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Discovery of a novel plant-derived agent against *Ralstonia solanacearum* by targeting the bacterial division protein FtsZ

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ABSTRACT

Ralstonia solanacearum (*R. solanacearum*) is one of the most devastating bacterial pathogens and leads to serious economic losses in crops worldwide. In this study, the antibacterial activities of novel plant-derived coumarins against *R. solanacearum* and their underlying mechanisms were initially investigated. The bioactivity assay results showed that certain coumarins had significant in vitro inhibitory effects against *R. solanacearum*. Notably, 6-methylcoumarin showed the best in vitro antibacterial activity with 76.79%. Interestingly, 6-methylcoumarin was found to cause cell elongation, disrupt cell division, and suppress the expression of the bacterial division protein coding genes *ftsZ*. Compared with the control treatment, the Δ*ftsZ* mutant inhibited bacterial growth and caused the bacteria to be more sensitive to 6-methylcoumarin. The application of 6-methylcoumarin effectively suppressed the development of tobacco bacterial wilt in pot and field experiments, and significantly reduced the bacterial population in tobacco stems. The control efficiency of 6-methylcoumarin treatment was 35.76%, 40.51%, 38.99% at 10, 11, and 12 weeks after tobacco transplantation in field condition. All of these results demonstrate that 6-methylcoumarin has potential as an eco-friendly and target specificity agent for controlling tobacco bacterial wilt.

1. Introduction

Bacterial wilt is a lethal systemic vascular disease caused by *Ralstonia solanacearum* (*R. solanacearum*), one of the world's most devastating plant bacterial pathogens, and heavily infects more than 250 plant species (Genin and Denny, 2012; Mansfield et al., 2012). Recently, plant bacterial wilt has caused huge annual economic losses to the world's agricultural production (Álvarez et al., 2010; Peeters et al., 2013). Bacterial wilt has been widely observed in tobacco-producing provinces in the southern of China, and had a vast impact on tobacco production (Jiang et al., 2017; Liu et al., 2017b). *R. solanacearum* invades host plants through root wounds, colonizes xylem vessels, grows to high cell densities and produces large masses of extracellular polysaccharides, resulting in host wilting and death (Genin, 2010; González and Allen, 2003). The fight between plant disease and humankind has been ongoing since the dawn of agriculture. Fortunately, pesticides discovered and produced by the agrochemical industry have developed powerful strengths for disease control management. However, the long-term unreasonable use of bactericides has led to increased bacterial resistance and exerts few positive effects in the field (Li et al., 2015; Xu et al., 2012). Therefore, considering the goal of developing healthy and sustainable agriculture, exploring and developing novel, efficient, and eco-friendly pesticides is necessary for crop protection.

Biopesticides are considered remarkable alternatives to classic agrochemicals and have played a crucial role in plant disease control. Renewable plant bioresources containing a variety of plant-derived compounds, such as plant essential oils, phenolic compounds, flavonoids, alkaloids, and terpenoids, have provided a source and inspiration for the discovery of novel pesticides (Lee et al., 2020; Paret et al., 2010; Seiber et al., 2014; Sparks et al., 2017; Yang et al., 2016b). Currently, a variety of plant-derived compounds have been developed as lead compounds to design biopesticides (Lee et al., 2020; Zhang et al., 2020; Zhang et al., 2018). In addition, considering their effective suppressive effects on bacteria and inducing plant defence, the use of plant-derived compounds may be a promising strategy for the management of plant disease (Wang et al., 2020; Zhang et al., 2018).

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Coumarins are natural secondary metabolites composed of fused benzene and α-pyrone rings produced via the phenylpropanoid pathway and accumulate in response to infection by bacteria, fungi, viruses and oomycetes (Barot et al., 2015; Gnonlonfin et al., 2012; Stringlis et al., 2019). *Nicotiana tabacum* cv. Petit Havana resistance to *Botrytis cinerea* is due to the accumulation of scopoletin and PR proteins (El Oirdi et al., 2010). Moreover, in the wild tobacco *Nicotiana attenuata*, the content of the phytoalexin scopoletin in roots is enhanced after infection by the fungus *Alternaria alternata* (Santhanam et al., 2019). Because of their excellent antimicrobial activities against plant and animal pathogens, coumarins have attracted extensive interest (Silva et al., 2016). Recently discovered coumarins exert strong antibacterial activity against *Escherichia coli* (*E. coli*) by reducing biofilm formation (Lee et al., 2014). Coumarins induce strong non-receptor mediated membrane lysis as their primary microbicide strategy (Azelmat et al., 2015; Chen et al., 2016). Certain coumarins derived from plant sources, exerted antibacterial activity against *R. solanacearum*, and the hydroxylation of these coumarins at C-6, C-7 and C-8 enhanced this activity (Yang et al., 2016a). Taken together, these facts highlight the value and necessity of studying the antibacterial activities of plant-derived coumarins against *R. solanacearum*.

In most bacteria, bacterial cell division protein FtsZ exerts a constrictive force on the membrane by using the chemical energy of guanosine triphosphate hydrolysis, which is the key step of bacterial cytokinesis (Li et al., 2013). Plant-derived compound alkyl gallates display antibacterial activity against *Xanthomonas citri* subsp. *citri* by disrupting the Fts*Z*-ring in vivo and causing cell elongation (Krol et al., 2015). Resveratrol inhibits bacterial cell growth of *E. coli* by suppressing FtsZ expression and Z-ring formation (Hwang and Lim, 2015). Indeed, scopoletin and daphnetin have been proven to be promising inhibitors of the bacterial cell division protein FtsZ, and hydroxyl, diethyl, or dimethyl amino substituents on the 7th carbon of scopoletin enhances this inhibitory activity, halting the first step of bacterial cell division (Duggirala et al., 2014). Therefore, we hypothesis certain coumarins inhibit *R. solanacearum* growth via suppressing FtsZ protein.

In this study, we demonstrated the antibacterial activity of ten coumarins against the plant pathogen *R. solanacearum*. Notably, 6-methylcoumarin showed strong bioactivity against *R. solanacearum*, and the antibacterial mechanism of 6-methylcoumarin via inhibition of the bacterial division-associated gene *ftsZ* was further investigated. The control efficiency of 6-methylcoumarin on tobacco bacterial wilt and the suppression of the bacterial population in tobacco stems were evaluated.

2. Materials and methods

2.1. Materials and bacterial strains

The plant pathogen *R. solanacearum* CQPS-1 (phylotype I, race 1, biovar 3) utilized in this study was collected by the Laboratory of Natural Products Pesticides, Southwest University, Chongqing, China (accession number NZ_CP016914.1) (Liu et al., 2017a). *R. solanacearum* and mutants were grown in nutrient-rich medium (B medium) at 28 °C for 48 h.

Coumarins (HPLC ≥98%) used in the study were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). The compounds were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 50 mg/mL.

2.2. Construction of the Δ*ftsZ* mutant

The Δ*ftsZ* (GenBank ID: CP016914.1) mutant of *R. solanacearum* CQPS-1 was generated through pK18mobsacB-based homologous recombination (Zhang et al., 2015). As described in a previous study, two DNA fragments flanking of *ftsZ* were conjugated with joint PCR and subcloned into pK18mobsacB to generate *pK18-ftsZ* plasmids. After the sequence was validated, *pK18-ftsZ* was transferred into *R. solanacearum* by conjugation with S17–1. The Δ*ftsZ* mutant was constructed and confirmed by colony PCR using the primers listed in Table S1.

2.3. Antibacterial activity of coumarins against R. solanacearum

The culture amendment assay used to investigate the antibacterial activity of coumarins against *R. solanacearum* was performed as in a previous study with minor modifications (Yang et al., 2016a). Briefly, an overnight-cultured suspension of *R. solanacearum* was inoculated in B medium supplemented with 100 mg/L coumarins. Then, the cell density of *R. solanacearum* was detected by measuring the optical density (OD) at 600 nm after incubation for 12 h. 0.1% DMSO treatment was used as a control. The average OD_{600} was used to calculate the antibacterial activity of coumarins against *R. solanacearum*. All assays were carried out with four biological replicates.

The same assay mentioned above was used for measuring the growth curve of Δ*ftsZ* mutant and wild type (CQPS-1). The bacterial OD_{600} was measured every two hours during 24 h of cultivation. The experiment was repeated three times.

2.4. General microscopic analysis

R. solanacearum suspension was inoculated in B medium supplemented with 100 mg/L coumarins for 12 h. The bacterial suspensions were smeared on clean glass slides and fixed by gentle heating on a spirit lamp flame. Then, aqueous crystal violet solution (0.5%) was spread over the smears for 30 s followed by washing with sterilized water. Twenty microliters of Gram's iodine solution was added to the smear for 1 min followed by washing with sterilized water. Later, the samples were decolorized with 95% ethanol until color runoff, washed twice, treated with safranin stain for 1 min, washed with water and air dried. *R. solanacearum* cells were observed under an optical microscope at 100× using oil immersion.

2.5. Effects of 6-methylcoumarin on cell division associated gene expression

Total RNA isolation and quantitative real-time PCR (qRT-PCR) were performed as previously described with minor modifications (Wu et al., 2015). *R. solanacearum* was inoculated in B medium supplemented with 100 mg/L 6-methylcoumarin for 6 h and 12 h. 0.1% DMSO was used as a control treatment, and the samples were harvested by centrifugation at 5000 rpm for 10 min at 4 °C. The total RNA of the collected bacterial cells was extracted by using TRIzol reagent according to the manufacturer's instructions (Tiangen Biotech Co. Ltd., Beijing, China) and was subsequently treated with RNase-free DNase I (Tiangen Biotech Co. Ltd., Beijing, China) to remove contaminating genomic DNA. First-strand cDNA was synthesized using the iScript gDNA clear cDNA synthesis kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. qRT-PCR was performed on a C1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA). The primers for the tested genes were designed using the primer program from NCBI and synthesized by BGI Technologies (Shenzhen, Guangzhou, China) (Table S2). All qRT-PCR analyses were performed in 96-well plates in a 20-μL reaction system. Three technical replicate reactions were used for each sample. The experiment was performed four times.

2.6. Virulence assay

The control efficiency of 6-methylcoumarin on tobacco bacterial wilt was evaluated by the naturalistic soil drench assay as described in a previous study with minor modifications (Yang et al., 2018). Briefly, six-week-old tobacco plants (*Nicotiana tabacum* Yunyan87) were pre-inoculated with 15 mL of 100 mg/L 6-methylcoumarin without wounding the roots. After 24 h of pre-inoculation, individual plants were inoculated by pouring 15 mL of *R. solanacearum* CQPS-1 suspension (1

$\times 10^8$ CFU/mL) into the soil. All plants were placed in climate cabin at 28 °C with a 14/10 h light/dark cycle. Bacterial wilt symptoms were scored daily using a disease index scale from 0 to 4 (0, no symptoms appeared; 1, 1–25% of leaves wilted; 2, 26–50% of leaves wilted; 3, 51–75% of leaves wilted; 4, 76–100% of leaves wilted). Individual treatments contained 16 plants for each independent experiment, and the assay was repeated three times. The disease index was calculated as a weighted average.

To measure the bacterial population in the tobacco stems, four samples of each treatment were destructively harvested at the base of the stems 4, 7, and 10 d after inoculation with *R. solanacearum*. The tissues were disinfected in 75% alcohol for 1 min, rinsed twice with sterile water, transferred into a 2.5 mL sterile centrifuge tube, and grinded with sterile glass beads on an MP Biomedicals FastPrep (FastPrep-24™, M. P. Biomedicals, Santa Ana, California, USA). Next, the bacterial suspension was serially diluted, and the bacterial suspensions were plated on SMSA medium. Each treatment contained 8 samples. The assay was repeated twice.

2.7. Field experiment

The field trial was carried out to determine the control efficacy of 6-methylcoumarin on tobacco bacterial wilt at Zhengan county, Guizhou Province, China, during 2018. Tobacco plants (susceptible cv. *Nicotiana tabacum* Yunyan87) were used to transplant into the field. 6-methylcoumarin was applied to the plants at 4 weeks after transplanting. Thiodiazole copper was used as control treatment. The treatments were set as follows: Treatment 1: untreated (Mock); Treatment 2: 100 mg/L 6-methylcoumarin; Treatment 3: 200 mg/L thiodiazole copper. Different bactericidal agents were continuously applied by root irrigated for three times. After transplanted for 10, 11, 12, 13, 14 weeks, bacterial wilt symptoms were scored using a disease index scale. Control efficacy of season-long protection for each treatment was calculated as the mean of the disease index.

2.8. Molecular docking

Molecular docking was performed using AutoDock 4.2 as previously described (Zhou et al., 2021). The three-dimensional (3D) model of LpxB and its binding pocket were generated by the I-TASSER server (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) (Yang et al., 2015). The 3D model of the ligands and their energy minimization were established by ChemOffice 2004. The model results were analysed by Discovery Studio Visualizer 4.5 (Accelrys Software Inc., San Diego, CA, USA) (Patil et al., 2010).

2.9. Statistical analysis

The data were analysed with the SPSS 17.0 statistical software program (SPSS Inc. Chicago, IL) using ANOVA and Student's *t*-test under significance levels of 0.05 and 0.01 (P-value < 0.05 and P-value < 0.01).

3. Results

3.1. In vitro antibacterial activity of coumarins against R. solanacearum

The preliminary in vitro antibacterial activities of ten plant-derived coumarins against *R. solanacearum* at 100 mg/L were tested through turbidimetry (Fig. 1). As shown in Fig. 2A, all coumarins implied moderate to good in vitro activity against *R. solanacearum*. In particular, compared with positive control (thiodiazole copper), compounds C1 (coumarin), C3 (esculetin), C6 (3-aminocoumarin), C7 (3-acetylcoumarin), C8 (7-methoxycoumarin) and C10 (6-methylcoumarin) exhibited excellent antibacterial activity against *R. solanacearum* and had inhibition rates of 71.62%, 68.73%, 72.02%, 76.01%, 74.28% and 76.79%, respectively. Bacterial cells were investigated by Gram staining supplemented with 100 mg/L coumarins, *R. solanacearum* is a gram-negative strain and the pink bacterial cells were imaged under an optical microscope. Compared with the control, most coumarins-treated *R. solanacearum* cells formed bacterial polymers that eventually lysed. Interestingly, after C10 (6-methylcoumarin) treatment, the bacterial

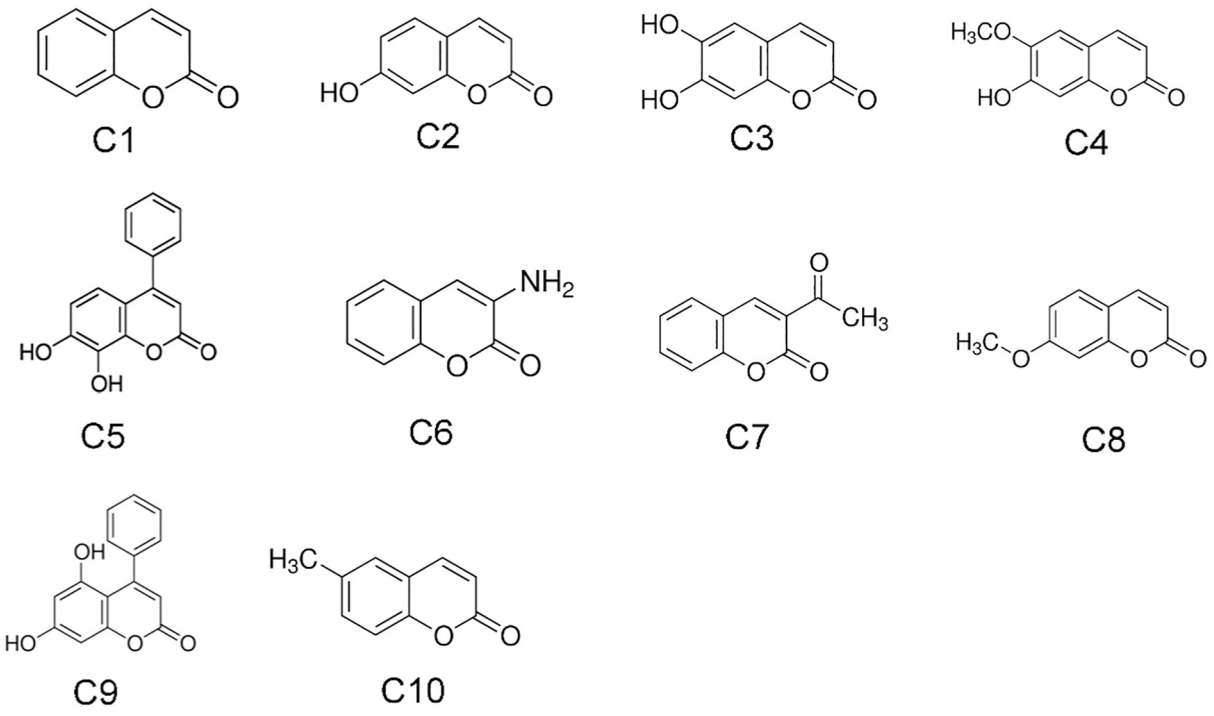

Fig. 1. The chemical structures of the studied compounds. Coumarin (C1), umbelliferone (C2), esculetin (C3), scopoletin(C4), 7,8-dihydroxy-4-phenylecoumarin (C5), 3-aminocoumarin (C6), 3-acetylcoumarin (C7), 7-methoxycoumarin (C8), 5,7-dihydroxy-4-phenylecoumarin (C9), and 6-methylcoumarin (C10).

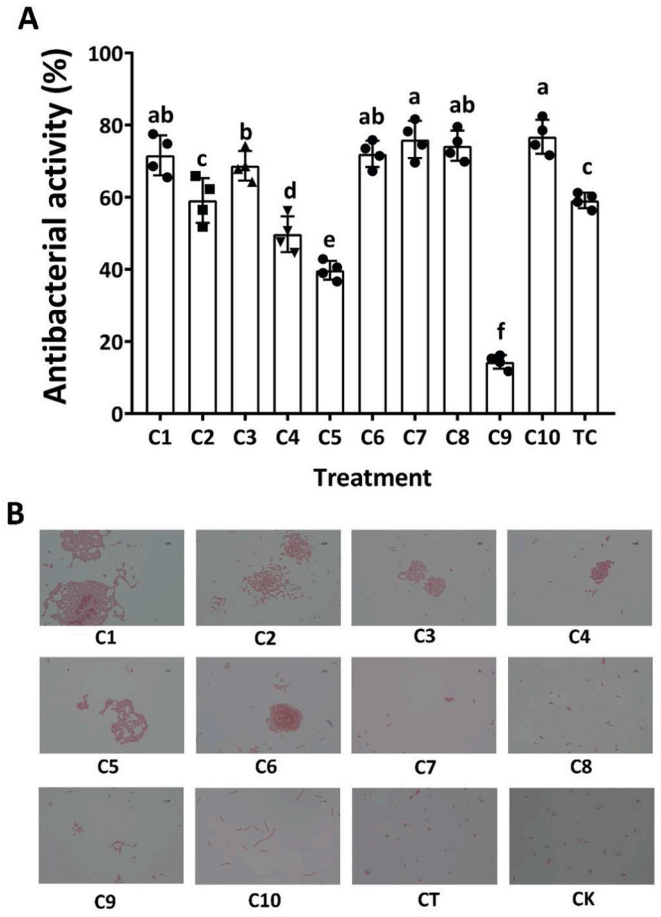

Fig. 2. Antibacterial activity of coumarins against *R. solanacearum*. (A) The antibacterial activity of the studied coumarins at 100 mg/L against *R. solanacearum* after 12 h of inoculation. Each bar represents the mean ± SE of independent experiments with four replicates. Different letters indicate significant differences between different treatments according to one-way ANOVA with Duncan's test ($P < 0.05$). (B) Gram staining of *R. solanacearum* supplemented with coumarins at a concentration of 100 mg/L.

cells grew into long filaments by suppressing bacterial division (Fig. 2B).

3.2. 6-Methylcoumarin suppresses the expression of the bacterial division genes *ftsZ* and *ftsQ*

The essential cytoskeletal cell division protein FtsZ (named after the filamenting temperature-sensitive mutant Z) forms a ring-like structure at the site of division and is highly conserved in bacteria (Fig. 4A). We investigated the mRNA levels of key cell division genes, such as *ftsZ*, *ftsQ* and *ftsW*, in *R. solanacearum* cells treated with 6-methylcoumarin. The results showed that the cell division genes *ftsZ* and *ftsQ* were significantly suppressed by 6-methylcoumarin treatment (Fig. 3). Compared with DMSO treatment, the mRNA levels of *ftsZ* significantly decreased by 39.08% and 65.18% after 6-methylcoumarin treatment for 6 h and 12 h, respectively (Fig. 3A). However, *ftsW* was similarly expressed in DMSO and 6-methylcoumarin-treated cells (Fig. 3C). These results indicated that 6-methylcoumarin implied antibacterial activity against *R. solanacearum* by inhibiting the expression of the bacterial division gene *ftsZ*.

3.3. The bacterial cell division gene *ftsZ* is necessary for the growth and virulence of *R. solanacearum*

On rich nutrient medium plates supplemented with D-glucose and

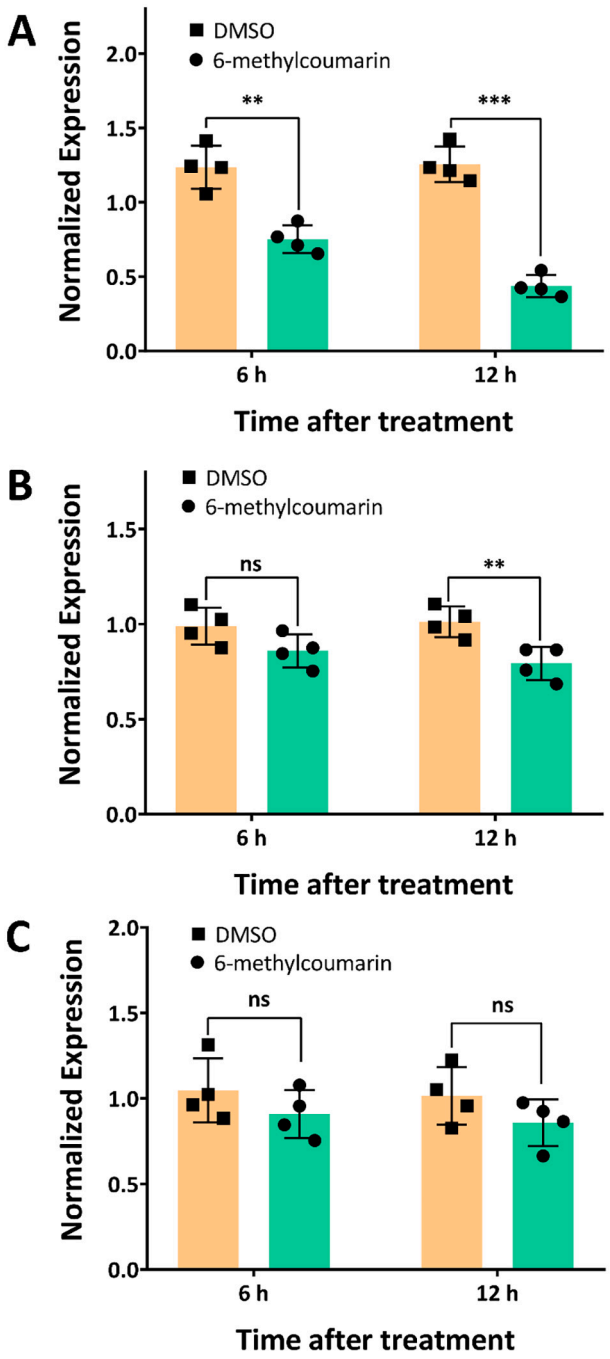

Fig. 3. The effect of 6-methylcoumarin on the expression of cell division associated genes in *R. solanacearum*. (A) *ftsZ*, (B) *ftsQ*, (C) *ftsW*. Each bar represents the mean ± SE of four replicates. Asterisks indicate significant differences between the 6-methylcoumarin treatment and control treatment according to Student's *t*-test (* indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$).

triphenyl tetrazolium chloride, the *R. solanacearum* wild-type (WT) strain CQPS-1 formed fluidal and pink-centred colonies. We observed that the Δ*ftsZ* mutant formed small red colonies and grew slower than the WT (Fig. 4C). The cell morphologies of the WT and Δ*ftsZ* mutant were observed under an optical microscope using Gram staining. The results showed that the Δ*ftsZ* mutant did not divide and grew into long filaments that eventually lysed (Fig. 4D), resulting in significantly suppressed bacterial growth in liquid medium (Fig. 4B). Compared with the WT treatment, we observed that the disease index of the *ftsZ* mutant was

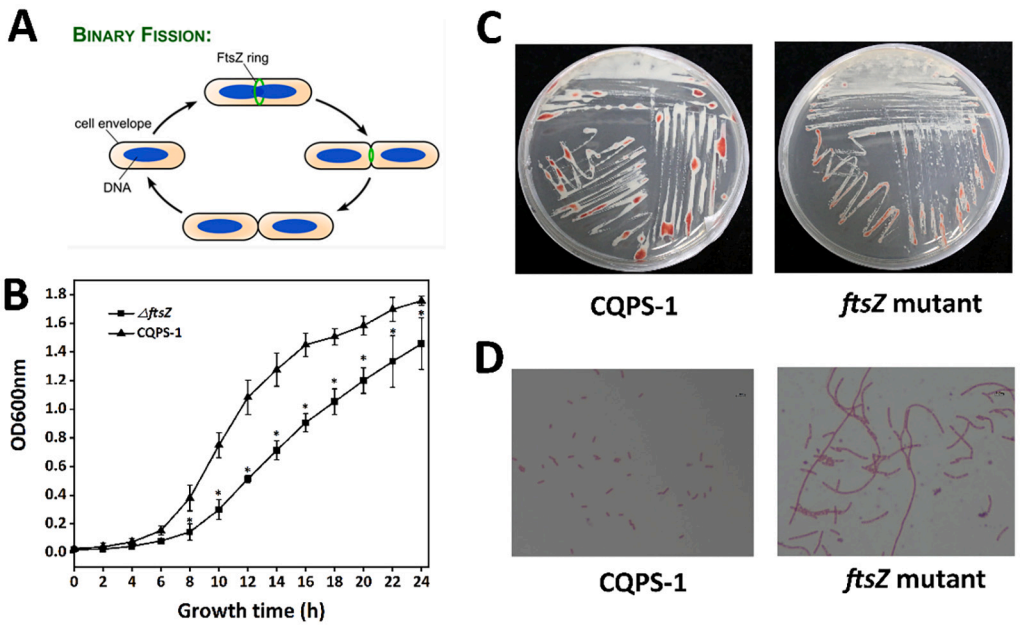

Fig. 4. The bacterial cell division gene *ftsZ* is necessary for the growth and virulence of *R. solanacearum*. (A) The action of *ftsZ* on the bacterial division process. (B) The growth curve of the Δ*ftsZ* mutant in B medium after 24 h of inoculation. Each bar represents the mean ± SE of three replications. Asterisks indicate significant differences between the Δ*ftsZ* mutant and WT according to Student's *t*-test (* indicates $P < 0.05$). (C) The strain of the Δ*ftsZ* mutant and wild type (CQPS-1) grown on B medium. (D) Gram staining of Δ*ftsZ* mutant and CQPS-1.

significantly reduced, particularly reducing bacterial populations at the base of tobacco plant stems (Fig. 6A and B).

3.4. *ftsZ* silencing increased the sensitization of *R. solanacearum* to 6-methylcoumarin

The mode of action of 6-methylcoumarin against *R. solanacearum* was examined using an antibacterial activity sensitization assay. We applied the Δ*ftsZ* mutant through homologous recombination. The results implied that the antibacterial activity of 6-methylcoumarin against the Δ*ftsZ* mutant significantly increased compared with WT (CQPS-1) (Fig. 5A). Moreover, silencing *ftsZ* caused the bacteria to be more sensitive to 6-methylcoumarin. The half maximal inhibitory concentration (IC_{50}) of 6-methylcoumarin against the Δ*ftsZ* mutant was 53.93 mg/L, which was significantly lower than that of CQPS-1 (77.99 mg/L) (Fig. 5B).

3.5. 6-Methylcoumarin reduces the virulence of *R. solanacearum* on tobacco plants

Based on the strong antibacterial activity of 6-methylcoumarin against *R. solanacearum*, the effects of 6-methylcoumarin on bacterial wilt disease progression was evaluated. As shown in Fig. 6A, compared with the mock control, 100 mg/L 6-methylcoumarin significantly suppressed the disease progression of bacterial wilt ($P < 0.05$). Moreover, 6-methylcoumarin significantly reduced the population of *R. solanacearum* in the base stem of tobacco plants. Compared with the mock control, the population of tobacco stems supplemented with 100 mg/L 6-methylcoumarin was significantly reduced by 4.76-fold, 13.12-fold, and 19.37-fold at 4 d, 7 d and 10 d after inoculation, respectively (Fig. 6B).

In the field trials, 6-methylcoumarin at 100 mg/L significantly reduced disease severity compared with the untreated control ($P < 0.05$). The control effect of 6-methylcoumarin was similar to that of positive control thiodiazole copper treatment (Fig. 6C). The control efficiency of 6-methylcoumarin treatment was 35.76%, 40.51%, 38.99% at 10, 11, and 12 weeks after tobacco transplantation in field condition (Table S3). These findings showed that 6-methylcoumarin may exhibit

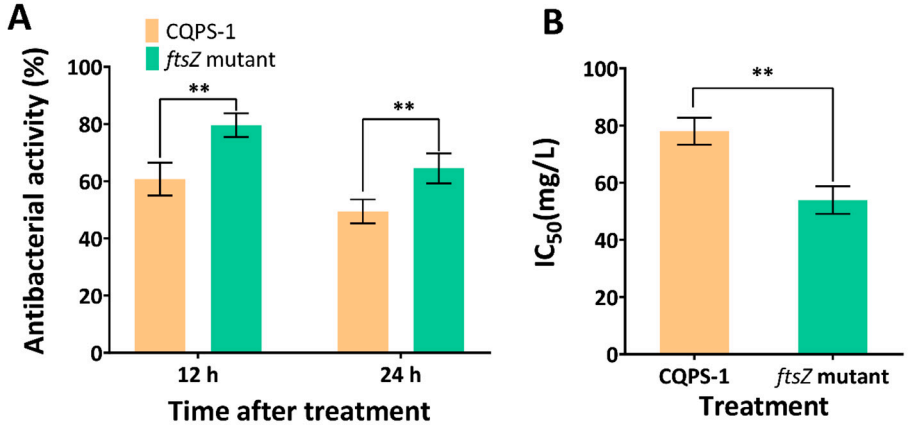

Fig. 5. Effect of the Δ*ftsZ* mutant on *R. solanacearum* growth and susceptibility to 6-methylcoumarin. (A) The antibacterial activity of 6-methylcoumarin against the *ftsZ* mutant and wild type (CQPS-1). (B) The IC_{50} of 6-methylcoumarin on both strains. Each bar represents the mean ± SE of three replications. Asterisks indicate significant differences between the 6-methylcoumarin treatment and control treatment according to Student's *t*-test (* indicates $P < 0.05$, ** indicates $P < 0.01$).

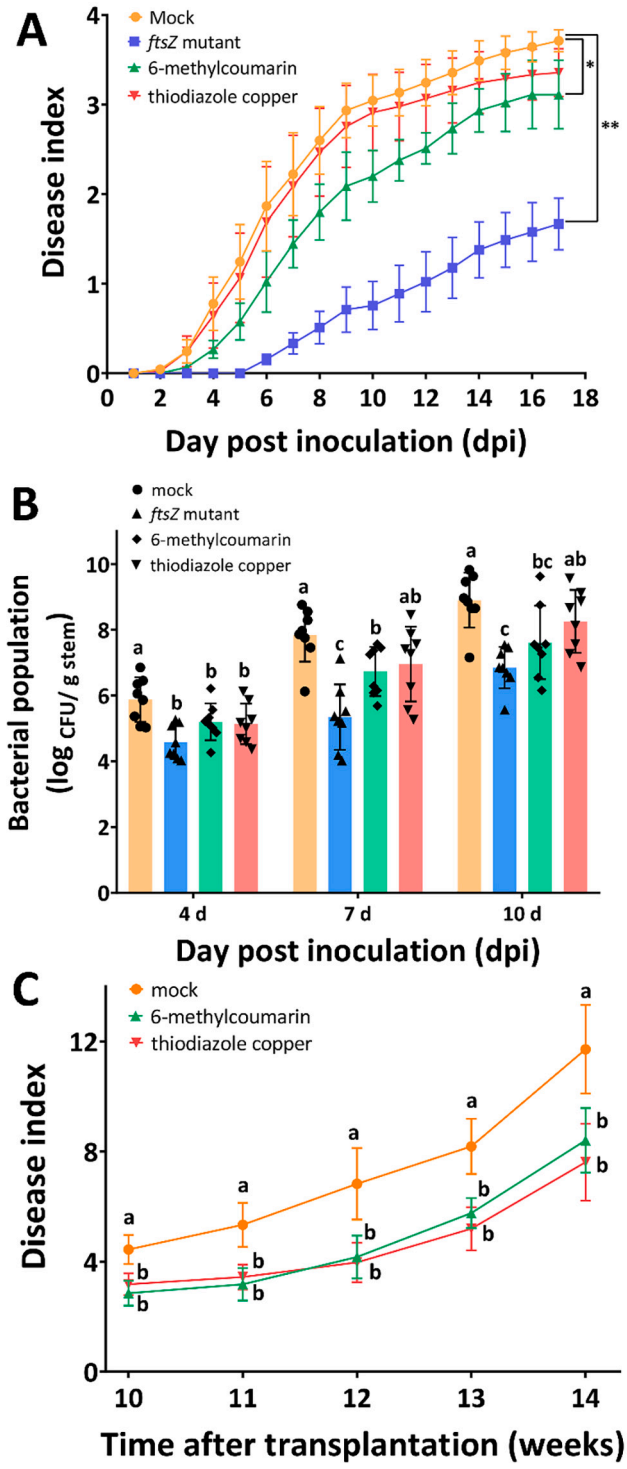

Fig. 6. Control effects of 6-methylcoumarin against tobacco bacterial wilt. (A) The disease index of tobacco bacterial wilt pre-treated with 100 mg/L 6-methylcoumarin. After 24 h of application, the *R. solanacearum* suspension was poured around the plant soil. Thiodiazole copper was used as positive control. Error bars indicate the standard errors. (B) 6-Methylcoumarin significantly reduced the bacterial population of *R. solanacearum* in the tobacco stem. Each bar represents the mean ± SE of eight replicates. (C) Control effects of 6-methylcoumarin against tobacco bacterial wilt under field conditions. Different letters indicate significant differences between different treatments according to one-way ANOVA with Duncan's test ($P < 0.05$).

great potential applications for controlling tobacco bacterial wilt.

3.6. Molecular docking

To examine the interaction between 6-methylcoumarin and FtsZ and evaluate the structure-activity relationship, molecular docking was performed to analysis the binding mode of 6-methylcoumarin within the binding pocket of FtsZ. The docking results of 6-methylcoumarin binding to FtsZ were shown in Table S4. The binding energy of 6-methylcoumarin was calculated to be −4.59 kcal mol^{-1}, which indicates that 6-methylcoumarin can be considered a specific ligand of FtsZ. The binding modes and orientations of 6-methylcoumarin with FtsZ were showed in Fig. 7(D,E). Six key amino acids (ALA319, LYS222, MET224, ARG320, VAL252 and LEU315) were interacted with 6-methylcoumarin via hydrogen bonding and hydrophobic interactions in the binding pocket of FtsZ (Fig. 7(F). The hydrogen atoms of the benzene ring form a hydrogen bond (3.25) with ALA319. In addition, the acidic residues LYS133, VAL162, ALA318, ARG317, ASP253, and GLY251 interact with 6-methylcoumarin via Van der Waals interactions in the binding pocket of FtsZ.

4. Discussion

Biopesticides are considered remarkable alternatives to classic agrochemicals and have played a crucial role in plant disease control. However, certain pesticides have been recognized as capable of gathering in the human body through the food chain and causing further risks (Spochacz et al., 2018). Recently, the effects of plant derived coumarins on human diseases, including the suppression of anti-inflammatory and antibacterial activity against *E. coli*, was investigated (Azelmat et al., 2015; Grover and Jachak, 2015; Lee et al., 2014). Some coumarins have been developed as antibacterial agents for commercial use (Veselinović et al., 2016). However, plant-derived coumarins with antibacterial properties against plant disease and the mechanism of 6-methylcoumarin against *R. solanacearum* have rarely been reported.

In this study, 6-methylcoumarin was explored as an antibacterial agent against *R. solanacearum* in vitro, and further insight into its mechanism was investigated for the first time. According to our results, certain coumarins display strong antibacterial activity against *R. solanacearum*. Interestingly, 6-methylcoumarin showed antibacterial activity by inhibiting bacterial division (Fig. 2). qRT-PCR analysis found that 6-methylcoumarin could suppress the bacterial division genes *ftsZ* and *ftsQ* (Fig. 3). Several studies have proven that diverse coumarins show antimicrobial activities against plant and animal pathogens. Recently discovered coumarins from plant sources, have been shown to exert antibacterial activity against *R. solanacearum*, and hydroxylation at C-6, C-7 and C-8 enhances this activity (Yang et al., 2016a). Indeed, scopoletin and daphnetin are proven to be promising inhibitors of the bacterial cell division protein FtsZ, and hydroxyl, diethyl, or dimethyl amino substituents on the 7th carbon enhanced this inhibitory activity (Duggirala et al., 2014). Moreover, coumarins inhibit the proliferation of *Mycobacteria* by targeting the assembly of *MtbFtsZ* (Sridevi et al., 2017). Hydroxycoumarins have also displayed antibacterial activity by inhibiting isoleucyl-transfer RNA (tRNA) synthetase gene expression (Veselinović et al., 2016). In addition to their above-mentioned role in damaging the cell membrane, coumarins might efficiently traverse the cell membrane and bind to DNA or RNA ligases to reduce the biosynthesis of these molecules. These actions could then control the expression of genes encoding transcriptional regulators and other downstream genes (Silva et al., 2016). More importantly, to some extent, the application of 6-methylcoumarin inhibited the bacterial population in plant tissues and suppressed tobacco bacterial wilt progress in the greenhouse and field conditions (Fig. 6).

In most bacteria, cytokinesis is initiated by the localization of the essential bacterial protein FtsZ, a guanosine triphosphatase (GTPase)

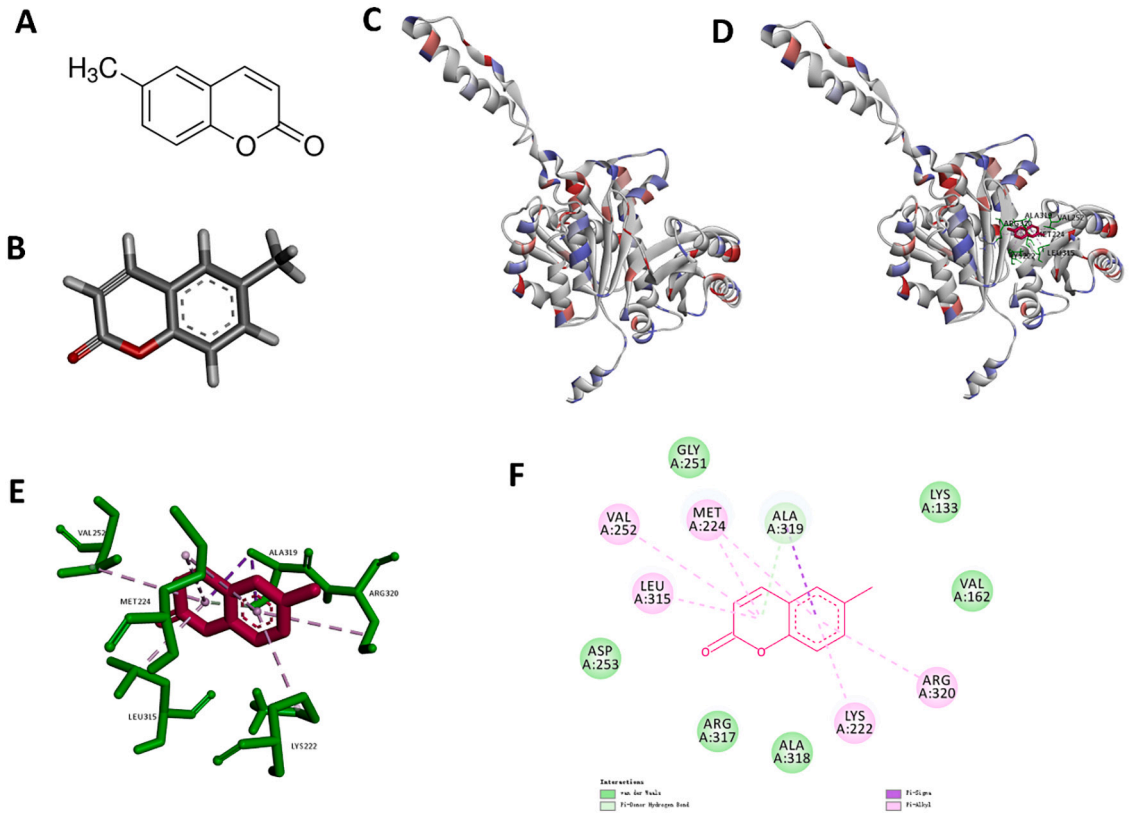

Fig. 7. Molecular docking of FtsZ protein to 6-methylcoumarin. (A) Chemical structural formula of 6-methylcoumarin. (B) The cartoon representation of 6-methylcoumarin. Red regions represent oxygen atoms; gray region indicate carbon atoms. (C) Homology modeling 3D-structure of FtsZ. (D) Best conformation of 6-methylcoumarin docked to binding pocket of FtsZ. (E) The recognized binding modes and molecular interactions of 6-methylcoumarin in the active site of FtsZ. (F) The two-dimensional interactions scheme of 6-methylcoumarin to FtsZ.

and tubulin homologue that can self-assemble into a structure at the division site termed the Z ring. During cytokinesis, the Z ring exerts a constrictive force on the membrane by using the chemical energy of guanosine triphosphate hydrolysis (Erickson et al., 2010; Li et al., 2013). Owing to its important role in bacterial cell division and largely unexploited as a new target for antibacterial agents, FtsZ has aroused great interest among many researchers involved in developing new a generation of novel antibacterial agents (Tripathy and Sahu, 2019). Plant-derived compound alkyl gallates display antibacterial activity against *Xanthomonas citri* subsp. *citri* bi disrupting the Fts*Z*-ring in vivo and causing cell elongation (Krol et al., 2015). Resveratrol inhibits bacterial cell growth of *E. coli* by suppressing FtsZ expression and Z-ring formation (Hwang and Lim, 2015). Indeed, scopoletin and daphnetin have been proven to be promising inhibitors of the bacterial cell division protein FtsZ, and hydroxyl, diethyl, or dimethyl amino substituents on the 7th carbon of scopoletin enhances this inhibitory activity, halting the first step of bacterial cell division (Duggirala et al., 2014). Difluorobenzamide derivatives exhibited antimicrobial activity against *Staphylococcus aureus* by targeting FtsZ (Chai et al., 2020). In this study, we found that 6-methylcoumarin suppressed the transcriptional level of *ftsZ* in *R. solanacearum*. Furthermore, the Δ*ftsZ* mutant did not divide, grew into long filaments that eventually lysed, and inhibited bacterial growth in vitro (Fig. 4). More importantly, the antibacterial activity of 6-methylcoumarin against the Δ*ftsZ* mutant increased significantly compared with that of the WT (CQPS-1). Moreover, silencing *ftsZ* caused the bacteria to be more sensitive to 6-methylcoumarin (Fig. 5). Compared with mock treatment, we observed that the *ftsZ* mutant significantly reduced the pathogenicity of *R. solanacearum* on tobacco, and particularly inhibited bacterial invasion in tobacco stem tissues (Fig. 6). This is the first study focused on the essential bacterial protein FtsZ in *R. solanacearum* and developed a new plant-derived coumarin targeting FtsZ. Therefore, 6-methylcoumarin might have the potential to develop new bactericides for controlling plant bacterial wilt.

Coumarins have been considered natural secondary compounds against several plant diseases, including bacteria, fungi and viruses. The young leaves of *N. attenuata* displayed higher resistance to *A. alternata*, which was associated with a high accumulation of scopoletin (Sun et al., 2014). Several coumarins derived from different plant species exhibited strong antibacterial activity against pathogens, including *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Gnonlonfin et al., 2012; Shakeel et al., 2010). Recent studies have found that scopoletin induced the accumulation of reactive oxygen species (ROS) in Arabidopsis against Asian soybean rust (Beyer et al., 2019). Coumarins might be regarded as plant resistance elicitors that induce plant systemic defence to control plant disease. In this study, 6-methylcoumarin might be considered as an antibacterial agent against plant bacterial disease by inhibiting the bacterial cell division protein FtsZ. Our study provides an environmentally friendly and effective strategy for controlling plant bacterial wilt, and might be extended to improve plant defence with potential applications to control plant diseases in the future.

5. Conclusions

In summary, certain coumarins in this study presented potential antibacterial activity against *R. solanacearum*, and 6-methylcoumarin could induce bacterial cells to grow into long filaments by suppressing the bacterial division genes *ftsZ* and *ftsQ*. The Δ*ftsZ* mutant displayed slower bacterial growth and virulence on tobacco plants, and increased the sensitization of *R. solanacearum* to 6-methylcoumarin. Finally, 6-methylcoumarin inhibited bacterial invasion in the stem and

suppressed tobacco bacterial wilt progression. This study suggests that the 6-methylcoumarin is a very promising compound for the development of a botanical bactericidal pesticide for preventing tobacco bacterial wilt.

Author contributions

Conceived and designed the experiments: LY, WD. Performed the experiments: LY, YW, XH, QX, SH, ZJ. Analysed the data: LY, SL, ZJ. Wrote the paper: LY, WD.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pestbp.2021.104892.

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