for 30 min. Five replicate plates were used per treatment in a single experiment, and the experiment was repeated three times independently. The antifungal activity of the temperature-treated strain P11 was evaluated using the dual culture assay on PDA plates, with plates inoculated only with *B. dothidea* serving as the control (CK). The inhibition rate (%) was calculated using the formula described above.

2.5.4. Effects of different pH on the growth and antifungal activity of strain P11

LB medium was used as the fermentation medium, with pH values adjusted to 5.0, 7.0, 9.0, and 11.0, respectively. After sterilization (LB medium), strain P11 suspension was inoculated into the pH-adjusted LB medium and cultured at 28 °C with shaking at 180 rpm for 12 h. Five replicate plates were used per treatment in a single experiment, and the experiment was repeated three times independently. The antifungal activity of strain P11 cultures grown under different pH conditions was evaluated using the dual culture assay on PDA plates, with plates inoculated only with *B. dothidea* serving as the control (CK). The inhibition rate (%) was calculated using the formula described above.

2.6. Population dynamics of strain P11 in apple fruit

This parameter was carried out according to the protocol described by Khadiri et al. (2023). Apple fruits were disinfected and dried, followed by wounding at four equidistant sites (1 mm diameter, 2 mm depth). Each wound was inoculated with 20 µL of strain P11 (1×10^8 CFU/mL), and the fruits were incubated at 28 °C under light conditions. At designated time points (0, 1, 2, 3, 4, and 5 days post-inoculation), tissue samples were collected. Then, each sample was homogenized in sterile water and dilute to 10^4 , and 100 µL aliquot of the diluted suspension was spread onto LB agar plates. After incubation for 48 h, bacterial colonies were counted, and colony counts were converted to Log CFU per gram of fresh weight (FW). For each time point, three replicates of five fruits were used, and the entire experiment was

performed three independently.

2.7. Biocontrol efficacy of strain P11 on *B. dothidea*

Biocontrol assays were conducted according to the method described by Feng et al. (2021) with slight modifications. Briefly, a sterile blade was used to create a 5 mm × 5 mm wound at the equator of each apple fruit. Four treatment groups were established as follows: (1) injection of 20 µL sterile water (control, CK); (2) injection of 20 µL strain P11 antagonistic suspension (P11); (3) co-injection of 20 µL strain P11 antagonistic suspension and a 2-mm-diameter *B. dothidea* inoculum (P11 + B); (4) inoculation with a 2-mm-diameter *B. dothidea* inoculum alone (B). The inoculated fruit were placed in plastic containers lined with moist filter paper to maintain humidity and incubated at 28 °C. Three replicates of five fruits were used for each incubation period. At 7 d post-inoculation (DPI), the fruit were photographed, and disease incidence and lesion areas were recorded.

2.8. Control efficacy of different concentrations of strain P11 against apple ring rot

One artificial wound was created on each apple fruit as described in Section 2.6. Subsequently, 20 µL aliquots of strain P11 suspension at different concentrations (1×10^5 , 1×10^6 , 1×10^7 , and 1×10^8 CFU mL⁻¹) were administered into each wound. Wounds treated with 20 µL sterile water served as the control (CK). Two hours post-inoculation, *B. dothidea* inoculum was introduced into each wound. The treated apples were incubated in a growth chamber at 28 °C and 95% relative humidity. Three replicates of five fruits were used for each incubation period. Five days post-inoculation (DPI), the lesion diameter at each wound site was measured along two perpendicular axes using a vernier caliper, and the average diameter was calculated.

2.9. The effect of strain P11 on apple fruit quality

Soluble sugar content was determined using the phenol-sulfuric acid method (Huang et al., 2024). Briefly, 1 g of ground sample was extracted twice with distilled water by boiling (30 min, then 10 min). The combined filtrate was adjusted to 100 mL. For analysis, 0.5 mL of extract was mixed with 1.5 mL of water, 1 mL of phenol (0.09 g mL⁻¹), and 5 mL of concentrated sulfuric acid. After color development, absorbance was measured. All measurements were performed in triplicate.

Titrateable acid content was measured by titration (Huang et al., 2024). A 10 g sample was ground, diluted to 100 mL with water, and allowed to stand for 30 min before filtration. An aliquot of 20 mL filtrate was titrated against standard NaOH using 1% phenolphthalein as indicator until a faint pink color persisted for 30 s (pH 8.1 – 8.3). A distilled water blank was included. All measurements were performed in triplicate.

Soluble solids content was determined using a handheld refractometer (Atago) (Huang et al., 2024). Juice from 5 g of fruit sample was collected after centrifugation (4000 r min⁻¹, 10 min) or filtration. Results were expressed as mass percentage (%), with three replicates per sample.

Vitamin C content was quantified by iodometric titration (Huang et al., 2024). A 10 g sample was ground with 2% HCl in an ice bath, diluted to 100 mL with 2% HCl, extracted for 10 min and filtered. The reaction mixture contained 0.5 mL KI (10 g L⁻¹), 2.0 mL starch (5 g L⁻¹), 5.0 mL extract, and 2.5 mL water. This was titrated with KIO₃ until a faint blue color persisted for 30 s. A blank using 5.0 mL of 2% HCl was included. Each sample was analyzed in triplicate.

2.10. Effect of strain P11 on mycelial growth of *B. dothidea*

Freshly cultured *B. dothidea* mycelial discs and 20 µL of strain P11 suspension were co-inoculated into potato dextrose broth (PDB)

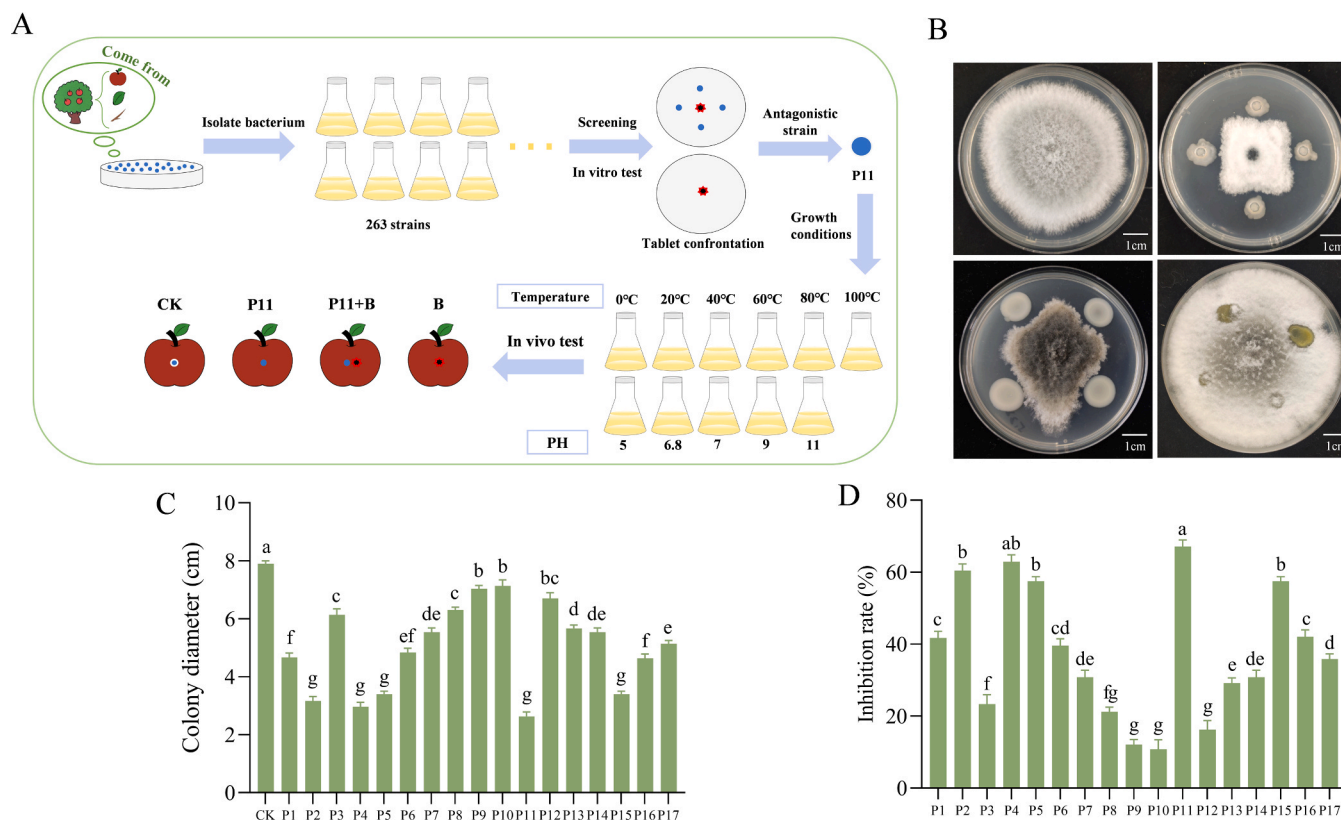


Fig. 1. Screening and evaluation of antagonistic bacterial strains against *B. dothidea*. (A) Workflow for the screening of antagonistic bacteria. CK: sterile water; P11: strain P11; P11 + B: Co-inoculation with both *B. dothidea* and strain P11; B: *B. dothidea*. (B) Confrontation culture assays of candidate strains against *B. dothidea*. Scale bars = 1 cm. (C) Mycelial diameter of *B. dothidea* under treatment with different candidate strains. (D) Antifungal rate of candidate strains against *B. dothidea*. Bars represent the mean $\pm$ standard deviation of values from three biological replicates. Different letters above the bars (based on LSD analysis) indicate statistically significant differences at $P < 0.05$.

medium. The co-cultures were incubated at 28 °C for 5 days. Mycelial morphological changes were observed and recorded at sequential time points (Days 1, 3, and 5) under a light microscope. A control group consisting of PDB medium inoculated only with *B. dothidea* mycelial discs (without P11) was included for comparison.

2.11. Scanning electron microscopy (SEM)

The apple fruit were applied with *B. dothidea* or *B. dothidea* + strain P11. The inoculated fruit were incubated at 28 °C under controlled humidity conditions for 5 d. After incubation, 0.5 cm² fruit were excised from the junction of symptomatic and asymptomatic tissues. All samples were individually fixed in 2.5% (v/v) glutaraldehyde solution at 4 °C for 12 h. Following rinsing with phosphate-buffered saline (0.1 M PBS, pH 7.2–7.4), stepwise dehydration, critical point drying, and gold sputtering, the samples were observed using a JSM-7500F scanning electron microscope (SEM; Tokyo, Japan), and micrographs were captured to document morphological features.

2.12. Membrane permeabilization assay

2.12.1. Propidium iodide (PI) staining

The plasma membrane permeability of *B. dothidea* mycelia was evaluated using PI staining according to a previously method (Fan et al., 2023). Briefly, *B. dothidea* mycelia were collected and resuspended in 2 mL of PDB medium, followed by the addition of 20 μ L of strain P11 suspension. The co-cultures were incubated at 28 °C for 3 h, 6 h, and 12 h, respectively. A control group consisting of *B. dothidea* mycelia resuspended in PDB medium alone (without strain P11) was included. Subsequently, *B. dothidea* mycelia were submerged in equal volumes of

20 mg L⁻¹ PI solution in the dark for 10 min and observed under a fluorescence microscope. Fluorescence images were captured to assess the extent of PI penetration, which reflects the degree of plasma membrane damage.

2.12.2. Measurement of nucleic acid and protein release

The effect of strain P11 on the release of nucleic acids and proteins from *B. dothidea* was evaluated according to a previously described method (Cai et al., 2015). Briefly, *B. dothidea* mycelia were collected and resuspended in 30 mL of PDB medium, followed by the addition of 1 mL of strain P11 suspension. The co-cultures were incubated in a shaker at 28 °C for 0, 3, 6, 12, 24, and 48 h, respectively. Three experimental groups were established: (1) *B. dothidea* mycelia resuspended in 30 mL of PDB medium alone; (2) *B. dothidea* mycelia co-cultured with P11; (3) 1 mL of strain P11 suspension in 30 mL of PDB medium. At each time point, samples were collected and centrifuged at 12,000 $\times$ g at 4 °C for 5 min. The supernatant was collected, and its optical density (OD) values at 260 nm (OD₂₆₀) and 280 nm (OD₂₈₀) were measured using a spectrophotometer.

2.13. Statistical analysis

All statistical analyses were performed using SPSS 19.0 software (Chicago, USA). One-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test was used for multiple comparisons between groups, with a significance level set at $P < 0.05$. An independent samples *t*-test was applied to compare the mean values between the control and treatment groups, with statistical significance defined as $P < 0.05$.

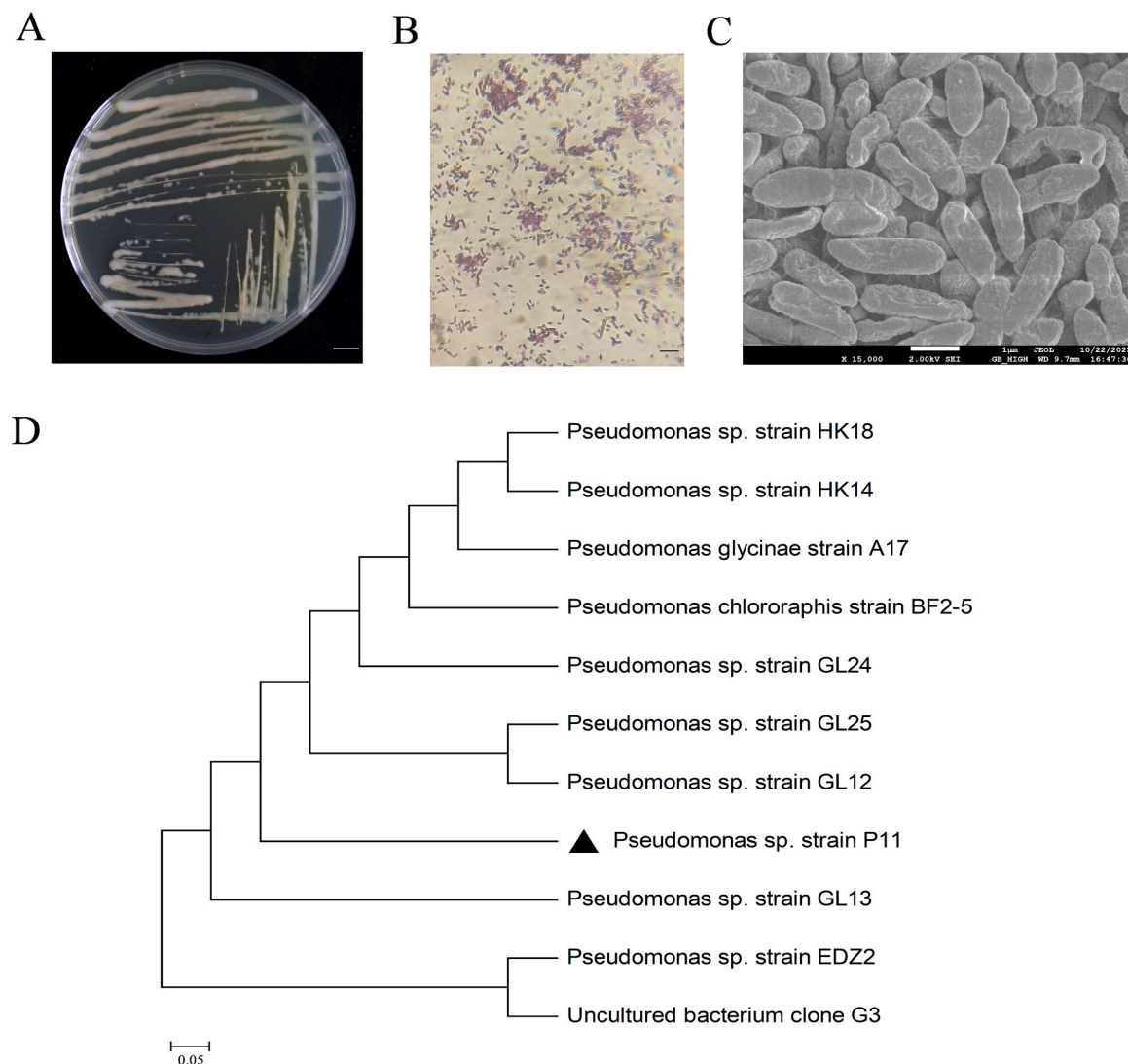


Fig. 2. Characterization of endophytic strain P11. (A) Single colony morphology. Scale bars = 1 cm. (B) Gram staining. Scale bars = 10 μ m. (C) Scanning electron microscopy (SEM) morphology. Scale bars = 1 μ m. (D) Phylogenetic tree based on 16S rRNA gene sequences. The triangle represents the endophytic bacteria P11.

3. Results

3.1. Identification and antifungal activity of strain P11

To identify beneficial endophytic microorganisms, culturable microbes were isolated and purified from the tissues of healthy apple. Potential core strains were initially selected in accordance with pre-defined screening criteria, and the associated screening workflow is depicted in Fig. 1A. A total of 263 candidate strains were isolated from the sterilized of healthy apple. The antagonistic activities of these strains against *B. dothidea* were initially assessed using confrontation culture assays conducted in petri dishes (Fig. 1B). Then, the in vitro biocontrol efficacy of individual endophytic microorganisms against *B. dothidea* was assessed in vitro. Seventeen strains of endophytic bacteria that exhibited antagonistic effects against *B. dothidea* were preliminarily screened and designated as P1 to P17. Among these strains, P11 exerted the most robust inhibitory effects, suppressing fungal growth in vitro (Fig. 1C and D). Additionally, P2, P4, P5, and P15 showed significantly greater antifungal activity relative to other strains. Based on these findings, strain P11 was selected for subsequent research.

3.2. Characterization of endophytic strain P11

Strain P11 formed white, round, opaque single colonies with smooth edges and a raised center (Fig. 2A). Gram staining revealed that P11 stained purple, indicating it is a Gram-positive bacterium (Fig. 2B). Scanning electron microscopy (SEM) observations showed that strain P11 exhibited a rod-shaped morphology (Fig. 2C). Physiological and biochemical characterization indicated that strain P11 could utilize glucose but not arabinose, sucrose, or lactose. Furthermore, strain P11 was capable of producing protease but unable to hydrolyze starch (Table S1).

A 1450-bp 16S rRNA gene sequence of strain P11 was amplified by polymerase chain reaction (PCR). Sequence alignment against the National Center for Biotechnology Information (NCBI) and EzBioCloud databases showed that strain P11 shared similarity with *Pseudomonas* sp. Phylogenetic analysis was further performed by constructing a neighbor-joining tree, which demonstrated that strain P11 clustered closely with *Pseudomonas* sp. strain GL12 (Fig. 2D). Combining cultural, morphological, physiological, and biochemical characteristics with molecular identification results, strain P11 was identified as *Pseudomonas* sp.

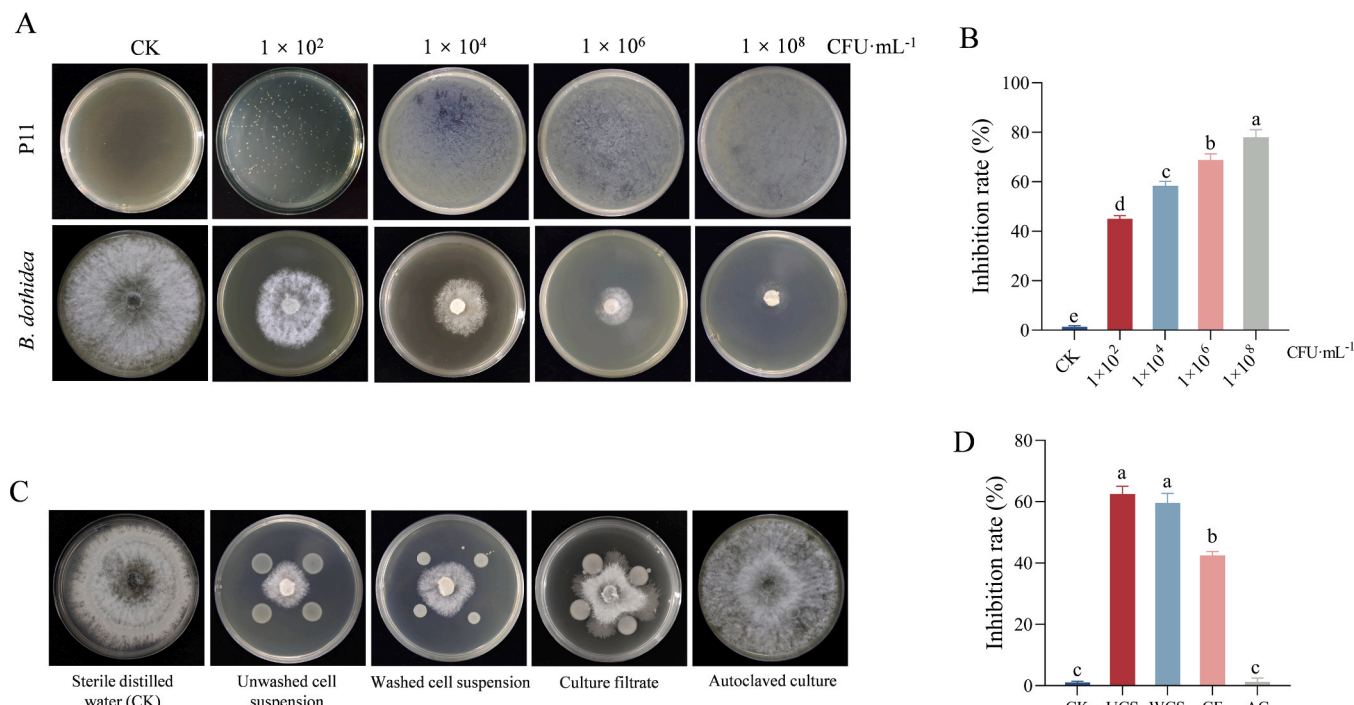


Fig. 3. Inhibitory activity of strain P11 against *B. dothidea* mycelial growth. (A) Colony morphology of *B. dothidea* after treatment with different concentrations of strain P11 cell suspension, fumigation with blank LB agar medium served as the control (CK). (B) Antifungal rate of strain P11 at different concentrations. (C) Inhibitory effects of different forms of strain P11 treatments on *B. dothidea*. (D) Antifungal rates of different forms of strain P11 treatments. CK, sterile distilled water; UCS, unwashed cell suspension; WCS, washed cell suspension; CF, culture filtrate; AC, autoclaved culture. Bars represent the mean $\pm$ standard deviation of values from three biological replicates. Different letters above the bars (based on LSD analysis) indicate statistically significant differences at $P < 0.05$.

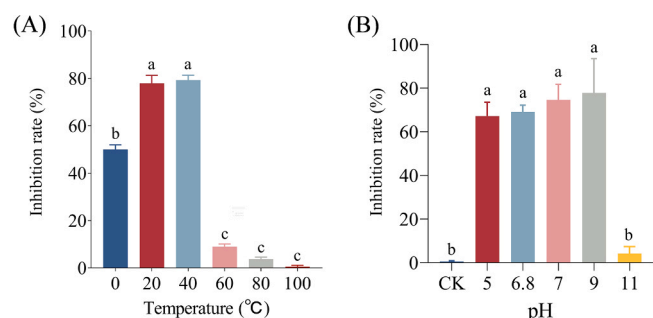


Fig. 4. Effects of temperature and pH on the antifungal activity of strain P11. (A) Relative antifungal rate of strain P11 under different temperature treatments. (B) Relative antifungal rate of strain P11 under different pH treatments. Bars represent the mean $\pm$ standard deviation of values from three biological replicates. Different letters above the bars (based on LSD analysis) indicate statistically significant differences at $P < 0.05$.

3.3. Effect of strain P11 on the growth of *B. dothidea* in vitro

Petri dish assays revealed that strain P11 effectively suppressed the mycelial growth of *B. dothidea* in a concentration-dependent manner (Fig. 3A). When *B. dothidea* was treated with strain P11 at concentrations of 1×10^2 , 1×10^4 , 1×10^6 , and 1×10^8 CFU mL⁻¹, the resulting colony diameters were recorded as 4.40, 3.33, 2.50, and 1.76 cm, respectively—all significantly smaller than the control (CK, 7.90 cm) (Fig. 3A). Correspondingly, the inhibitions were calculated to be 45.00%, 58.33%, 68.75%, and 77.92% (Fig. 3B). Furthermore, in vitro bioassays demonstrated that different forms of strain P11 varied in their ability to inhibit *B. dothidea* growth. Specifically, significant inhibition of *B. dothidea* was observed when the fungus was exposed to the culture filtrate, washed cell suspension, or unwashed cell suspension of strain P11. In contrast, minimal to no inhibition was detected in the autoclaved

culture or sterile water control groups (Fig. 3C and D).

Bacterial activity is influenced by environmental conditions such as temperature and pH. To investigate the effects of these factors on the antifungal activity of strain P11, bacterial cultures were subjected to varying temperatures and pH levels, and their activity was assessed via plate confrontation assays. For temperature, the antifungal rate of strain P11 increased gradually when cultured at temperatures ranging from 0 °C to 40 °C, with the maximum rate reaching approximately 80%. In contrast, at higher temperatures (60 °C to 100 °C), the antifungal activity decreased sharply to less than 10% (Fig. 4 A and S1). These results indicate that the optimal temperature range for strain P11 to exert antifungal activity is 20 °C to 40 °C. For pH, the results showed that pH 9.0 was optimal for the antifungal activity of strain P11, yielding a rate of approximately 80%. In contrast, the activity was markedly reduced to ~2% at pH 11 (Fig. 4B and S2). The antifungal activity increased steadily from pH 5.0 up to the optimal pH of 9.0.

3.4. Biocontrol efficacy of strain P11 against *B. dothidea*

To first assess the colonization potential of strain P11 on apple fruits, we monitored its population dynamics (Fig. 5A). The number of strain P11 cells increased rapidly from an initial 9.5 log CFU/FW on 0-day to a peak of 11.7 log CFU/FW on day 1, suggesting that strain P11 can successfully colonize and proliferate on apple fruits. During the 5-day storage period, apple fruit treated with sterile water (control, CK) or strain P11 suspension (P11 alone) maintained a healthy appearance, with no lesion initiation. In contrast, apple fruit inoculated exclusively with *B. dothidea* (pathogen alone, B) exhibited rapid lesion expansion over time, culminating in severe rot by the 5th day (Fig. 5B). Notably, pre-inoculation with strain P11 suspension 48 h prior to *B. dothidea* challenge (P11 + B) markedly retarded lesion expansion (Fig. 5B). Quantitative assessment and statistical analysis of lesion diameters confirmed these phenotypic observations (Fig. 5C). On days 3, 4, and 5 post-inoculation (DPI), the lesion diameters in the *B. dothidea* alone

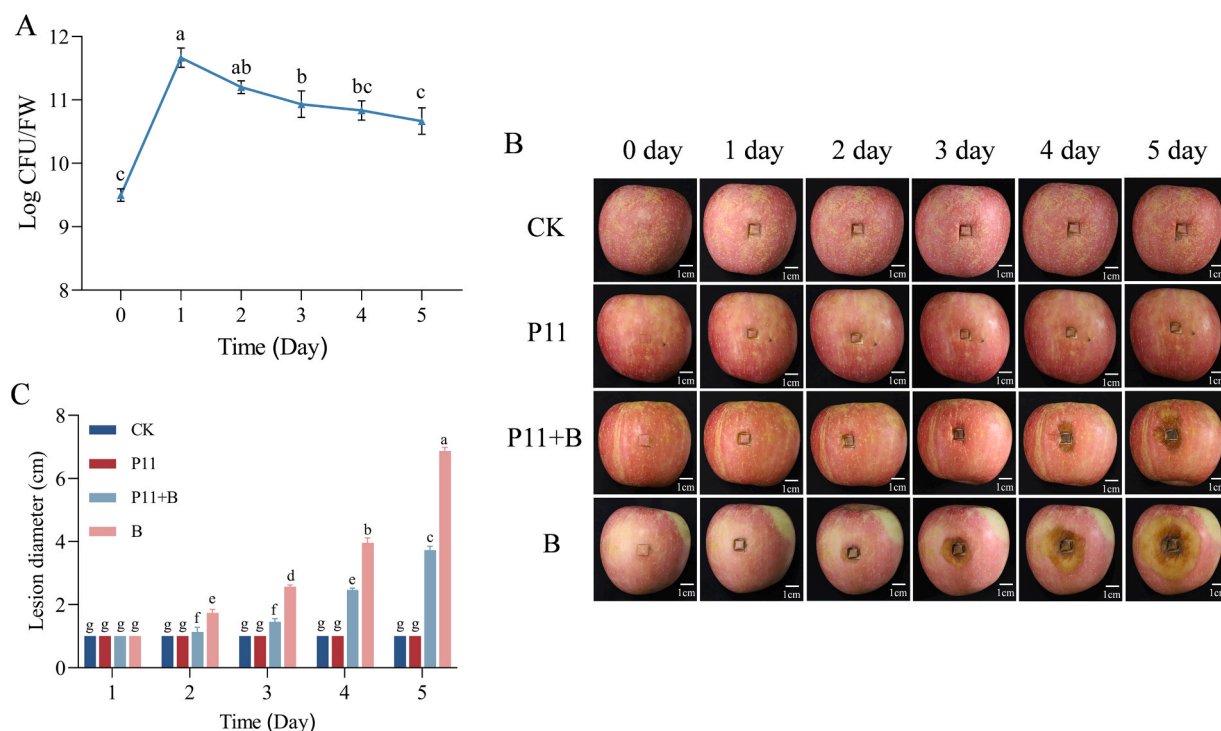


Fig. 5. Biocontrol efficacy of strain P11 against *B. dothidea* on apple fruits. (A) Population densities of of strain P11 on apple fruits. (B) Inhibitory effect of strain P11 on *B. dothidea* infection of apple fruits ("Fuji"). (C) Lesion diameter of apple fruits after different treatments. CK: sterile water; P11: strain P11; P11 + B: Co-inoculation with both *B. dothidea* and strain P11; B: *B. dothidea*. Bars represent the mean $\pm$ standard deviation of values from three biological replicates. Different letters above the bars (based on LSD analysis) indicate statistically significant differences at $P < 0.05$.

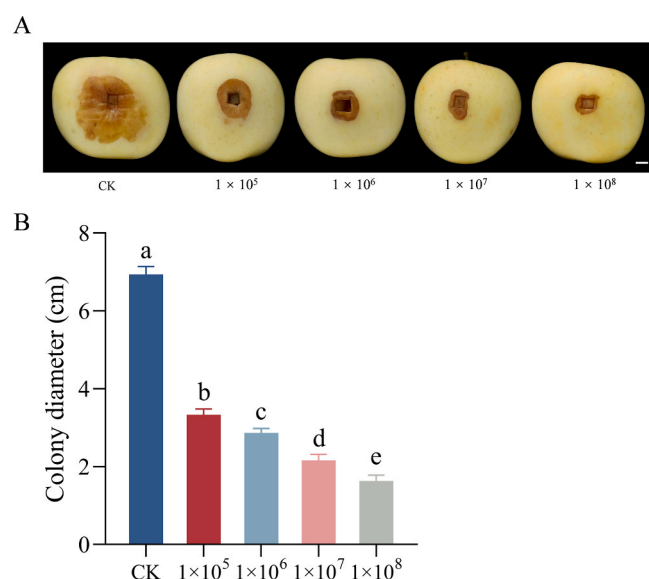


Fig. 6. Biocontrol efficacy of different concentrations of strain P11 against *B. dothidea* on apple fruits. (A) Control effect of different concentrations of strain P11 on postharvest decay of apple fruits ("Fuji"). Scale bar = 1 cm. (B) Lesion diameter of apple fruits under different treatments. Bars represent the mean $\pm$ standard deviation of values from six biological replicates. Different letters above the bars (based on LSD analysis) indicate statistically significant differences at $P < 0.05$.

group reached 2.41 cm, 4.56 cm, and 6.78 cm, respectively. By comparison, the P11 + B treatment group exhibited substantially smaller lesions, measuring 1.32 cm, 2.58 cm, and 3.64 cm on the same days. The results demonstrates that strain P11 effectively suppresses apple ring rot and significantly impairs the infectivity of *B. dothidea* on apple fruit.

Furthermore, no lesions were detected in the P11 alone treatment group across all sampling days, confirming that strain P11 is non-pathogenic to apple fruit.

3.5. Control efficacy of different concentrations of strain P11 against *B. dothidea*

To investigate whether the biocontrol effect of strain P11 on apple fruit in a concentration-dependent, the control efficacy of different concentrations of strain P11 against *B. dothidea* was conducted. Apple fruit inoculated with *B. dothidea* and sterile water (CK) developed extensive brown lesions around the inoculation sites, with dimensions significantly larger than those observed in wounds treated with varying concentrations of strain P11 (Fig. 6A). In contrast to the control (CK), where the lesion diameter measured approximately 6.93 cm, apple fruit subjected to strain P11 treatment exhibited lesion diameters of 3.33 cm (1×10^5 CFU mL⁻¹), 2.86 cm (1×10^6 CFU mL⁻¹), 2.16 cm (1×10^7 CFU mL⁻¹), and 1.63 cm (1×10^8 CFU mL⁻¹) (Fig. 6B). Above all, the biocontrol efficacy of strain P11 showed a positive correlation with its applied cell concentration.

3.6. Effect of strain P11 on apple fruit quality

At 7 d post-treatment, the decay rate of the *B. dothidea* treatment group had increased to 90.83%. In comparison, apple fruit co-treated with strain P11 and *B. dothidea* exhibited a significantly lower decay rate of 31.63% (Fig. 7A). In addition to its antifungal activity, strain P11 treatment effectively preserved apple fruit quality metrics compared to *B. dothidea* treatment. Compared with the control group (CK) inoculated with sterile water, apple fruit inoculated with strain P11 alone showed no significant differences in soluble solids, soluble sugar, titratable acid, or vitamin C contents (Fig. 7B–E). However, apple fruit inoculated with *B. dothidea* showed a significant decrease compared to the CK, the soluble solids content was 8.5%, the soluble sugar content was 1.75 g kg⁻¹, the

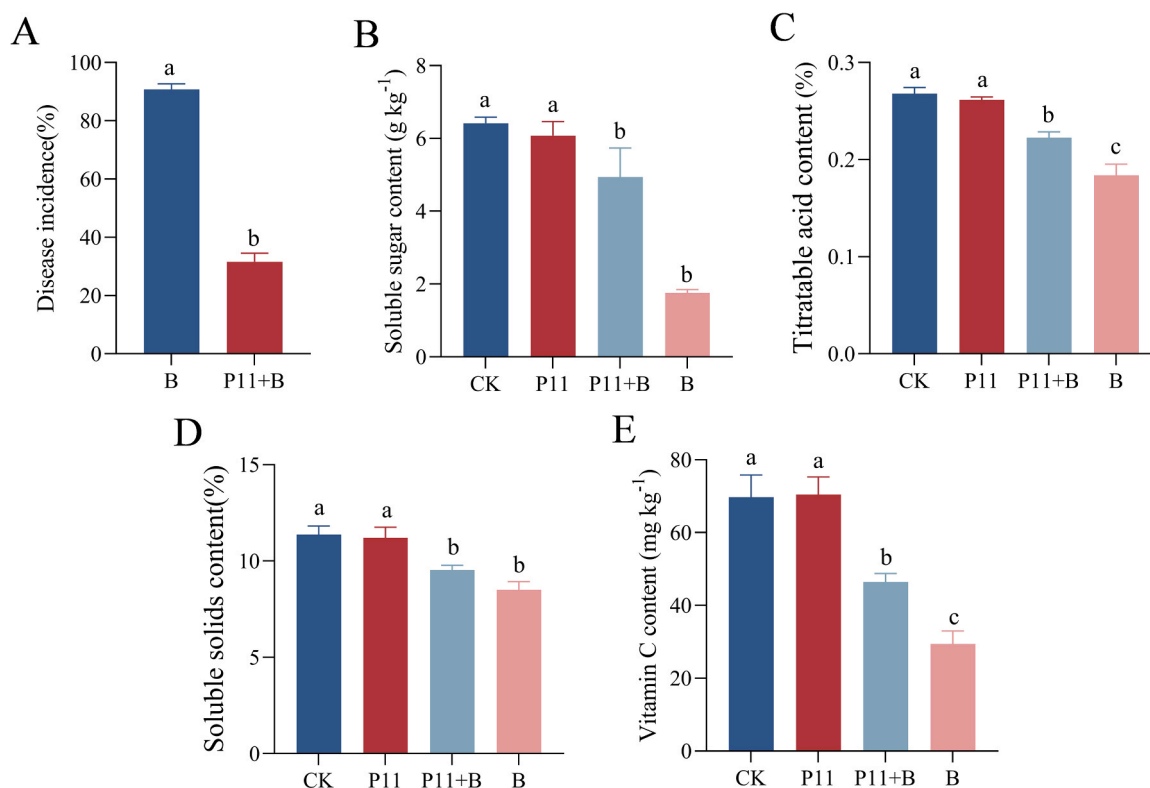


Fig. 7. Disease incidence and fruit quality indices of apple fruits after different treatments. (A) Disease incidence on the 7th day after apple inoculation with different treatments. (B–E) Physiological quality indices of apple fruits under different treatments: Soluble sugar content (B), Titratable acid content (C), Soluble solids content (D), Vitamin C content (E). CK: sterile water; P11: strain P11; P11 + B: Co-inoculation with both *B. dothidea* and strain P11; B: *B. dothidea*. Bars represent the mean $\pm$ standard deviation of values from three biological replicates. Different letters above the bars (based on LSD analysis) indicate statistically differences at $P < 0.05$.

titratable acidity was 0.18%, and the vitamin C content was 29.42 mg kg^{-1} . Importantly, co-inoculation with both *B. dothidea* and strain P11 improved these physiological indices relative to the *B. dothidea* alone group: soluble solids content was 9.53%, soluble sugar content was 4.94 g kg^{-1} , titratable acid content was 0.22%, and vitamin C content was 46.45 mg kg^{-1} (Fig. 7B–E).

3.7. Effect of strain P11 on the mycelial morphology of *B. dothidea*

To further elucidate the physiological mechanism underlying the inhibitory effect of strain P11 on *B. dothidea*, the mycelial morphology of *B. dothidea* was examined at sequential time points (Days 1, 3, and 5) using light microscopy. For *B. dothidea* grown in monoculture (B), the mycelia exhibited a smooth surface, no structural breakage, and non-protruding tips throughout the 5-day incubation period (Fig. 8A). In contrast, when co-cultured with strain P11, *B. dothidea* exhibited progressive morphological abnormalities over time. As early as Day 3, the hyphal surface became slightly rough and irregular. By Day 5, severe structural damage was observed, including extensive hyphal breakage and prominent tip protrusions (Fig. 8A). Furthermore, scanning electron microscopy (SEM) observations confirmed distinct morphological differences in *B. dothidea* hyphae between the *B. dothidea* treatment and strain P11-treated groups. In the *B. dothidea* treatment group (B), the fungal hyphae displayed a smooth surface and maintained structural integrity with no signs of damage. In contrast, the fungal hyphae exposed to strain P11 exhibited obvious abnormalities: their surfaces were wrinkled and collapsed into a spiral morphology (Fig. 8B). These morphological alterations directly demonstrate that strain P11 exerts a targeted inhibitory effect on *B. dothidea* by disrupting the fungal mycelial structure.

3.8. Effect of strain P11 on membrane permeabilization of *B. dothidea*

The integrity of *B. dothidea* cell membranes was assessed via propidium iodide (PI) staining. As illustrated in Fig. 9A, no detectable fluorescence signal was observed in the *B. dothidea* treatment group (B) at any of the tested time points. In contrast, the group co-treated with strain P11 and *B. dothidea* (P11 + B) exhibited strong fluorescence, and within the 12 h treatment period, the fluorescence intensity increased significantly as the treatment duration extended. Nucleic acids and proteins exhibit maximum absorption peaks at 260 nm and 280 nm, respectively, which enables the quantification of nucleic acid and protein leakage using optical density (OD) measurements at 260 nm (OD_{260}) and 280 nm (OD_{280}) (Cai et al., 2015). Furthermore, throughout the experimental period, the OD_{260} and OD_{280} values in the *B. dothidea* treatment group (B) increased slowly and maintained stable growth rates. After 48 h of incubation, these values remained at relatively low levels in the *B. dothidea* treatment group (B) (Fig. 9B, C). In contrast, the OD_{260} and OD_{280} values of the strain P11-treated group were significantly higher than those of the *B. dothidea* treatment group (B) at all tested time points. Notably, the difference between the two groups became more pronounced after 6 h of incubation (Fig. 9B, C). These observations confirm that strain P11 induces the leakage of nucleic acids and proteins from *B. dothidea* mycelia.

4. Discussion

Apples are among the most widely cultivated and consumed fruit globally. However, apple ring rot caused by *B. dothidea* poses a severe threat to apple production, with yield losses ranging from 30% to 50% in infected orchards (Tang et al., 2012). The over-reliance on chemical fungicides for disease control has led to environmental pollution, fungicide resistance, and potential risks to human health, highlighting

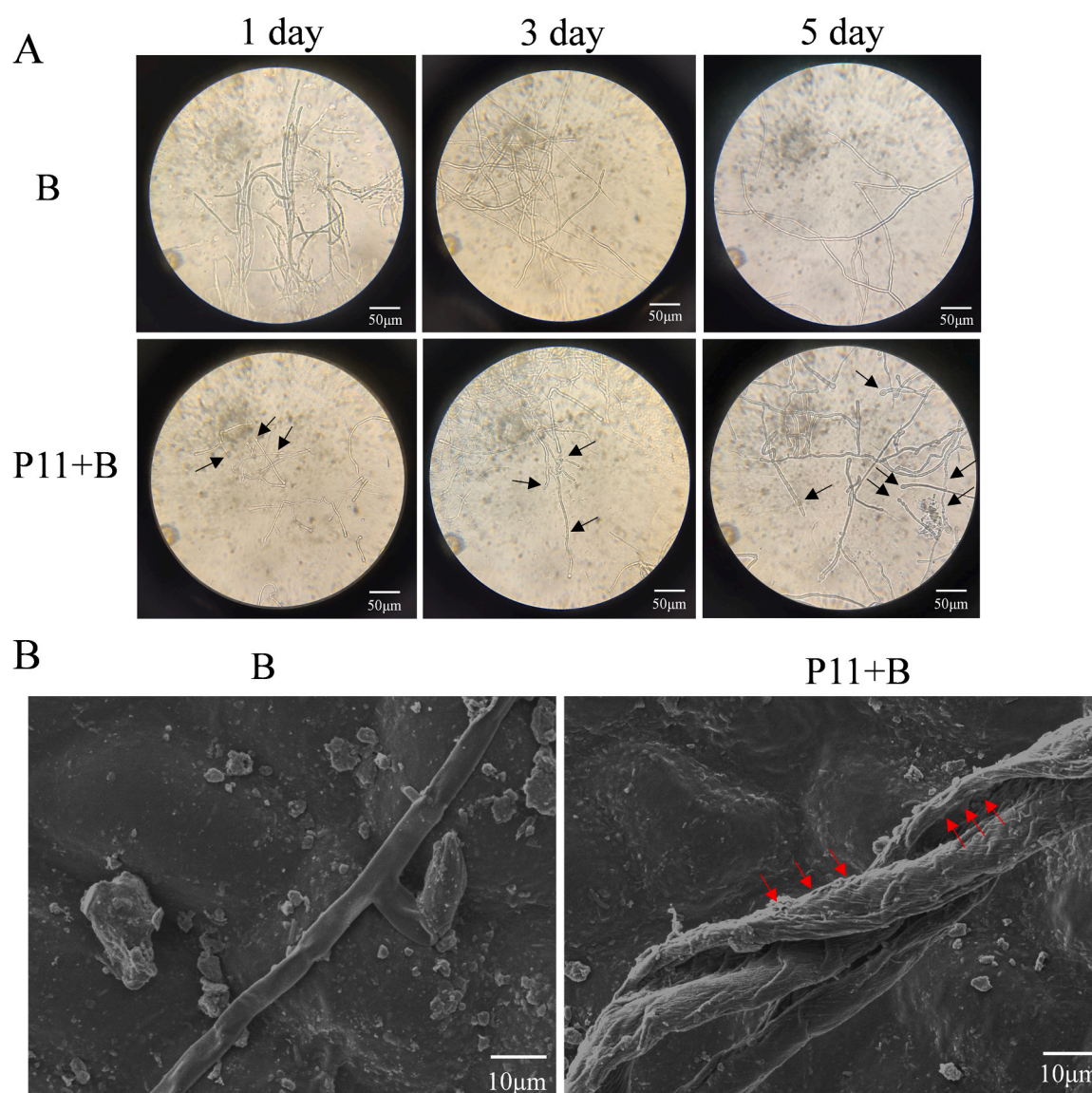


Fig. 8. Antifungal activity of strain P11 against *B. dothidea*. (A) Dynamic inhibitory effect of strain P11 on mycelial morphological changes of *B. dothidea*. Scale bars = 50 μm. (B) Scanning electron micrographs of the interaction between *B. dothidea* and strain P11 on apple fruits (indicated by red arrows). Scale bar = 10 μm.

the urgent need for sustainable and eco-friendly alternative strategies (Wei et al., 2024). In recent years, biological control has emerged as a pivotal approach for managing postharvest diseases of fruit and vegetables, with microbial biocontrol agents being increasingly recognized as promising alternatives to chemical fungicides (Seo et al., 2023). Thus, exploring more effective and stable biocontrol agents, coupled with elucidating their antifungal mechanisms, is critical for advancing their practical application in the field (Yuan et al., 2025). In this study, we isolated strain P11 that showed a significant inhibitory effect on apple *Botryosphaeria dothidea*, providing an effective and environmentally friendly measure to control the disease.

As a promising class of natural biocontrol resources, plant endophytes colonize plant tissues or intercellular spaces, establishing mutualistic, symbiotic, or commensal relationships with their hosts. They are non-pathogenic to hosts and can protect hosts from biotic stresses by enhancing plant resistance to pathogens (Singh et al., 2017). The biocontrol mechanisms of endophytes typically involve niche competition, synthesis of antimicrobial metabolites, induction of host resistance, and promotion of plant growth (Ahmed et al., 2020). Among these beneficial endophytes, *Pseudomonas* sp. have been widely reported as effective antagonists against a range of plant pathogens. For instance,

Pseudomonas palleroniana strain B-BH16-1 disrupts fusaric acid production by emitting volatile organic compounds (VOCs), thereby directly inhibiting the growth and sporulation of seven major foliar pathogens of *Pseudostellaria heterophylla* (Qing et al., 2024). A synthetic microbial community (SynCom) of *Pseudomonas* enhances the resistance of willow trees to the insect pest *Plagioderma versicolora* (Wang et al., 2024). The endophytic bacterium *Pseudomonas fluorescens* strain BRZ63, derived from rapeseed (*Brassica napus* L.), exhibits broad-spectrum antifungal activity against pathogens including *Rhizoctonia solani* W70, *Colletotrichum dematium* K, *Sclerotinia sclerotiorum* K2291, and *Fusarium avenaceum* (Chlebek et al., 2020). These findings collectively underscore the significant biocontrol potential of *Pseudomonas* species across diverse plant–pathogen systems. In line with these reports, we isolated the endophytic bacterium strain P11 from apple tissues, which exhibited strong in vitro inhibition of hyphal growth of *B. dothidea* (Fig. 5). Based on morphological characterization, molecular phylogeny, and whole-genome analysis, P11 was identified as a member of the genus *Pseudomonas* (Fig. 1). Our results further support the role of *Pseudomonas* endophytes as effective biocontrol agents and highlight their potential in sustainable plant protection strategies.

Mechanistically, microscopic and SEM analysis revealed that strain

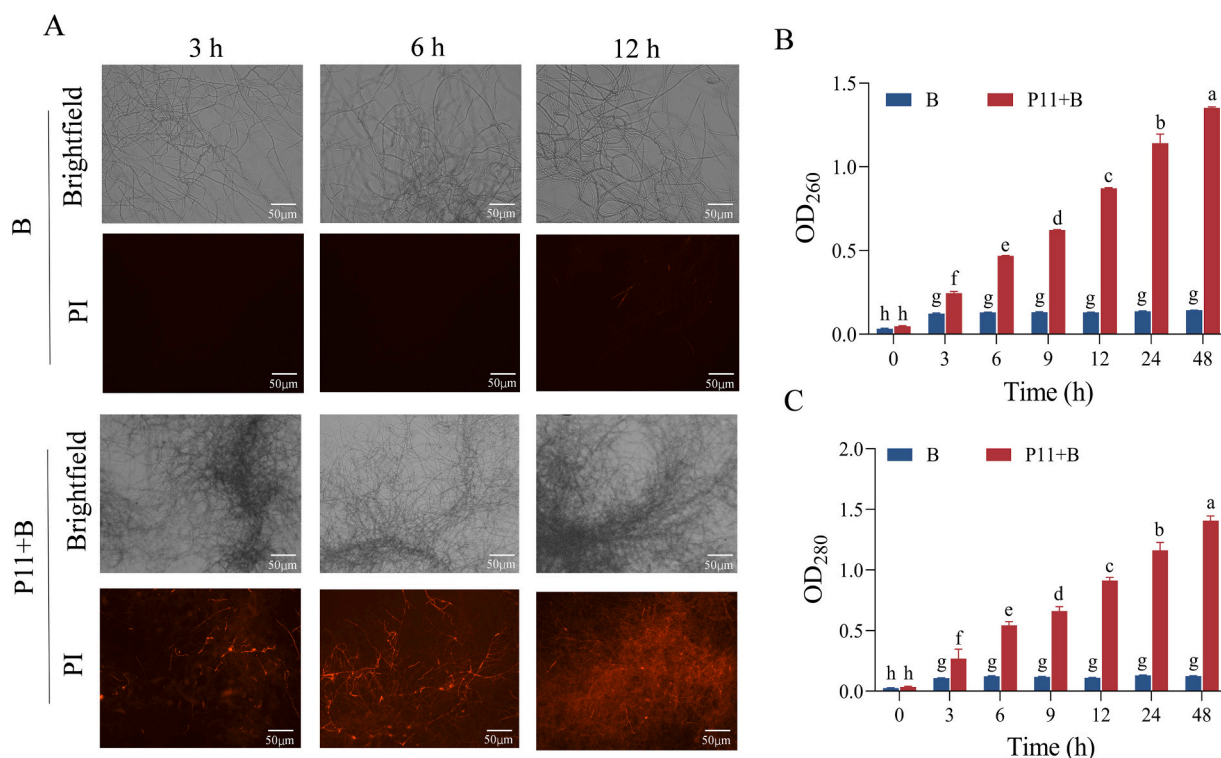


Fig. 9. Effect of strain P11 on the cell membrane integrity of *B. dothidea* mycelia. (A) Propidium iodide (PI) staining of *B. dothidea* mycelia. Scale bar = 50 µm. (B) Determination of OD₂₆₀ value (nucleic acid leakage). (C) Determination of OD₂₈₀ value (protein leakage). Bars represent the mean ± standard deviation of values from three biological replicates. Different letters above the bars (based on LSD analysis) indicate statistically significant differences at $P < 0.05$.

P11 induced significant morphological alterations in the mycelia of *B. dothidea*, including surface breakage, tip protrusion, and loss of hyphal smoothness (Fig. 8). These abnormalities contrasted sharply with the uniform, intact, and smooth mycelial structures observed in the control group. Such structural disruptions are indicative of compromised cell membrane integrity, a critical factor for maintaining fungal cellular homeostasis, viability, and normal growth (Doralicia et al., 2019; Zou et al., 2022). Impairment of the cell membrane represents a well-documented antifungal strategy employed by antagonistic microorganisms (Fan et al., 2023). Supporting this, propidium iodide (PI) uptake assays and increased extracellular leakage of nucleic acids and proteins (measured by OD₂₆₀/OD₂₈₀) confirmed that P11 treatment damages the plasma membrane of *B. dothidea* (Fig. 9). Together, these findings demonstrate that the antifungal action of strain P11 is mediated, at least in part, by disrupting hyphal morphology and membrane integrity—a conserved mechanism among many effective biocontrol agents and one that underpins the robust inhibitory activity of strain P11 in vitro.

Beyond its antifungal performance in vitro, strain P11 also effectively suppressed disease progression caused by *B. dothidea* in fruit-based assays. Notably, fruit co-inoculated with strain P11 and the pathogen maintained significantly higher levels of soluble solids, soluble sugars, titratable acids, and vitamin C compared to those inoculated with *B. dothidea* alone (Fig. 7). These parameters are key determinants of fruit quality, flavor, and nutritional value, underscoring their importance in postharvest management. Consistent with this observation, previous studies have reported that endophytes can enhance sugar accumulation, water content, and single-fruit biomass in Honeycrisp apples (Hyunmin et al., 2020), while *Pseudomonas* protegens has been shown to improve postharvest quality of peach fruit (Shan et al., 2024). The ability of strain P11 not only to reduce disease severity but also to preserve postharvest fruit quality distinguishes it from biocontrol agents that solely focus on pathogen inhibition. This dual functionality enhances the practical relevance of P11 for integrated postharvest

management, offering a strategy that aligns with both disease control and quality preservation goals in apple storage systems.

In addition to efficacy, safety represents a critical criterion for evaluating potential biocontrol agents. Notably, strain P11 was originally isolated as an endophyte from healthy apple tissues (Figs. 5 and 7), suggesting a natural compatibility with its host and a favorable safety profile for both human health and the environment. This intrinsic origin underscores its potential for sustainable agricultural applications. In summary, our findings demonstrate that the endophytic strain P11 holds considerable promise for the biocontrol of *B. dothidea* in apples. However, several important aspects warrant further investigation. First, the specific antimicrobial metabolites responsible for its antifungal activity remain to be identified; elucidating the chemical basis of its efficacy will be essential for formulation and mode-of-action studies. Second, while strain P11 showed significant protective effects under controlled greenhouse conditions, its performance must be validated in field trials, where environmental variability and complex microbial interactions may influence its stability and effectiveness. Collectively, this study provides both a theoretical foundation and a valuable microbial resource for the integrated management of apple ring rot and the development of novel biocontrol strategies.

5. Conclusion

This study isolated and identified an endophytic *Pseudomonas* sp. strain P11 from apple tissues, which potently inhibits *B. dothidea* via disrupting mycelial structure and membrane integrity. strain P11 exhibits concentration-dependent biocontrol efficacy, preserves apple quality, and maintains activity under relevant environmental conditions. These findings highlight strain P11 as a promising eco-friendly biocontrol agent, providing strain resources and theoretical basis for apple ring rot management.

CRediT authorship contribution statement

Zijun Yan: Resources, Investigation. **Jinhua Dou:** Writing – review & editing, Investigation. **Xiaodong Zheng:** Writing – review & editing, Resources, Methodology, Conceptualization. **Yuqing Zhu:** Resources, Investigation. **Qiang Zhao:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Ying Zhang:** Writing – review & editing, Writing – original draft, Software, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (32272684), Shandong Province Key Research and Development Plan (2024CXGC010908), Funds for Modern Agricultural Industry Technology System in Shandong Province (SDAIT-06–06).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.postharvbio.2026.114450](https://doi.org/10.1016/j.postharvbio.2026.114450).

Data availability

Data will be made available on request.

References

- Ahmed, A., Munir, S., He, P.F., Li, Y.M., He, P.B., Wu, Y.X., He, Y.Q., 2020. Biocontrol arsenals of bacterial endophyte: an imminent triumph against clubroot disease. *Microbiol. Res.* 241, 126565. <https://doi.org/10.1016/j.micres.2020.126565>.
- Beneduzi, A., Ambrosini, A., Passaglia, M.L., 2012. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genet. Mol. Biol.* 35, 1044–1051. <https://doi.org/10.1590/s1415-47572012000600020>.
- Cai, J., Chen, J., Lu, G., Zhao, Y., Tian, S., Qin, G., 2015. Control of brown rot on jujube and peach fruits by trisodium phosphate. *Postharvest Biol. Technol.* 99, 93–98. <https://doi.org/10.1016/j.postharvbio.2014.08.003>.
- Carreras, C., Zamorano, A., González, V.L., Pimental, P., Pizarro, L., Beltrán, M.F., Cui, W., Pinto, M., Figueroa, F., Hernández, C.R., Llanes, A., Bertaccini, A., Fiore, N., 2024. *Pseudomonas syringae* pathovar *syringae* infection reveals different defense mechanisms in two sweet cherry cultivars. *Plants* 14, 87. <https://doi.org/10.3390/plants14010087>.
- Chlebek, D., Pinski, A., Zur, J., Michalska, J., HupertKocurek, K., 2020. Genome mining and evaluation of the biocontrol potential of *Pseudomonas fluorescens* BRZ63, a new endophyte of oilseed rape (*Brassica napus* L.) against fungal pathogens. *Int. J. Mol. Sci.* 21, 8740. <https://doi.org/10.3390/ijms21228740>.
- Dong, X.L., Cheng, Z.Z., Leng, W.F., Li, B.H., Xu, X.M., Lian, S., Wang, C.X., 2021. Progression of symptoms caused by *Botryosphaeria dothidea* on apple branches. *Phytopathology* 111. <https://doi.org/10.1094/phyto-12-20-0551-r>.
- Doralicia, C., Panlo, V.E., Catalina, A.R., 2019. Membrane lipid composition: effect on membrane and organelle structure, function and compartmentalization and therapeutic avenues. *Int. J. Mol. Sci.* 20, 2167. <https://doi.org/10.3390/ijms20092167>.
- Fan, L., Wei, Y.Y., Chen, Y., Jiang, S., Xu, F., Zhang, C.D., Wang, H.F., Shao, X.F., 2023. Epinecidin-1, a marine antifungal peptide, inhibits *Botrytis cinerea* and delays gray mold in postharvest peaches. *Food Chem.* 403, 134419. <https://doi.org/10.1016/j.foodchem.2022.134419>.
- Feng, S., Lu, W., Jian, Y.F., Chen, Y., Meng, R., Deng, J., Liu, Q., Yu, T.T., Jin, L., Yang, X. Y., Li, Z.G., Jian, W., 2021. Biocontrol effect and possible mechanism of food-borne sulfide 3-Methylthio-1-Propanol against *Botrytis cinerea* in postharvest tomato. *Front. Plant Sci.* 12, 763755. <https://doi.org/10.3389/fpls.2021.763755>.
- Gao, Y., Ning, Q., Yang, Y.Z., Liu, Y., Niu, S.Q., Hu, X.C., Pan, H.R., Bu, Z.G., Chen, N., Gao, J.Y., Yu, J.L., Cao, L.D., Qin, P., Xing, J.J., Liu, B., Liu, X.M., Zhu, Y.H., 2021. Endophytic *Streptomyces hygroscopicus* OsiSh-2-mediated balancing between growth and disease resistance in host rice. *MBio* 12, e01566-21. <https://doi.org/10.1128/mbio.01566-21>.
- Hong, Y., Pang, W., Feng, J., Liang, Y., 2025. Identification of a *Pseudomonas brassicacearum* strain as a potential biocontrol agent against clubroot in cruciferous plants through endophytic bacterial community analysis and culture-dependent isolation. *Pest. Manag. Sci.* 82, 442–454. <https://doi.org/10.1002/ps.70209>.
- Huang, Y.H., Li, J.H., Shan, X.Y., Wang, H.T., Duan, Y.X., 2024. Bioactivities evaluation of an endophytic bacterial strain *Bacillus tequilensis* QNF2 inhibiting apple ring rot caused by *Botryosphaeria dothidea* on postharvest apple fruits. *Food Microbiol.* 123, 104590. <https://doi.org/10.1016/j.fm.2024.104590>.
- Huang, Y., Sun, C.S., Guan, X.N., Lian, S., Li, B.H., Wang, C.X., 2021. Biocontrol efficiency of *Meyerozyma guilliermondii* Y-1 against apple postharvest decay caused by *Botryosphaeria dothidea* and the possible mechanisms of action. *Int. J. Food Microbiol.* 338, 108957. <https://doi.org/10.1016/j.ijfoodmicro.2020.108957>.
- Hyunmin, R., Victor, V.E., Soo-Hyung, K., Sharon, L.D., 2020. Endophytes increased fruit quality with higher soluble sugar production in honeycrisp apple (*Malus pumila*). *Microorganisms* 8, 699. <https://doi.org/10.3390/microorganisms8050699>.
- Jiang, M.Y., Wang, Z.R., Chen, K.W., Kan, J.Q., Wang, K.T., Zalan, Z., Hegyi, F., Takacs, K., Du, M.Y., 2019. Inhibition of postharvest gray mould decay and induction of disease resistance by *Pseudomonas fluorescens* in grapes. *Acta Aliment.* 48, 288–296. <https://doi.org/10.1556/066.2019.48.3.2>.
- Khadiri, M., Boubaker, H., Askarne, L., Ezrari, S., Radouane, N., Farhaoui, A., Hamss, H. E., Tahiri, A., Barka, E.A., Lahlali, R., 2023. *Bacillus cereus* B8W8 an effective bacterial antagonist against major postharvest fungal pathogens of fruit. *Postharvest Biol. Technol.* 200, 112315. <https://doi.org/10.1016/j.postharvbio.2023.112315>.
- Liu, B., Qiao, H.P., Huang, L.L., Heinrich, B., Han, Q.M., Kang, Z.S., Gong, Y.F., 2009. Biological control of take-all in wheat by endophytic EIR-j and potential mode of action. *Biol. Control* 49, 277–285. <https://doi.org/10.1016/j.biocontrol.2009.02.007>.
- López-Orona, A.C., Hernández-Verdugo, S., Velarde-Félix, S., Garzón-Tiznado, J.A., Sy, O., Retes-Manjarrez, J.E., 2019. Cross pathogenicity of *Fusarium oxysporum* isolated from peppers. *Eur. J. Plant Pathol.* 154, 1111–1123. <https://doi.org/10.1007/s10658-019-01732-z>.
- Mazzola, M., Funnell, D.L., Raaijmakers, J.M., 2004. Wheat cultivar-specific selection of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* species from resident soil populations. *Microb. Ecol.* 48, 338–348. <https://doi.org/10.1007/s00248-003-1067-y>.
- Monaco, C., Bello, D.G., Rollan, C.M., Ronco, L., Lampugnani, G., Arteta, N., Abramoff, C., Aprea, A., Larran, S., Stocco, M., 2009. Biological control of *Botrytis cinerea* on tomato using naturally occurring fungal antagonists. *Arch. Phytopathol. Plant Prot.* 42, 729–737. <https://doi.org/10.1080/03235400701390646>.
- Mu, Y.P., Yue, Y.Y., Gu, G.R., Deng, Y.M., Jin, H., Tao, K., 2020. Identification and characterization of the *Bacillus atrophaeus* strain J-1 as biological agent of apple ring rot disease. *J. Plant Dis. Prot.* 127, 1–12. <https://doi.org/10.1007/s41348-020-00309-x>.
- Parsa, S., García-Lemos, A.M., Castillo, K., Ortiz, V., López-Lavalle, L.A.B., Braun, J., Vega, F.E., 2016. Fungal endophytes in germinated seeds of the common bean, *Phaseolus vulgaris*. *Fungal Biol.* 120, 783–790. <https://doi.org/10.1016/j.funbio.2016.01.017>.
- Qing, S.Y., Gao, Y.P., Wang, L., Wang, X.A., Wang, L.L., Ran, J.Y., Ou, X.H., Wang, Y.H., Xiao, C.H., Jiang, W.K., Guo, L.P., Tao, Z., Huang, L.Q., 2024. Pathogen-driven *Pseudomonas* reshaped the phyllosphere microbiome in combination with *Pseudostellaria heterophylla* foliar disease resistance via the release of volatile organic compounds. *Environ. Micro* 19, 61. <https://doi.org/10.1186/s40793-024-00603-3>.
- Reynolds, J., Moyes, R., Breakwell, D.P., 2009. Differential staining of bacteria: endospore strain. *Cur. Protoc. Microbiol.* <https://doi.org/10.1002/9780471729259.mca03js15>. Appendix 3:3J.
- Seo, H.H., Chaw, E.H.M., Jun, S.N., Jeong, Y.C., Kil, Y.K., 2023. Butyl succinate-mediated control of *Bacillus velezensis* ce 100 for apple anthracnose caused by *Colletotrichum gloeosporioides*. *J. Appl. Microbiol.* 134, lxad247. <https://doi.org/10.1093/jambio/lxad247>.
- Shan, X.Y., Zhang, C.F., Li, J.H., Duan, Y.X., Huang, Y.H., 2024. *Pseudomonas protegens* controlled brown rot (*Monilinia fructicola*) on peach fruit in postharvest by suppressing fungal development and inducing plant defense-related genes. *Postharvest Biol. Technol.* 213, 112971. <https://doi.org/10.1016/j.postharvbio.2024.112971>.
- Singh, M., Kumar, A., Singh, R., Pandey, K.D., 2017. Endophytic bacteria: a new source of bioactive compounds. *3 Biotech* 7, 1–14. <https://doi.org/10.1007/s13205-017-0942-z>.
- Sun, M., Liu, J.P., Li, J.H., Huang, Y.H., 2022. Endophytic bacterium *Serratia plymuthica* from chinese leek suppressed apple ring rot on postharvest apple fruit. *Front. Microbiol.* 12, 802887. <https://doi.org/10.3389/fmicb.2021.802887>.
- Tang, W., Ding, Z., Zhou, Z.Q., Guo, L.Y., 2012. Phylogenetic and pathogenic analyses show that the causal agent of apple ring rot in China is *Botryosphaeria dothidea*. *Plant Dis.* 96, 486–496. <https://doi.org/10.1094/pdis-08-11-0635>.
- Wang, H.T., Zhang, F.J., Zhang, Y.L., Wang, M.N., Zhang, Y.Q., Zhang, J., 2024. Enrichment of novel entomopathogenic *Pseudomonas* species enhances willow resistance to leaf beetles. *Microbiome* 12, 169. <https://doi.org/10.1186/s40168-024-01884-z>.
- Wei, J., Yu, C., Liu, S.Y., Ou, X.G., Sun, L.X.Y., Gong, H.M., Mu, Q.T., Li, Z.G., Zhao, L., W., 2024. Biocontrol efficacy and mechanism of endophytic *Bacillus velezensis* HE-23 from *Aucklandia lappa* roots against the gray mold of postharvest tomato. *Postharvest Biol. Technol.* 217, 113104. <https://doi.org/10.1016/j.postharvbio.2024.113104>.
- Yuan, H.B., Shi, B.K., Wang, L., Huang, T.X., Zhou, Z.Q., Hou, H., Tu, H.T., 2022. Isolation and characterization of *Bacillus velezensis* strain P2-1 for biocontrol of apple postharvest decay caused by *Botryosphaeria dothidea*. *Front. Microbiol.* 12, 808938. <https://doi.org/10.3389/fmicb.2021.808938>.
- Yuan, H., Shi, B., Wang, Z., Qin, G., Hou, H., Wang, L., 2023. Exploration of the biocontrol activity of *Bacillus atrophaeus* strain HF1 against pear valsa canker caused by *valsa pyri*. *Int. J. Mol. Sci.* 24, 15477. <https://doi.org/10.3390/ijms242015477>.
- Yuan, H.B., Wang, L., Hou, H., Qin, G.H., Shi, B.K., Zhou, B.B., Chen, Y., Tu, H.T., 2025. Antifungal activity and mechanism of volatileorganic compounds produced by

- Bacillus velezensis* strain P2-1 against *Botryosphaeria dothidea*-induced postharvest decay in apples. *Postharvest Biol. Technol.* 222, 113405. <https://doi.org/10.1016/j.postharvbio.2025.113405>.
- Zheng, X., Zheng, L., Xia, F., Li, J., Zhou, W., Yuan, L., Rao, S., Yang, Z., 2023. Biological control of blue mold rot in apple by *Cluyveromyces marxianus* XZ1 and the possible mechanisms of action. *Postharvest Biol. Technol.* 196, 112179. <https://doi.org/10.1016/j.postharvbio.2022.112179>.
- Zou, X.R., Wei, Y.Y., Jiang, S., Xu, F., Wang, H.F., Zhan, P.P., Shao, X.F., 2022. ROS stress and cell membrane disruption are the main antifungal mechanisms of 2-phenylethanol against *Botrytis cinerea*. *J. Agric. Food Chem.* 70, 6187. <https://doi.org/10.1021/acs.jafc.2c06187>.