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Original Article

Endophytic *Bacillus subtilis* P10 from *Prunus cerasifera* as a biocontrol agent against tomato Verticillium wilt

Bacillus subtilis P10 endofítico de *Prunus cerasifera* como agente de biocontrole contra murcha de Verticillium de tomate

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Abstract

Endophytic bacteria serve key roles in the maintenance of plant health and growth. Few studies to date, however, have explored the antagonistic and plant growth-promoting (PGP) properties of *Prunus cerasifera* endophytes. To that end, we isolated endophytic bacteria from *P. cerasifera* tissue samples and used a dual culture plate assay to screen these microbes for antagonistic activity against *Verticillium dahliae*, *Botryosphaeria dothidea*, *Fusarium oxysporum*, *F. graminearum*, and *F. moniliforme*. Of the 36 strains of isolated bacteria, four (strains P1, P10, P16, and P20) exhibited antagonistic effects against all five model pathogens, and the P10 strain exhibited the strongest antagonistic to five pathogens. This P10 strain was then characterized in-depth via phenotypic assessments, physiological analyses, and 16s rDNA sequencing, revealing it to be a strain of *Bacillus subtilis*. Application of a P10 cell suspension (1×10^8 CFU/mL) significantly enhanced the seed germination and seedling growth of tomato in a greenhouse setting. This P10 strain further significantly suppressed tomato Verticillium wilt with much lower disease incidence and disease index scores being observed following P10 treatment relative to untreated plants in pot-based experiments. Tomato plants that had been treated with strain P10 also enhanced defense-related enzymes, peroxidase, superoxide dismutase, and catalase activity upon *V. dahliae* challenge relative to plants that had not been treated with this endophytic bacterium. The results revealed that the P10 bacterial strain has potential value as a biocontrol agent for use in the prevention of tomato Verticillium wilt.

Keywords: Endophytes, *Bacillus subtilis*, *Prunus cerasifera*, Verticillium wilt, Biocontrol.

Resumo

As bactérias endofíticas desempenham papel fundamental na manutenção da saúde e do crescimento das plantas. Poucos estudos até o momento, no entanto, exploraram as propriedades antagônicas e promotoras de crescimento de plantas (PGP) de endófitos de *Prunus cerasifera*. Para esse fim, isolamos bactérias endofíticas de amostras de tecido de *P. cerasifera* e usamos um ensaio de placa de cultura dupla para rastrear esses micróbios quanto à atividade antagonista contra *Verticillium dahliae*, *Botryosphaeria dothidea*, *Fusarium oxysporum*, *F. graminearum* e *F. moniliforme*. Das 36 cepas de bactérias isoladas, quatro (cepas P1, P10, P16 e P20) exibiram efeitos antagônicos contra todos os cinco patógenos modelo, e a cepa P10 exibiu o antagonista mais forte para cinco patógenos. Essa cepa P10 foi então caracterizada em profundidade por meio de avaliações fenotípicas, análises fisiológicas e sequenciamento de rDNA 16s, revelando ser uma cepa de *Bacillus subtilis*. A aplicação de uma suspensão de células P10 (1 × 108 UFC / mL) aumentou significativamente a germinação das sementes e o crescimento das mudas de tomate em casa de vegetação. Essa cepa P10 suprimiu ainda mais a murcha de Verticillium do tomate com incidência de doença muito menor e pontuações de índice de doença sendo observadas após o tratamento com P10 em relação a plantas não tratadas em experimentos baseados em vasos. As plantas de tomate que foram tratadas com a cepa P10 também aumentaram as enzimas relacionadas à defesa, peroxidase, superóxido dismutase e atividade da catalase após o desafio de *V. dahliae* em relação às plantas que não foram tratadas com essa bactéria endofítica. Os resultados revelaram que a cepa bacteriana P10 tem valor potencial como agente de biocontrole para uso na prevenção da murcha de Verticillium em tomate.

Palavras-chave: endófitos, *Bacillus subtilis*, *Prunus cerasifera*, murcha de Verticillium, Biocontrole.

1. Introduction

Endophytic bacteria and fungi are microbial species that asymptomatically colonize specific plants and that can regulate a range of host physiological responses. These endophytes exhibit mutualistic relationships with their hosts such that plants provide these microbes with nutrients necessary for growth and survival, and these

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microbes in turn produce nutrients or modulate host processes through the production of specific metabolites. Endophytes can bolster plant defense responses, thereby enhancing resistance to both biotic and abiotic stressors (Di Fiore and Del Gallo, 1995; Giauque et al., 2019; Wani et al., 2015; Amatuzzia et al., 2018). Over 120 species of endophytic bacteria across 54 genera have been identified to date, including common soil microbes of the *Bacillus*, *Pseudomonas*, *Enterobacter*, and *Agrobacterium* genera (Afzal et al., 2019; Sturz et al., 2000).

The most commonly characterized endophytic bacteria are *Bacillus* species (Lilley et al., 1996; Viana et al., 2020), with *B. subtilis* being particularly well known for its ability to promote plant growth (Poonguzhali et al., 2006) and to antagonize a range of plant pathogens, including *Ceratocystis ulrni* (Schreiber et al., 1988), *Fusarium moniliforme* (Hinton and Bacon, 1995), *Xanthomonas oryzae* (Lin et al., 2001), *X. campestris* pv. *Campestris* (Singh et al., 2010), *Colletotrichum* spp. (Ruangwong et al., 2012), *F. graminearum* (Zhao et al., 2014), *Sclerotinia sclerotiorum* (Sun et al., 2017), and *Scleromitrula shiraiana* (Xu et al., 2019). *B. subtilis* strains can also be isolated from individual hosts and used to inoculate other plants in order to enhance their growth and pathogen resistance (Bacon and Hinton, 2002).

Verticillium wilt, which is caused by the soil-borne *Verticillium dahliae* fungus, is a severe vascular disease capable of affecting over 200 species of plants, including many key food crops (Inderbitzin and Subbarao, 2014). Tomatoes (*Solanum lycopersicum* L.) are the most widely grown species of fruit vegetable, and tomato plants are highly susceptible to Verticillium wilt, resulting in major reductions in crop yield and quality. While fungicides can be used to control disease outbreaks, these chemical control measures can disrupt normal ecological homeostasis, leave toxic residues, and facilitate the development of fungicide-resistant pathogens. Biological control measures, in contrast, represent a novel approach to preventing disease outbreaks without incurring substantial health or environmental risks. As they produce antimicrobial compounds, induce host plant resistance, and compete for limited space and nutrients, endophytes represent a promising approach to the biological control of *Verticillium* wilt (Chen et al., 2014).

Prunus cerasifera is a deciduous tree that is most commonly cultivated for its ornamental and medicinal value (Song et al., 2018). No studies to date have examined the use of endophytes derived from *P. cerasifera* to protect against *V. dahliae* infections. As such, in this report, a range of endophytic bacteria exhibiting varying levels of antagonistic activity against *V. dahliae* and other pathogens were isolated from *P. cerasifera.* The growth-promotion and biocontrol potential of these endophytic bacteria were also assessed.

2. Materials and methods

2.1. Endophytic bacteria isolation and purification

Endophytic bacteria were isolated from leaves of *P. cerasifera* as detailed in a study previously published by Miller et al. (2012). Isolates were then streaked on appropriate culture plates and were assessed via microscopy. Pure culture isolates were stored at 4°C for short term use and at -80°C in a 30% (v/v) glycerol solution for long term maintenance.

2.2. Assessment of the antagonistic activity of endophytic bacteria

A dual culture plate assay was used to screen for the ability of isolated endophytes to antagonize the growth of specific plant pathogens (*V. dahliae*, *Botryosphaeria dothidea*, *F. oxysporum, F. graminearum* and *F. moniliforme*). Mycelial pathogen plugs (5 mm in diameter) corresponding to each of these pathogens were isolated following one week of culture on potato dextrose agar (PDA), and were transferred to the center of a fresh PDA plate. Individual endophytic bacterial strains were grown overnight in Luria-Bertani (LB) broth at 37°C while shaking at 170 rpm, after which a 1.5 mL volume of each culture was collected, washed two times using fresh LB, resuspended in 100 µL of LB, and applied to three separate locations surrounding the mycelial plug on prepared PDA plates (25 µL/spot). The antifungal activity of these endophytic species was then monitored via measuring inhibition zones at 72 h post-incubation at 28°C. As a control, plates that had not been inoculated with any endophytic bacteria were used.

2.3. Endophytic bacterial identification

2.3.1. Assessment of bacterial strain morphology, physiology, and biochemistry

Bergey's Manual of Determinative Bacteriology was used to guide all analyses of the physiology and morphology of these isolates (Buchanan and Gibbons, 1994). Assays used in this context included analyses of indole production, the Voges-Proskauer reaction, lactose utilization, nitrate reduction, gelatin liquefaction, methyl red reaction, glucose utilization, and starch hydrolysis. In addition, cell shape, cell size, colony properties, spore production, and Gram staining were evaluated.

2.3.2. PCR-based phylogenetic analyses

A DNA extraction kit (Takara Bio Inc., Dalian) was used to isolate total genomic DNA from endophytic bacterial strains. A PCR approach was then employed to amplify the 16S rDNA gene using the fD1 5'-AGAGTTTGATCCTGGCTCAG-3' universal forward primer and the rP1 5'-ACGGTTACCTTGTTACGACTT-3' reverse primer. All PCR reactions were conducted in a 50 µL volume containing 25 µL 2×PCR Mix, 15µL ddH2O, 5µL template DNA, and 2.5µL of forward and reverse primers. Thermocycler settings were as follows: 94°C for 4 min; 35 cycles of 94°C for 30 s, 60°C for 45 s, and 72°C for 1 min; 72°C for 10 min. Amplified PCR products were then sequenced by Sangon Biotech (Shanghai) Co., Ltd. using the same primers described above, and the resultant sequences were aligned with those in GenBank using the BLAST tool (Johnson et al., 2008). The neighbor-joining method was then used to construct a phylogenetic tree with the MEGA 7.0 software. The 16s rDNA sequence generated in this study were deposited in the NCBI GenBank.

2.4. The impact of the P10 strain on seed germination and seedling growth

After a 24 h culture period in liquid LB medium, the P10 endophytic bacterial strain was suspended at different concentrations (1.0×10^7, 1.0×10^8, 1.0×10^9 CFU/mL) in sterilized distilled water (SDW). Tomato seeds (Bai Guo Qiang Feng) were disinfected for 30 s with 70% ethanol, after which they were sterilized for 5 minutes with 5% NaClO, washed thrice using SDW, and soaked in the prepared P10 suspensions at 25°C for 24 h. After treatment, seeds were germinated in Petri dishes at 26°C with 16 h of light per day. After five days, germination rates were determined. After seven days, radicle and plumule length were measured, and fresh seedling weight was determined. Disinfected seeds that had been soaked in SWD served as controls. Twenty replicate seeds were used per treatment, and experiments were repeated thrice.

2.5. Assessment of the biocontrol properties of the P10 strain

For these pot-based experiments, SDW-treated tomato seedlings were prepared as above and were individually transplanted into 8 × 8 cm diameter pots containing autoclaved soil. Pots were then transferred to a greenhouse (26°C, 70% relative humidity) with a 16 h photoperiod. After seedlings exhibited 4 leaves, 20 mL suspensions of water, P10 (1.0×10^8 CFU/mL), or carbendazim (1g/L) were added to individual pots. In total, 100 seedlings per treatment condition were analyzed. On day 10 post-inoculation, a 20 mL suspension of *V. dahliae* spores (1.0×10^8CFU/mL) was applied to each pot via a root-drenching approach. At 15 days post-spore application, disease incidence, disease index values, and biocontrol efficacy were assessed using the formulas detailed in Chen et al. (2014).

A 5-point grading scale was used to evaluate Verticillium wilt severity in these tomato plants (grades 0-4) as follows: Grade 0, no evidence of disease; Grade 1, < 25% of leaves exhibit symptoms, with faint yellow mesophyll cells; Grade 2, 26-50% of leaves exhibit symptoms, with most plants exhibit yellow or tawny leaves with curly edges; Grade 3, 51-75% of leaves exhibit disease symptoms, with partial defoliation; Grade 4, > 76% of leaves exhibit symptoms. In cases of particularly severe disease, all leaves may fall off because stems are bare, and plants may wither to death.

2.6. Antioxidant enzyme activity and MDA content analyses

On days 2, 6, 10, 14, and 18 of P10 or carbendazim treatment upon *V. dahliae* challenge, antioxidant enzyme activity levels and MDA (malondialdehyde) contents were assessed, with plants that had only been inoculated with *V. dahliae* serving as controls. Briefly, freshly isolated leaf samples were ground in 50 mM phosphate buffer (pH 7.8) on ice, after which the homogenates were centrifuged for 15 minutes at 10,000 g at 4°C. Supernatants were then collected for subsequent analysis. SOD (Superoxide Dismutase) activity was measured based upon nitroblue tetrazolium (NBT) photoreduction, with one unit of SOD activity being defined as the amount of SOD required to achieve a 50% inhibition of this NBT photoreduction reaction. POD (Peroxidase) activity was assessed via a guaiacol approach, with a unit of POD enzymatic activity being defined as a 0.01 increase in A470 per minute. CAT (Catalase) activity was assessed via UV absorption, with one unit of CAT activity being the amount necessary to reduce the A240 value by 0.1 per minute. MDA (Malondialdehyde) levels were assessed via a thiobarbituric acid assay. Three replicate samples were analyzed for each treatment condition, and a UV Spectrophotometer (Ultrospec 3300 pro, Amersham Biosciences) was used for all spectrophotometric readings.

2.7. Statistical analysis

SPSS v17.0 (SPSS Inc., IL, USA) and Microsoft Excel were used for all statistical testing. Data were compared via one-way analyses of variance (ANOVAs) and least-significant difference (LSD) tests, with $P < 0.05$ as the significance threshold.

3. Results

3.1. Isolation of endophytic bacteria with antagonistic activity against pathogenic fungi

In total, we isolated 36 strains of endophytic bacteria from *P. cerasifera* (strains P1-P36), with 11, 9, and 16 strains being isolated from the roots, stems, and leaves of these plants. The ability of these endophytes to antagonize the growth of five strains of pathogenic fungi (*V. dahliae*, *B. dothidea*, *F. graminearum*, *F. oxysporum*, and *F. moniliforme*) was then assessed. Of these isolates, four strains (P1, P10, P16, and P20) strongly suppressed fungal growth relative to control (Table 1, Figure 1). Strain P10 exhibited the most robust inhibition of *B. dothidea* (68.8%), *F. oxysporum* (52.9%), *V. dahliae* (51.5%), *F. moniliforme* (48.4%), and *F. graminearum* (40.8%) growth. As such, strain P10 was the subject of all further experiments.

3.2. Characteristics of the P10 endophytic bacterial strain

P10 cells were off-white, rod-shaped, Gram-positive, V-P test-positive, spore-positive, methyl red test-negative, and without indole production. These bacteria also utilized lactose and glucose, and were able to reduce nitrate to

Table 1. Inhibition of pathogenic fungi by endophytic bacteria.

strains	source	*B. dothidea*	*F. moniliforme*	*F. graminearum*	*F. oxysporum*	*V. dahliae*
P1	leaf	60.8	48.4	33.8	51.5	51.3
P10	leaf	68.8	48.4	40.8	52.9	51.5
P16	root	34.1	38.5	37.5	40.8	35.4
P20	leaf	47.0	45.8	48.4	40.7	43.1

Inhibition ratio for the indicated pathogenic fungi (%) = (Control colony diameter – treatment colony diameter) 100/Control colony diameter.

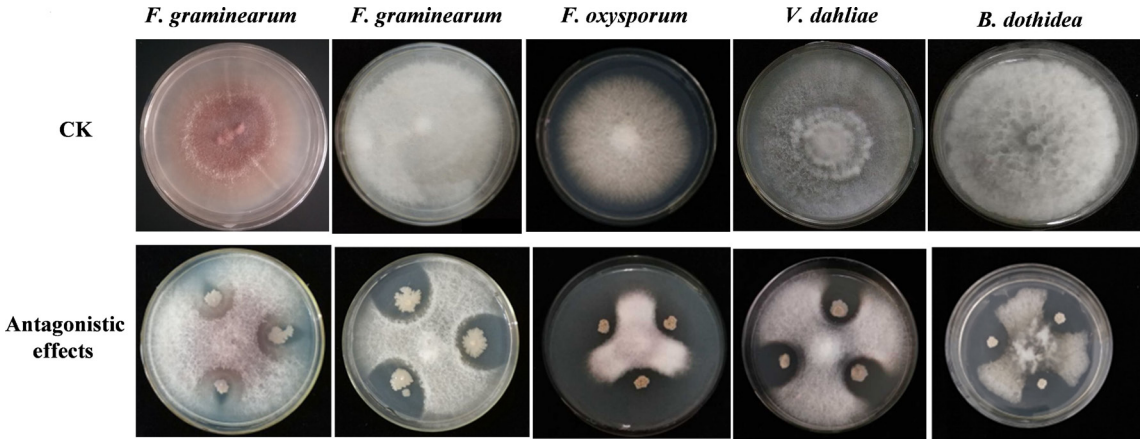

Figure 1. Antagonistic effects of the endophytic bacteria strain P10 on the growth of five fungal pathogens. CK, control.

nitrite. These bacteria also hydrolyzed starch and gelatin in corresponding assays. Overall, the results of these biochemical and physiological analyses suggested that P10 may be a strain of *B. subtilis* (Table 2).

3.3. P10 16S rDNA sequencing and phylogenetic classification

We next conducted 16S rDNA sequencing in order to definitively identify strain P10. The resultant 16s rDNA sequence (deposited as GenBank accession No. MT830869) was 1415 nucleotides in length, and BLAST alignment revealed 99.23% identity to *B. subtilis* KCTC13429. A phylogenetic tree incorporating strain P10 was additionally constructed (Figure 2). Given that these phylogenetic findings are consistent with the above biochemical and morphological findings, this confirmed the taxonomical classification of this *B. subtilis* strain.

3.4 B. subtilis strain P10 enhances tomato seed germination and seedling growth

Relative to control treatment, P10 suspensions (1.0×10^8 CFU/mL) significantly enhanced tomato seed germination ($P \leq 0.05$; Table 3). Seedlings treated with P10 suspensions also exhibited increased radicle length, plumule length, and fresh weight relative to control seedlings, with the maximal effect being observed for the 1.0×10^8 CFU/mL suspension. Relative to control treatment, the application of 1.0×10^8 CFU/mL P10 increased radicle length from 2.57 cm to 4.28 cm, plumule length from 0.84 cm to 3.68 cm, and fresh weight from 0.025 g to 0.110 g (Table 3).

3.5. Biocontrol efficacy of B. subtilis strain P10 as a means of controlling Verticillium wilt in pot experiments

Pot-based experiments revealed that P10 application markedly reduced Verticillium wilt disease incidence and disease index values in tomato seedlings relative to control treatment (Table 4). The overall biocontrol efficacy of strain P10 was 58.45%. While this value was lower than that observed in seedlings treated with the fungicide carbendazim (72.68%), these differences were insignificant.

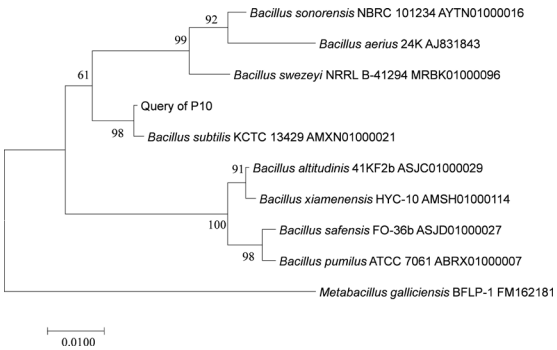

Figure 2. A neighbor-joining phylogenetic analysis of strain P10.

Table 2. Physiological and biochemical characteristics of the P10 endophytic bacterial strain.

	P10	*B.s*	*B.c*
Gelatin liquefaction	+	+	+
methyl red reaction	–	–	–
Voges-Proskauer reaction	+	+	+
indole production	–	–	–
nitrate reduction	+	+	+
D-glucose utilization	+	+	+
D-lactose utilization	+	+	+
starch hydrolysis	+	+	+
Gram staining	+	+	+

"+" indicates positive, "-" indicates negative. *B. s: Bacillus subtilis, B. c: Bacillus cereus* correspond to utilized control strains.

3.6. The impact of P10 treatment on antioxidant enzyme activity and MDA contents

Lastly, we evaluated the impact of P10 treatment on antioxidant enzyme activity and MDA levels in potted tomato seedlings. SOD, POD, and CAT activity levels initially increased and then decreased. The SOD, POD, and CAT activity of P10-treated plants reached the maximum values on days 10, 10, and 6, respectively, at which time SOD, POD,

Table 3. The impact of strain on tomato seed germination and seedling growth.

Treatments (cfu/mL)	Germination rate±SE (%)	Fresh weigh±SE (g)	Radicle length±SE (cm)	Plumule length±SE (cm)
10^7	78.33±1.67 b	0.067±0.004 b	3.95±0.42 a	2.27±0.08 b
10^8	90.00±2.89 a	0.110±0.011 a	4.28±0.13 a	3.68±0.29 a
10^9	76.67±1.67 b	0.050±0.005 b	2.71±0.23 b	1.43±0.07 c
Control	71.67±1.67 b	0.025±0.003 c	2.57±0.10 b	0.84±0.06 d

SE, standard error of the mean. Identical letters within the same treatment condition indicate a lack of significant difference at the P < 0.05 threshold, as determined via LSD multiple range test.

Table 4. *B. subtilis* strain P10 biocontrol efficacy on *V. dahliae* in pot experiments.

Treatment	Disease incidence±SE (%)	Disease index±SE (%)	Biocontrol efficacy (%)
Bacillus subtilis	69.33±3.84 b	34.67±1.92 b	58.45±0.92 a
Carbendazim	45.67±3.48 c	22.83±1.74 c	72.68±1.04 a
Control	100.00±0.00 a	83.33±3.18 a	— —

SE, standard error of the mean. Data are means of three replicates of 100 plants each. Identical letters within the same treatment condition indicate a lack of significant difference at the P < 0.05 threshold, as determined via LSD multiple range test.

and CAT activity levels in P10-treated plants were 21.3%, 77.4%, and 103.3% higher than in control plants, whereas in carbendazim-treated plants these values were increased by 69.4%, 115.0%, and 206.6%, respectively. All differences between treatment and control groups were significant. Overall, SOD, POD, and CAT activity levels were higher in P10-treated plants relative to untreated controls, and were these levels were even higher in carbendazim-treated plants (Figures 3-5).

MDA levels in analyzed plants tended to increase after an initial decrease. These levels were 36.7% lower in P10-treated plants relative to untreated controls, while levels in carbendazim-treated plants were 44.0% lower than that in control groups on day 10 of the analysis period. These differences were significant. Over the course of this period, MDA levels in all treated samples were lower than those in control samples, and these levels were substantially lower in carbendazim-treated plants relative to P10-treated plants (Figure 6).

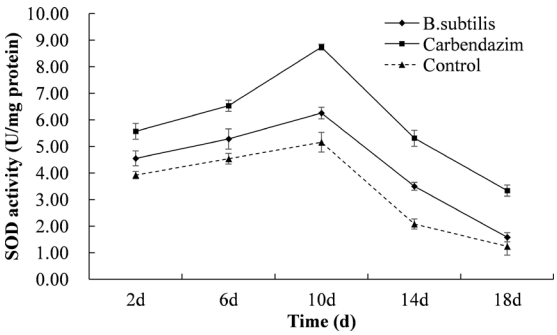

Figure 3. The impact of strain P10 application on the induction of SOD activity.

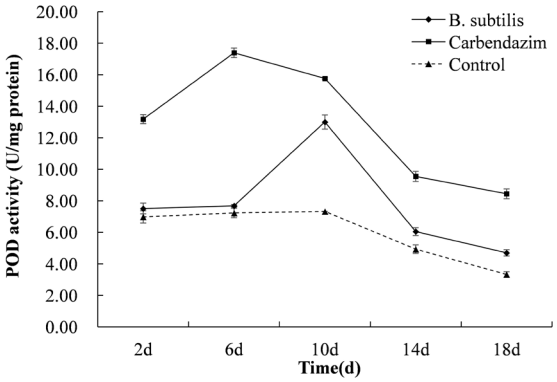

Figure 4. The impact of strain P10 application on the induction of POD activity.

4. Discussion

The biological control of pathogenic infections represents an attractive alternative to chemical control strategies, as the former is associated with lower health risks and a lower risk of environmental damage. Specific *B. subtilis* strains exhibiting a high degree of antagonistic activity towards phytopathogens represent ideal agents for the biocontrol of a range of fungal diseases (Chen et al., 2014; Shrestha et al., 2016; Sun et al., 2017), including fungal diseases such as wheat root rot caused by *F. graminearum* (Moussa et al., 2013), apple ring rot caused by *B. dothidea* (Fan et al., 2017), tomato gray mold and leaf mold caused by *Botrytis cinerea* and *Cladosporium fulvum* respectively (Wang et al., 2018), pea wilt disease caused by *F. oxysporum* (Khan et al., 2018), pepper seedling wilt disease and rice sheath blight caused by *Rhizoctonia solani* (Jamali et al., 2020; Wu et al., 2019), bacterial diseases such as rice bacteria blight caused by *Xanthomonas oryzae pv. Oryzae* (Chithrashree et al., 2011), citrus canker caused by *X. axonopodis pv. Citri* (Huang et al., 2012), tomato bacterial wilt caused by *Ralstonia solanacearum* (Chen et al., 2013),

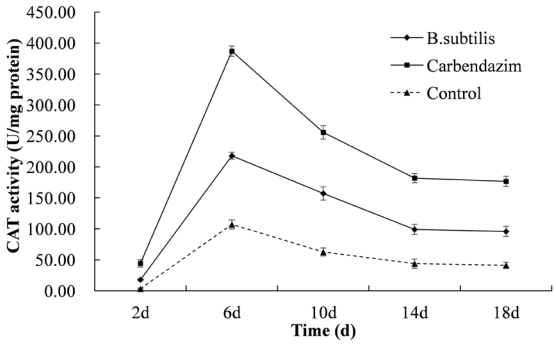

Figure 5. The impact of strain P10 application on the induction of CAT activity.

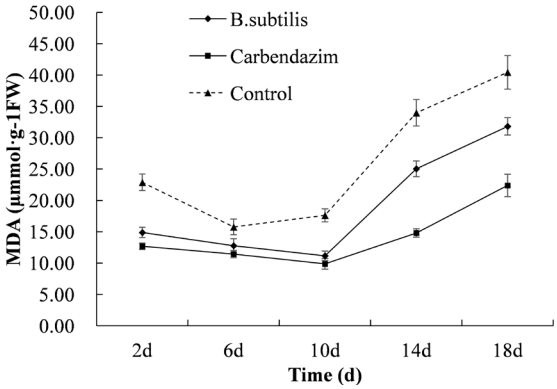

Figure 6. The impact of strain P10 application on MDA levels.

and melon bacterial fruit blotch caused by *Acidovorax citrulli* (Fan et al., 2017). *B. subtilis* strains are the dominant endophytic bacteria associated with many plant species, and they commonly exhibit the ability to enhance host plant resistance to pathogenic fungi and bacteria (Ansary et al., 2018; Kushwaha et al., 2020; Xu et al., 2019, 2020). These *B. subtilis* also do not pose a risk to humans, making them ideal for use in biocontrol applications (Earl et al., 2008).

Herein, we found that applying *B. subtilis* strain P10 to tomato seedlings was sufficient to suppress Verticillium wilt severity. *B. subtilis* is among the most commonly studied microbes in the context of biocontrol, but few studies to date have sought to control Verticillium wilt using *Bacillus* strains. Similar findings were also observed upon the application of endophytic *B. subtilis* BSD-2 isolated from cotton stems, which was able to control cotton *V. dahlia* infections (Liu et al., 2016). In this paper, the P10 strain was identified as one that may be well-suited to the biocontrol of Verticillium infections in modern agricultural systems.

B. subtilis species are well-suited to controlling a range of bacterial and fungal diseases via enhancing host plant resistance, producing antimicrobial compounds, or competing with pathogens for nutrients (Hashem et al., 2019). While the mechanisms whereby *B. subtilis* strain P10 protects against pathogenic infection were not clarified in the present study, this protective activity may be associated with protease secretion. Enzymes including POD, SOD, and CAT are responsible for scavenging ROS (Reactive Oxygen Species) in plants (Chandrasekaran and Chun, 2016). We found that *B. subtilis* strain P10 enhanced the activity of these enzymes in treated plants, with higher POD, SOD, and CAT activity being observed in P10-treated plants challenged with *V. dahliae*. *B. subtilis* CBR05-mediated alleviation of ISR (Induced Systematic Resistance) oxidative stress has also been linked to enhanced SOD, POD, CAT, PPO (Polyphenol Oxidase), and PAL (Phenylalanine Ammonia-lyase) activity in early defense responses against bacterial soft rot in tomato plants (Chandrasekaran and Chun, 2016), and *B. subtilis* SL-44 treatment increased the activity of defense-related enzymes (SOD, POD, CAT, PAL, and PPO) and activated cellular defense responses in pepper plants (Wu et al., 2019).

In addition to their ability to facilitate disease control, *Bacillus* species can indirectly and directly enhance plant growth via producing siderophores, phytohormones, and enzymes including proteases and cellulases (Ben Abdallah et al., 2018; Radhakrishnan and Lee, 2016). We found that *B. subtilis* strain P10 was able to enhance the germination and growth of tomato seedlings, particularly in response to treatment with 1.0×10^8 CFU/mL P10 (Table 4). These results thus suggested that *B. subtilis* strain P10 can simultaneously stimulate plant growth while inhibiting pathogenic infection.

In summary, the isolated *B. subtilis* strain P10 exhibited antagonistic activity against the plant pathogen strains *B. dothidea*, *V. dahliae*, *F. oxysporum, F. graminearum,* and *F. moniliforme*, indicating that this endophyte exhibits relatively broad-spectrum antagonistic activity. P10 application was sufficient to suppress Verticillium wilt disease severity by 58.45% while simultaneously decreasing MDA levels and enhancing SOD, POD, and CAT activity in treated plants. This *B. subtilis* isolate was also able to enhance tomato seed germination and seedling growth. Together, these data indicate that this endophytic isolate can enhance plant growth and stress resistance. However, the biocontrol efficacy of endophytic microbes may vary under field conditions. As such, future field-based efficacy tests of the ability of isolate P10 to protect against tomato Verticillium wilt infection are essential.

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