

Biocontrol and Growth Promotion Potential of *Bacillus subtilis* CTXW 7-6-2 against *Rhizoctonia solani* that Causes Tobacco Target Spot Disease

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Abstract

Fungal diseases form perforated disease spots in tobacco plants, resulting in a decline in tobacco yield and quality. The present study investigated the antagonistic effect of *Bacillus subtilis* CTXW 7-6-2 against *Rhizoctonia solani*, its ability to promote the growth of tobacco seedlings, and the expression of disease resistance-related genes for efficient and eco-friendly plant disease control. Our results showed that CTXW 7-6-2 had the most vigorous growth after being cultured for 96 h, and its rate of inhibition of *R. solani* growth *in vitro* was 94.02%. The volatile compounds produced by CTXW 7-6-2 inhibited the growth of *R. solani* significantly (by 96.62%). The fungal growth-inhibition rate of the *B. subtilis* CTXW 7-6-2 broth obtained after high-temperature and no-high-temperature sterile fermentation was low, at 50.88% and 54.63%, respectively. The lipopeptides extracted from the *B. subtilis* CTXW 7-6-2 fermentation broth showed a 74.88% fungal growth inhibition rate at a concentration of 100 mg/l. Scanning and transmission electron microscopy showed some organelle structural abnormalities, collapse, shrinkage, blurring, and dissolution in the *R. solani* mycelia. In addition, CTXW 7-6-2 increased tobacco seedling growth and improved leaf and root weight compared to the control. After CTXW 7-6-2 inoculation, tobacco leaves showed the upregulation of the *PDF1.2*, *PPO*, and *PAL* genes, which are closely related to target spot disease resistance. In conclusion, *B. subtilis* CTXW 7-6-2 may be an efficient biological control agent in tobacco agriculture and enhance plant growth potential.

Key words: *Bacillus subtilis*, tobacco target spot, *Rhizoctonia solani*, plant growth promotion, biocontrol potential

Introduction

Nicotiana tabacum is one of the world's most widely cultivated non-food crops. China is the world's largest tobacco producer, and Guizhou is China's second-largest tobacco-growing province (Xiang et al. 2024). The types of diseases affecting tobacco growth and development are increasing with the expansion of tobacco-growing areas. Among them, target spot disease caused by *Rhizoctonia solani* has become a notable threat to tobacco production in China, including Liaoning (Ahsan et al. 2017), Guizhou (Sun et al. 2022), and Yunnan (Wu et al. 2012) provinces. The disease mainly occurs in the tobacco growth and maturation periods and often breaks out over large areas during wet and humid seasons. The tobacco seedlings infected by *R. solani* usually desiccate, the stems rot, and the mature tobacco leaves develop disease spots (Sun et al.

2022). Typical symptoms of the disease are the appearance of concentric round spots with brittle centers that become perforated – these result in the decline of tobacco yield and quality and, consequently, substantial economic losses. Changes in the expression of pectin-degrading enzymes and cytochrome P450 genes were observed in tobacco after infection with *R. solani* (Tang et al. 2023), along with the production of the phytotoxin 3-methoxyphenylacetic acid (Li et al. 2023), which seriously impairs the normal growth of tobacco. *R. solani* is a ubiquitous soil-borne plant pathogen that can survive in soil for several years. *R. solani* infects tobacco and also causes rice sheath wilt (Yang et al. 2022), pepper root rot (El-Kazzaz et al. 2022), peanut pod rot (Chi et al. 2016), stem rot of *Phaseolus vulgaris* (Woodhall et al. 2020), potato with stem canker (Yang et al. 2019), tomato root rot (Heflish et al. 2021), and root rot of wheat (Özer et al. 2019). The primary method of

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controlling tobacco target spot disease is using chemical fungicides. Iprodione, mancozeb, phenazine-1-carboxylic acid valine, and other fungicides act against *R. solani* (Csinos and Stephenson 1999; Gonzalez et al. 2011; Zhu et al. 2022).

However, the long-term use of a single drug or chemical fungicides may lead to fungal resistance and increase environmental pollution risk. Biological control is considered the most effective and environmentally friendly method of controlling pathogens (Brimner and Boland 2003; Castaño et al. 2013). Biological control remains an excellent approach for controlling the growth of *R. solani*, despite environmental conditions affecting its efficacy (Almeida et al. 2007). *Bacillus* spp. of bacteria can form spores and strongly resist harmful factors (Piggot and Hilbert 2004) and are often used in agricultural cultivation as biofertilizers or antagonists of various plant pathogens. In addition, they are widely used to produce beneficial plant growth substances, such as siderophores and phytohormones (Akhtar et al. 2020; Miljković et al. 2020; Zerrouk et al. 2020). *B. subtilis* is used against several plant-pathogenic fungi with broad antifungal activity and may be an effective alternative to chemical fungicides (Abdelmoteleb et al. 2017).

The aim of the present study was to investigate the antagonistic effect of *B. subtilis* CTXW 7-6-2 on *R. solani*. In addition, the cause of tobacco target spot, along with the growth-promotion effect of *B. subtilis* CTXW 7-6-2 on tobacco and the expression of related disease resistance genes, were investigated.

Experimental

Materials and Methods

Plant, bacterial strain, and pathogens. The Guizhou Province Tobacco Company (Guiyang, Guizhou province, China) provided a tobacco variety Yunna 87. *B. subtilis* CTXW 7-6-2 was previously isolated from kiwifruit and strongly inhibits *R. solani* (GenBank accessions: OK287282) associated with tobacco target spot. The bio-control strain and pathogenic fungi were preserved at the Kiwifruit Engineering Technology Research Center of Guizhou University, stored in glycerol at -80°C . The isolation method and *in vitro* antifungal activity assay, as described by Chen et al. (2022), were employed in the study. The cell concentration of the CTXW 7-6-2 culture medium was adjusted to 1×10^7 CFU/ml before application. The culture media were nutrient broth NB: 5 g beef extract, 10 g peptone, 5 g NaCl, and 1000 ml water) and potato dextrose agar (PDA) (200 g potatoes, 10 g glucose, and 1,000 ml water).

Bacterial growth curve and in vitro antifungal test of *B. subtilis* CTXW 7-6-2. Domańska's (2019) method

of determining bacterial growth was used and modified slightly to 2% (v/v) of the 1.0×10^7 CFU/ml CTXW 7-6-2 bacterial suspension inoculation in NB medium. The cultivation conditions were as follows: fluid volume 100 ml/250 ml, temperature 28°C , and shaking at 180 rpm. The OD_{600} was measured using a Thermo Scientific (USA) UV-VIS spectrophotometer at 6, 12, 24, 48, 72, 96, and 120 h.

The CTXW 7-6-2 strain was activated, transferred to NB medium, and incubated at 28°C with shaking at 180 rpm for 48 h as a seed solution. *R. solani* of tobacco target spot was transferred to a PDA plate for cultivation for 4 d (cultured in the dark at 28°C). Subsequently, holes were created in the mycelium cake using a 6-mm hole punch placed in the PDA plate's center. Then, 5 μl CTXW 7-6-2 seed solution was inoculated at four points 25 mm from the plate. Plates containing *R. solani* alone were used as the control group. After culturing at 28°C for 4 d, the colony diameter was measured, and the inhibition rate was calculated using the formula below:

$$\text{Inhibition rate (\%)} = \frac{(\text{diameter of control colony} - \text{diameter of treated colony})}{\text{diameter of control colony}} \times 100$$

Antifungal activity of *B. subtilis* CTXW 7-6-2 metabolites against *R. solani*. Effect of CTXW 7-6-2 sterile fermentation broth on *R. solani*. The antagonistic effect of sterile fermentation broth against *R. solani* was determined according to the method described by Xia et al. (2017), with slight modifications. Briefly, 1 ml of CTXW 7-6-2 seed solution was inoculated in 150 ml NB medium in a conical flask (specification 250 ml), and the fermentation broth was obtained via shaking culture at 28°C and 220 rpm for 96 h. The 150-ml fermentation broth was centrifuged at 4,000 rpm for 30 min in a low-speed benchtop centrifuge, and the supernatant was filtered through a 0.22- μm filter to remove the bacteria to produce a sterile fermentation broth without high-temperature treatment. To further verify that the antifungal substances in the fermented broth are resistant to high temperatures, a sample fermentation broth was treated at a high temperature (120°C) for 20 min. The fermentation broth (5 ml) with or without high-temperature treatment was added to 45-ml PDA medium at 40°C , mixed, and poured into a sterile petri dish. After coagulation, a 6-mm diameter *R. solani* cake was placed in the center of the plate, and a PDA plate without fermentation broth was used as the control group. The experiment was repeated in triplicate. After the colonies in the control group covered $\frac{2}{3}$ of the culture dish, the colony diameter on each treatment plate was measured, recorded, and the inhibition rate was calculated.

Determination of the antifungal activity of crude lipopeptide against *R. solani*. The crude lipopeptide

was extracted using acid precipitation (Nair et al. 2020). Briefly, 600 ml of CTXW 7-6-2 sterile fermentation broth was added to 6 mol/l HCl acid, pH 2.0, sealed at 4°C, and allowed to stand for 16 h. The above mixture was centrifuged at 10,000 rpm at 4°C for 15 min, the precipitate was collected and dissolved using methanol solution, and the supernatant was collected via centrifugation at 10,000 rpm at 4°C for 15 min. The supernatant was then evaporated at 55°C and 60 rpm. The crude lipopeptide extract was collected, weighed, recorded, and stored in the refrigerator at 4°C until further use.

The mycelium growth rate method was used to determine the inhibitory effect of the crude lipopeptide on *R. solani*. Briefly, crude lipopeptide (0.12 g) was dissolved in 4-ml methanol solution, and the excess impurities were removed using a 0.22- μ m filter membrane to obtain 30,000 mg/l mother liquor. Then, 0.15 ml of mother liquor was added to 45 ml of PDA medium, mixed evenly, and poured into a sterile petri dish. The crude lipopeptide concentration was 100 mg/l, and the methanol content was 0.33%. A 6-mm diameter *R. solani* cake was placed in the center of the plate. A PDA medium plate containing 0.33% methanol was used as the control group, and the experiment was repeated in triplicate. After the colony in the control group covered 2/3 of the culture dish, the colony diameter on each treatment plate was measured, recorded, and the inhibition rate was calculated.

Determination of the antifungal activity of CTXW 7-6-2 volatile compounds against *R. solani*. Manna and Kim's protocol (2018), was used to determine the effects of CTXW 7-6-2 volatile compounds on fungal mycelia, with slight modifications. The specific protocol was as follows: a 6-mm hole punch was used to obtain the cake containing *R. solani*, which was placed in the center of a PDA plate for later use. A total of 150 μ l of CTXW 7-6-2 seed solution was coated evenly on nutrient agar plates, air-dried naturally, and the two plates were aligned and sealed together, with the solution containing no CTXW 7-6-2 being used as the control group. The experiment was repeated in triplicate. The colony diameters and inhibition rate were calculated.

The mycelial morphology and ultrastructure of *R. solani*. An antifungal area on the edge of the hyphae was selected to evaluate the restraining effect of CTXW 7-6-2. Hyphal morphology and cell ultrastructure were observed using the SU8100 scanning electron microscope (SEM) (Hitachi, Japan) and JEM1200EX transmission electron microscope (TEM) (Mo et al. 2021; Chen et al. 2022).

Tobacco growth and induction of tobacco disease-related genes. Yunyan 87 seeds were cultured in floating seedling trays with sterilized substrates. The seedlings with four leaves were selected as the test plants, with a consistent single spraying of CTXW 7-6-2 bacte-

rial suspension at a concentration of 1.0×10^7 CFU/ml. NB liquid medium was used as a blank group. Seven days after treatment, the maximum leaf length, leaf width, whole plant fresh weight, root length, root dry weight, and root number of the seedlings were measured. Each process was repeated thrice, each repeat containing five seedlings.

Real-time PCR was used to examine the tobacco's CTXW 7-6-2 treatment time course-related gene expression. Yunyan 87 was used as the test plant with six leaves at the seedling stage. The mycelium of *R. solani* was cultured in a 50-ml PD medium with shaking for four days, and the fresh mycelium was collected by filtering with gauze. Mycelial suspensions were collected by breaking the mycelium and filtering with two layers of gauze, and sterile water was used to adjust the mycelium to 15–20 strips (100 $\times$) under a light microscope. It was then sprayed uniformly onto the surface of tobacco leaves, and the suspension of CTXW 7-6-2 (1.0×10^7 CFU/ml) was sprayed after the onset of symptoms. Samples were collected at 0, 6, 12, 24, 48, and 72 h, with three replicates per treatment and three seedlings per replicate, to determine the disease-related genes, including pathogenesis-related gene1 (*NPRI*), plant defensin (*PDF1.2*), chitinase (*chit*), phenylalanine ammonialyase (*PAL*), peroxidase (*POD*), and polyphenol oxidase (*PPO*) genes.

Referring to the primer sequence published by Jiao et al. (2019), Shanghai Bioengineering Co., Ltd. (China) was commissioned to synthesize the required primers, and the *tubulin* gene was used as the reference gene for tobacco. Each tobacco sample was weighed, and 100 mg was placed in a pre-cooled mortar. Liquid nitrogen was added, and the tobacco sample was quickly ground into a powder. The ground sample was transferred to a 1.5-ml centrifuge tube. Total RNA was extracted using the RNApure Pure Plant Total RNA extraction kit (TIANGEN Biotech (Beijing) Co., Ltd., China), following the manufacturer's instructions. Genomic DNA was removed using DNase I. RNA integrity and DNA contamination were analyzed using 1.5% agarose gel electrophoresis. RNA was reverse transcribed according to the instructions of the FastKing one-step (TIANGEN Biotech (Beijing) Co., Ltd., China) method. The reaction system consisted of 2 μ g RNA, 4 μ l 5 $\times$ FastKing-RT SuperMix, and RNase-free ddH₂O up to a 20- μ l solution volume. The experiment was performed at 42°C for 15 min, 95°C for 3 min, and finally stored at –20°C. The RT-qPCR reaction system was configured according to the instructions described by the Talent Fluorescence Quantitative Detection kit (TIANGEN Biotech (Beijing) Co., Ltd., China), and the two-step reaction program was set for detection. Twenty microliters of the reaction mixture comprised 10 μ l 2 $\times$ Talent qPCR PreMix, 0.6 μ l reverse primers (10 μ M), 0.6 μ l forward

primers (10 μM), 0.5 μl cDNA template (0.1 μg/μl), and 8.3 μl RNase-free ddH₂O. Amplification was performed using a BIO-RAD (USA) Bole real-time PCR instrument with a reaction program of one cycle at 95°C for 3 min, 40 cycles at 95°C for 5 s, and 60°C for 15 s. Three technical replicates of the target gene were performed for each sample, and relative expression was calculated.

Data analysis. All experimental data were analyzed using SPSS[®] 17.0 (SPSS Inc., USA) and the Duncan multiple range test. Statistical significance was set at $p < 0.05$.

Results

Growth curve of CTXW 7-6-2 and antagonism of *R. solani* in vitro. As shown in Fig.1A, the OD₆₀₀ value of the CTXW 7-6-2 culture did not change substantially from 6 to 12 h, indicating that the antagonistic strain grew slowly during this time. The OD₆₀₀ value increased after cultivation for 12–24 h; however, the growth of the plant and the inhibitory action remained slow. From 24 to 48 h of culture, the CTXW 7-6-2 increased rapidly in

the logarithmic phase. The OD₆₀₀ value at 96 h of culture was significantly higher than that at the other time points, and CTXW 7-6-2 had the best growth at this time and, thus, was used as the subsequent culture time. The *in vitro* antibacterial test showed that the CTXW 7-6-2 notably inhibited the *R. solani* of tobacco target spot. The colony diameter of *R. solani* after treatment with CTXW 7-6-2 was 0.43 ± 0.03 cm, and the inhibition rate was $94.02 \pm 0.41\%$. The results are similar to those of a study by Chen et al. (2022).

Antifungal activity of CTXW 7-6-2 metabolites against *R. solani*. The CTXW 7-6-2 metabolites inhibited the tobacco target spot caused by *R. solani* (Table I). The high-temperature and no-heat-treatment sterile fermentation broths inhibited the growth of *R. solani*; however, no notable difference was found between the two treatments, with inhibition rates of $50.88 \pm 1.92\%$ and $54.63 \pm 0.89\%$, respectively. This finding indicates that the antifungal activity of CTXW 7-6-2 is resistant to high temperatures. Crude lipopeptide at 100 mg/l inhibited *R. solani* hypha growth; the inhibition rate was $74.88 \pm 4.03\%$. CTXW 7-6-2 volatile compounds

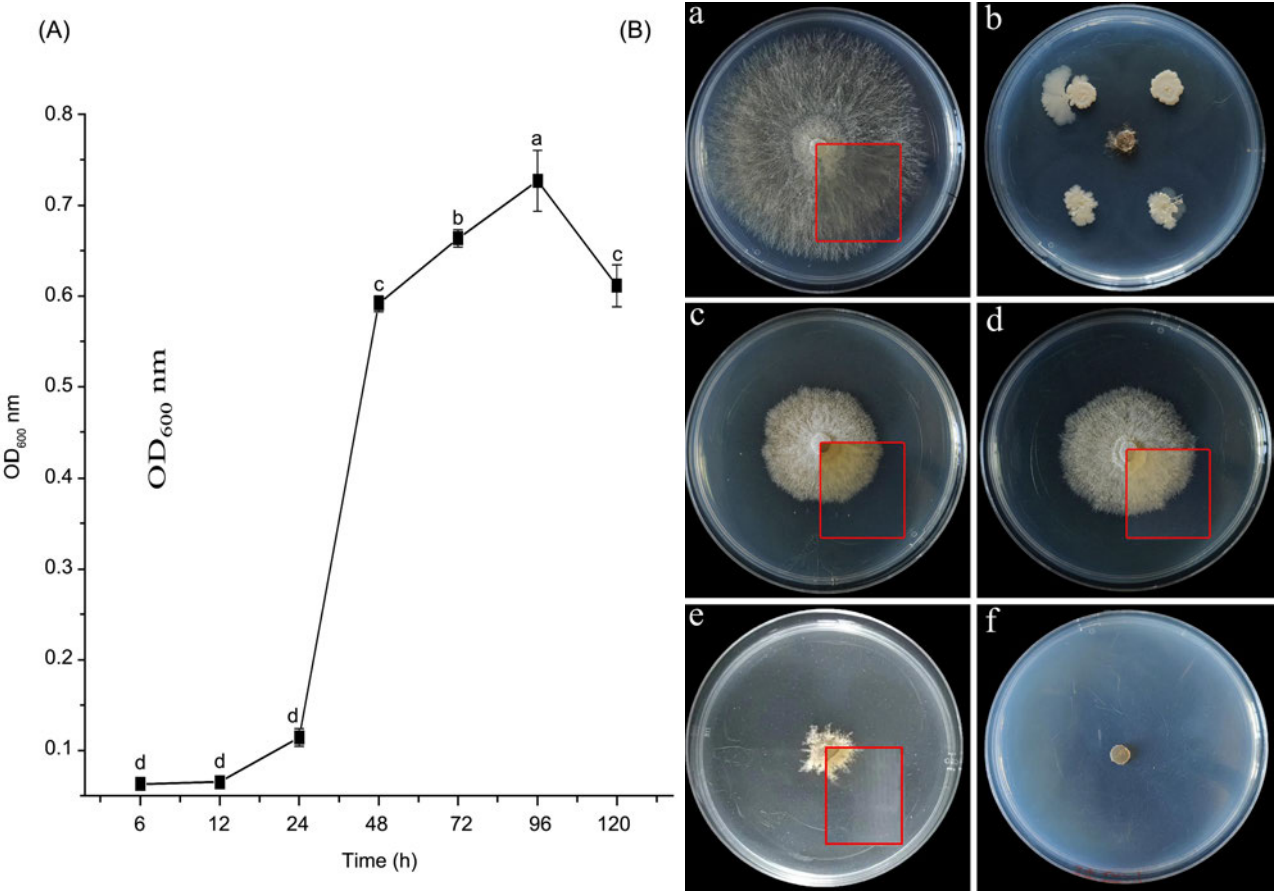


Fig. 1. Growth curve of *Bacillus subtilis* CTXW 7-6-2 and its antifungal activity against *Rhizoctonia solani*.

A) CTXW 7-6-2 growth curve;
B) a: colony morphology of the control group, b: colony morphology after confrontation with the bacteria, c: sterile fermentation broth treatment group without high-temperature treatment, d: high-temperature sterile fermented broth treatment group, e: crude lipopeptide treatment group, f: volatile compounds treatment group.
The red box indicates the back of the colony.

Table I
Antifungal activity of *Bacillus subtilis* CTXW 7-6-2 against *Rhizoctonia solani*.

Classes	Control (cm)	Treatment (cm)	Inhibition ratio (%)
Antagonistic bacteria confront pathogenic fungi	7.11 ± 0.13	0.43 ± 0.03	94.02 ± 0.41 ^a
Sterile fermentation broth without high-temperature treatment		3.23 ± 0.06	54.63 ± 0.89 ^c
High-temperature processing of sterile fermentation broth		3.49 ± 0.14	50.88 ± 1.92 ^c
100 mg/l crude lipopeptide	5.28 ± 0.01	0.23 ± 0.21	74.88 ± 4.03 ^b
Volatile compounds	6.66 ± 0.26	0.23 ± 0.12	96.62 ± 1.77 ^a

a, b, c – significant difference ($p < 0.05$)

inhibited *R. solani*, demonstrating significant antifungal effects, with an inhibition rate of up to 96.62 ± 1.77 %.

Fig. 1Ba shows the control group. The mycelia of *R. solani*, after confrontation and treatment with volatile compounds, grew slowly (Fig. 1Bb and 1Bf), and the aerial mycelia of the sterile fermentation broth with no high-temperature treatment, sterile fermentation broth with high-temperature treatment and crude lipopeptide treatment became denser (Fig. 1Bc, 1Bd and 1Be). These results indicated that the volatile compounds of CTXW 7-6-2 significantly inhibited the growth of *R. solani*, a key factor of its metabolite resistance to high temperature.

CTXW 7-6-2 inhibited mycelial morphology and ultrastructure of *R. solani*. SEM and TEM revealed that

the mycelium surface of the control group was complete, with no shrinkage and depression, and had uniform thickness. Compared with the control, the marginal hyphae, after the confrontation of the *R. solani* with CTXW 7-6-2 treatments, showed structural abnormalities, collapse, and shrinkage (Fig. 2A). The cell wall, membrane, and organelles of normal hyphal cells could be observed, whereas the hyphal organelles were blurred, and some organelles were dissolved in the treated group (Fig. 2B). The results show that abnormal structural changes occurred in the mycelium morphology of *R. solani* after CTXW 7-6-2 treatment, which affected the normal growth of *R. solani*.

CTXW 7-6-2 promoted growth and induced the expression of disease-related genes in tobacco. The

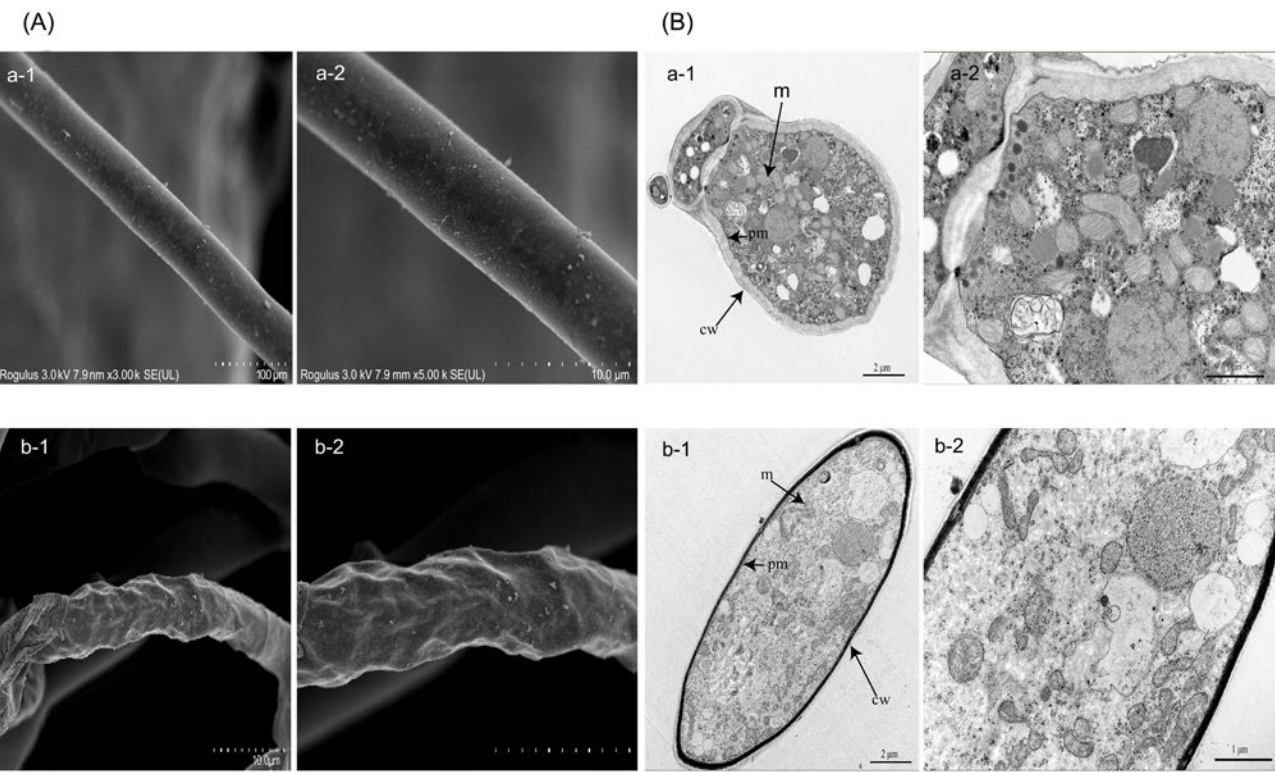


Fig. 2. Hypha morphology and cell ultrastructure of *Rhizoctonia solani* after treatment with *Bacillus subtilis* CTXW 7-6-2.
A) Scanning electron microscopy observation of mycelium morphology; B) Ultrastructure of hyphal cells observed using transmission electron microscopy. The arrows in the figure indicate cell wall (cw), plasma membrane (pm), and mitochondria (m).
a: the control hyphae, b: mycelia of the treated group

Table II
Effects of CTXW 7-6-2 fermentation on the growth of tobacco seedlings.

Treat	CTXW 7-6-2	Nutrient broth
Leaf length (cm)	7.59 ± 0.29 ^a	4.44 ± 0.28 ^b
Leaf width (cm)	5.41 ± 0.31 ^a	3.25 ± 0.23 ^b
Leaf number (piece)	4.67 ± 0.33 ^a	4.00 ± 0.00 ^a
Whole plant fresh weight (g)	2.01 ± 0.13 ^a	0.60 ± 0.0064 ^b
Root number	26.33 ± 0.88 ^a	24.33 ± 0.88 ^a
Root length (cm)	6.31 ± 0.37 ^b	10.41 ± 0.85 ^a
Dry weight of root (g)	0.17 ± 0.0025 ^a	0.091 ± 0.0041 ^b

^{a, b} – significant difference (*p* < 0.05)

maximum leaf length, maximum leaf width, whole plant fresh weight, and root dry weight of tobacco seedlings treated with CTXW 7-6-2 fermentation broth were considerably more significant than those of the NB control (Table II) and were increased by 70.95%, 66.46%, 141%, and 86.81%, respectively. No notable difference was observed in the number of leaves and roots compared with those in the control. In contrast, the root length of tobacco seedlings in the CTXW 7-6-2 fermentation broth treatment group was 39.39%, which was lower than that of the seedlings in the control group. The CTXW 7-6-2 fermentation broth promoted the growth of tobacco seedlings and enhanced leaf length, width, whole plant fresh weight, and root dry weight (Fig. 3).

CTXW 7-6-2 induced tobacco disease-related gene expression changes, as shown in Fig. 4. After 24 h of

treatment with CTXW 7-6-2, *NPR1* and *POD* expression levels increased; however, the change was insignificant, compared with the 0-h control group. Further, *chit* gene expression was significantly higher than in the 0-h control group. The expression of the *chit*, *NPR1*, and *POD* genes was downregulated at other times. Expression of the *PDF1.2* gene increased rapidly and peaked at 6 h, and notable differences were observed among the expression profiles of *PDF1.2* at 12, 48, and 72 h (Fig. 4). The expression of *PAL* changed over time, decreasing until 12 h and then peaking at 24 h, with a relative expression level of 6.41. The expression decreased with increased treatment time but consistently remained considerably higher than that at 0 h. Similarly, *PPO* expression was upregulated after CTXW 7-6-2 treatment, with a fold change of 4.44 at 24 h.

Discussion

Rhizoctonia solani causes perforation spots on tobacco leaves. It can persist in the soil for several years and become a primary infection source. The present study found that CTXW 7-6-2 could substantially inhibit the growth of *R. solani* hypha transformed into dry silk, leading to the formation of shriveled and dry mycelium and organelle dissolution. Pandey et al. (2023) also confirmed that *Bacillus* spp. could cause structural abnormalities in the hyphae of *R. solani*. The sterile fermentation broth, crude lipopeptide, and volatile compounds of CTXW 7-6-2 showed potent inhibitory effects against the growth of *R. solani*, mainly owing to the antagonistic action of the volatile compounds. Volatile compounds from other *Bacillus* species are potent against plant pathogens, including *Monilinia fructicola* in peach fruit (Liu et al. 2018), *Alternaria solani* in potatoes (Zhang et al. 2020), *Ceratocystis fimbriata* in postharvest sweet potatoes (Xu et al. 2021), *A. solani* in *Solanum lycopersicum* (Awan et al. 2023), *Ralstonia solanacearum* in tobacco (Tahir et al. 2017), an anthracnose pathogen in postharvest

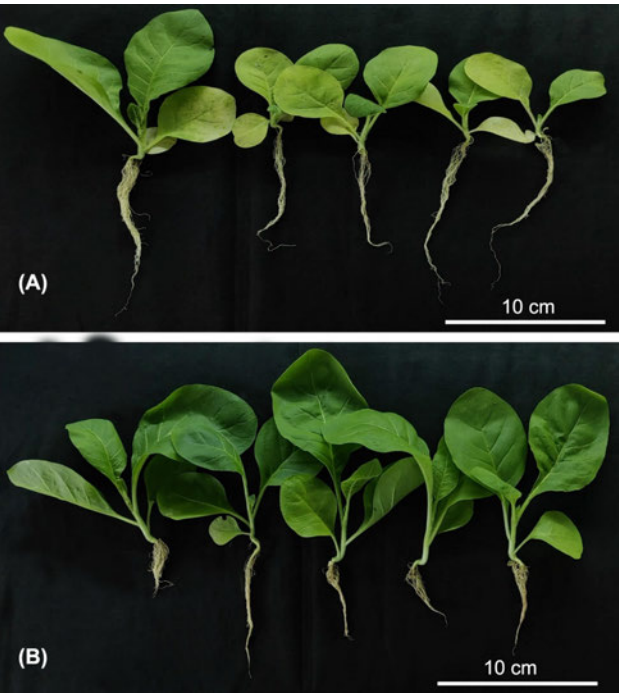


Fig. 3. Effects of A) nutrient broth medium and B) CTXW 7-6-2 fermentation broth on seedling growth of Yunyan no. 87.

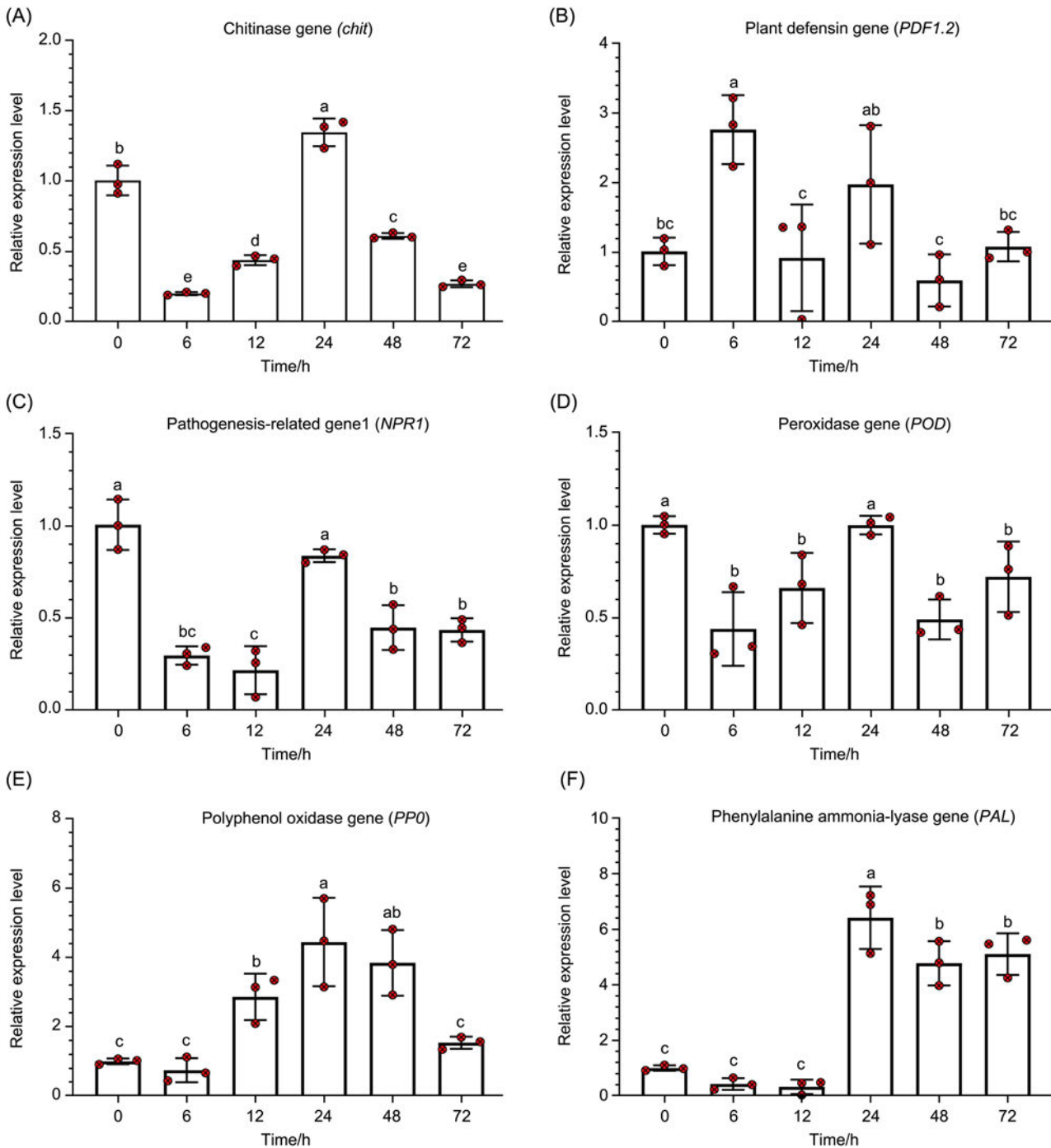


Fig. 4. Expression of genes involved in tobacco disease course induced by *Bacillus subtilis* CTXW 7-6-2.

mangos (Zheng et al. 2013), and *R. solani* web blight of bush cowpeas (Siva et al. 2023). It is evident that volatile compounds from biocontrol bacteria represent a good development prospect in the future and provide new ideas for controlling tobacco target spot disease. Lipopeptides are synthesized by non-ribosomal pathway and contain antibacterial active substances such as iturin, surfactin, and plipastatin (Wu et al. 2023). The different types of crude lipopeptides secreted by CTXW 7-6-2 can be further studied to improve the productivity of lipopeptides and provide a scientific basis for effi-

cient and green control of tobacco target spot disease. *B. subtilis* shows efficient colonization on the surfaces of various plants, including citrus fruit peels (Li et al. 2022) and cucumber rhizosphere (Li et al. 2018), which is advantageous for the long-term effective inhibition of pathogen propagation. This represents the possibility of using CTXW 7-6-2 as a biocontrol agent to manage tobacco target spot disease in the field.

In addition, members from the genus *Bacillus* produce siderophores, the extracellular lytic enzymes cellulase, phosphatase, pectinase, chitinase, and protease,

indole-3-acetic acid, and hydrogen cyanide (Alfiky et al. 2022; Wekesa et al. 2023), which promote plant growth. These enzymes' significant functions are to promote plant root development and increase plant dry weight (Liu et al. 2019; Deng et al. 2022). Consistent with these findings, the present study showed that CTXW 7-6-2 could promote tobacco seedling growth by increasing leaf length, leaf width, whole plant fresh weight, and root dry weight. However, the root length of tobacco seedlings did not increase and was, instead, slightly shortened. Therefore, the exact mechanism underlying the promotion of tobacco growth by CTXW 7-6-2 remains to be elucidated. In uninoculated and inoculated tobacco, CTXW 7-6-2 did not considerably upregulate the expression of *chit* and *NPR1* genes.

In contrast, the expression of *PDF1.2*, an essential gene specifically induced by jasmonate (JA)/ethylene (ET), reached the highest level (2.76-fold) at 6 h after inoculation. Treatment with CTXW 7-6-2 may have induced plant JA/ET signaling pathways to enhance tobacco resistance to target spots. Inoculation with biocontrol bacteria or other abiotic factors has been reported to increase the expression levels of the oxidase genes (*POD*, *PAL*, *PPO*, and *LOX*), which confer resistance against the invasion and growth of pathogens (Xu 2018). The results revealed that the defense enzyme genes *PPO* and *PAL* were upregulated in tobacco leaves after inoculation with CTXW 7-6-2. However, Jiao et al. (2019) observed *PPO* high expression and downregulation of *PAL* and *POD* in *Erysiphe cichoracearum*-infected tobacco after the inoculation of *Bacillus amyloliquefaciens*. Such results may be caused by differences in the types of antagonistic bacteria, antibacterials secreted, and the disease-causing fungal species. The field control effect of tobacco target spot disease and the processing of related dosage forms require further investigation. In conclusion, *B. subtilis* represents an important agent in the biological control of tobacco target spot disease, which has high research potential and development value.

Conclusions

B. subtilis CTXW 7-6-2 exhibited good inhibitory activity against the growth of *R. solani*, which causes target spot disease in tobacco plants. The high-temperature-treated sterile fermentation broth, crude lipopeptide, and volatile compounds of CTXW 7-6-2 had specific inhibitory effects on *R. solani* growth. After CTXW 7-6-2 treatment, the mycelia appeared wrinkled and shriveled and showed organelle dissolution. Thus, CTXW 7-6-2 can promote the growth of tobacco seedlings and provide high-quality strain resources for developing tobacco biofertilizers and biopesticides.

Availability of data and material

Research data will be shared by the corresponding authors upon request.

Author contributions

Methodology, N.H.; software, X.J.; validation, J.T.W., Y.F.Z. and X.Y.; formal analysis, X.Y.; investigation, G.Y.W.; data curation, N.H.; writing – original draft preparation, W.K.W.; writing – review and editing, M.Q.; visualization, Y.K.; supervision, N.H. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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