Biocontrol of tomato bacterial wilt by a new strain Bacillus velezensis FJAT-46737 and its lipopeptides

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Abstract

Background: There is an urgent need to discover alternative antimicrobial agents to control bacterial wilt. The objectives of this study were to report a new lipopeptide-producing biocontrol strain FJAT-46737 and its lipopeptides, and to investigate their antagonistic effects.

Results: Based on the whole genome sequence analysis, the new strain FJAT-46737 was finally identified as Bacillus velezensis and seven gene clusters that responsible for synthesis of bioactive secondary metabolite in the FJAT-46737 were predicted. Antimicrobial results demonstrated that FJAT-46737 exhibited broad-spectrum antimicrobial activities in vitro against bacteria and fungi . The pot experiments showed that the tomato bacterial wilt control efficiencies of the whole cultures, the 2-fold diluted supernatants and the crude lipopeptide of FJAT-46737 were 66.2%, 82.0%, and 96.2%, respectively. The above results suggested that one of the antagonistic mechanisms of FJAT-46737 was secretion of lipopeptides that consisted of iturins, fengycins and surfactins. The crude lipopeptides had significant antagonistic activities on several pathogen in a dosage-dependent manner, and the fengycins confirmed to play a major role in antibacterial abilities in vitro of the lipopeptides against Ralstonia solanacearum . Furthermore, it was found that the rich organic nitrogen sources (especially yeast extracts) in the media were beneficial for FJAT-46737 to produce fengycin and surfactin. The secretion of these two lipopeptides were also adjusted by the culture temperature: the content of the fengycins decreased by 96.6% and that of the surfactins ascended by 59.9% from 20 o C to 40 o C. The temperature of 20 o C~25 o C is the suitable temperature for FJAT-46737 to produce lipopeptide.

Conclusions: The B. velezensis strain FJAT-46737 and its lipopeptides would used as new sources of potential biocontrol agents against plant pathogens, especially the bacterial wilt pathogen R. solanacearum .

Background

Bacterial wilt caused by *Ralstonia solanacearum* is the devastating diseases of almost 250 plant species, which seriously threaten plant growth and lead to huge losses around the world [1, 2]. Traditional agricultural practices, like crop rotation, field sanitation, plant resistant varieties, and chemical bactericide have been widely applied to control the bacterial wilt but obtained limited success [3]. In factlly, the long-term or repeated using of chemical bactericide caused pathogen resistance and has its adverse effects on the environment, beneficial organisms, and human health [3]. To seek safe, effective and alternative methods for controlling plant pathogen diseases are become more and more important in improving the output and quality of agricultural products. Furthermore, biological control agents, the use of antagonistic microorganisms, including bacteria, yeasts, and filamentous fungi, fight plant pathogens, has been proved as a safe, effective and sustainable alternative method comparing to chemical bactericide [4-6]. Members of the genus *Bacillus* have been used as effectively biocontrol agents to reduce damage caused by bacterial wilt and their derived products occupy approximately half of the commercially available biopesticides [7]. However, the control of bacterial wilt becomes difficult and ineffective since

the high genetic variability, persistence in the environment and broad host range of *R. solanacearum*, highlighting the urgent need for discovering alternative antimicrobial agents [3, 8].

Studies on the suppression mechanism of the *Bacillus* agents indicated that the biocontrol effects of species primarily attribute to their production of various bioactive molecules [9]. Lipopeptide is one of the most important bioactive substances and exhibits excellent properties, such as broad-spectrum antibiotic activity, good stability, low toxicity, high biodegradability, and less prone to drug resistance [10]. *Bacillus* lipopeptides include three classes, namely iturin (bacillomycin D/F/L/Lc, iturin A/C/D/E, and mycosubtilin), fengycin (fengycin A/B, and plipastatin A/B) and surfactin (halobacillin, pumilacidin and surfactin) [11, 12, 13]. They all share a common amphiphilic molecule structure composed of a fatty acid side chain linking to a cyclic peptide ring (Figure S1). Among them, the iturins exhibit strong antifungal activities against various types of yeast and filamentous fungi, but with limited antibacterial activity; the fengycins have strong antifungal activities, especially on filamentous fungi, but with no effect on the inhibition of bacteria until the new finding reported recently by Villegas-Escobar *et al*. [14]; the surfactins display effectively bactericidal activities. Besides, surfactin can also effectively reduce the surface tension in plant root to facilitate the swimming ability and biofilm formation of the *Bacillus* strains to protect plants from attack by pathogens [15].

The well-studied lipopeptides were mainly focused on control of the plant fungal pathogens, such as *Rhizoctonia solani* [16], *Pythium ultimum* [4], *Botrytis cinerea* [17], *Podosphaera fusca* [17], *Fusarium graminearum* [18, 19], *Fusarium oxysporum* [19], and *Sclerotinia sclerotiorum* [20]. Few studies were devoted to bacterial pathogens of *R. solanacearum*. Zhu *et al*. [21] reported that the lipopeptide mixtures (surfactin and iturin A) produced by *B. amyloliquefaciens* XZ-173 could effectively inhibit growth of *R. solanacearum*. Furthermore, the lipopiptides secreted by XZ-173 were used to manufacture lipopeptide-mineral composites to achieve an 87.76% biocontrol efficacy on controlling bacterial wilt of tomato [22]. The suppression mechanism of lipopeptides against plant diseases is either by impeding the pathogens directly or acting as elicitors for the induced systemic resistance in the host plants [17, 23, 24]. A strong correlation between defense-inducing activity and content of the surfactins formed by the *Bacillus* strains have been reported [25]. In addition, the lipopeptide secretion of the *Bacillus* strains relies on not only the differences of the strains themselves but also of the culture medium components as well as fermentation conditions [26, 27, 28].

The present study is aim to report a new lipopeptide-producing biocontrol strain FJAT-46737. The strain FJAT-46737 was identified through the whole genome sequence analysis and the bioactive secondary metabolite gene clusters in the strain FJAT-46737 were predicted. The suppression effects of FJAT-46737 and its lipopeptides *in vitro* and *in vivo* were evaluated. Furthermore, the lipopeptide components were identified, and effects of culture conditions on antibacterial activity of the strain FJAT-46737 and its lipopeptides were investigated. This work would promote application of the strain FJAT-46737 and its lipopeptides as new sources of potential biocontrol agents against plant pathogens, especially the bacterial wilt pathogen *R. solanacearum*.

Methods

Strains and Chemicals

The strain FJAT-46737 was isolated from a soil sample of Huanggang Mountain, Fujian Province, China, which had been preserved at the China General Microbiological Culture Collection Center (CGMCC No. 14661). The indicator strains *R. solanacearum* FJAT-91 (CGMCC No. 10692, tomato pathogens), *R. solanacearum* FJAT-77 (peanut pathogens), *Escherichia coli* FJAT-301, *F. oxysporum* f. sp. *capsicum* FJAT-831, *F. oxysporum* f. sp. *niveum* FJAT-30265 and *F. oxysporum* f. sp. *melonis* FJAT-9230 were preserved at the Agricultural Bioresources Research Institute, Fujian Academy of Agricultural Sciences, Fujian, China. All the culture media used for bacteria and fungi growth were purchased from AoBoXing Biological Technology Co., Ltd. (Beijing, China).The cultivation substrates used for the pot experiments were purchased from Xiamen Jiang Ping biological Technology Co., Ltd. (Xiamen, China). The reference standards of iturin and surfactin that produced from *B. subtilis* were purchased from Sigma (St. Louis, MO, USA).

Antimicrobial spectra of the strain FJAT-46737

Using the agar disk diffusion method, the strain FJAT-46737 was evaluated for its *in vitro* potential to inhibit several animal and plant pathogens, including *R. solanacearum*, *E. coli*, *F. oxysporum* f. sp. *capsicum*, *F. oxysporum* f. sp. *niveum* and *F. oxysporum* f. sp. *melonis*. For fungal pathogen strains, a 7 mm-diameter block of mycelium was placed onto the centre of a new potato dextrose agar (PDA) plate, the cultures of the strain FJAT-46737 were then streaked with sterilised toothpicks at a distance 2.5 cm away from the marginal of the mycelia colony and cultivated at 28 ^{o}C for seven days.

The antagonistic effects of the strain FJAT-46737 against bacterial pathogen srains were carried out using a two layer plating method. The semi-solid (NA)/Luria–Bertani (LB) media containing pathogenic bacterial culture suspensions were poured in Petri plates (90 mm) coated with the corresponding solid media. After solidification, a colony of the strain FJAT-46737 was streaked on the plated, and then were co-cultivated at 30 ^{o}C for two days.

Identification of the strain FJAT-46737

The strain FJAT-46737 was streaked on the NA agar plates and cultured at 30 ^{o}C for 48 h for investigating the colony and cell morphology. The genomic deoxyribonucleic acid (DNA) of FJAT-46737 was extracted with the sodium dodecyl sulfate (SDS) method and detected by the agarose gel electrophoresis and quantified by Qubit. The strain FJAT-46737 was further identified through analysis of its 16*S rRNA* and partial gyrase subunit B (*gyrB*) gene sequences and whole-genome sequence. The 16*S rRNA* gene was amplified and sequenced using the universal primers 27F and 1492R. The *gyrB* gene was amplified using the specific primers F (5'-GAAGTCATCATGACC-3') and R (5'-AGCAGGGTACGGAT-3'). The whole-genome sequencing was performed on the Illumina HiSeq PE150 platform at the Beijing Novogene Bioinformatics Technology Co., Ltd. Average nucleotide identity (ANI) was calculted using the OrthoANIu algorithm [29].

The ANI value cut-off of 96% is recommended for species delineation [30, 31].The *16S rRNA*, *gyrB* gene and the whole-genome sequences of strain FJAT-46737 had been deposited in the GenBank with the accession number of MG924092, MH470338 and CP044133, respectively. The secondary metabolite gene clusters in the strain FJAT-46737 were predicted using the antibiotics Secondary Metabolite Analysis Shell (antiSMASH) tool.

Extraction and identification of the lipopeptides

The FJAT-46737 was grown at 30 °C in 50 mL of culture medium (Table S1) in a shaking incubator (170 rpm). After 48 h, the culture was centrifuged at 6000 g for 5 min to remove the cell. The supernatant was adjusted to pH 2.0 with 3 mol/L HCl to obtain the crude lipopeptides. The extract lipopeptides were dissolved in phosphate buffer and then were lyophilized. The obtained lipopeptide power were dissolved in methanol for for antimicrobial activity tests, qualitative and quantitative analyses, and dissolved in water for pot experiment in the further study.

The qualitative and quantitative analyses of lipopeptides using liquid chromatography quadrupole time-of-flight tandem mass spectrometry (LC-QTOF-MS/MS) technology were carried out using previous methods[32]. The structure of lipopeptide was estimated by their accurate mass and MS/MS fragmentation patterns according to literatures [33–37]. The content of each type lipopeptide was defined as: milligram of standard/litre of culture supernatant (mg/L).

Biocontrol evaluation of FJAT-46737 against tomato bacterial wilt

The strain FJAT-46737 was cultured at 30 °C for 48 h in medium E (Table S1) in a shaking incubator (170 rpm). The tomato seedlings with 3-4 emerged leaves were transplanted into 15 cm diameter pots at 30°C. After 2 days, two groups (each with thirty seedlings) were drenched with 100 mL/pot of the whole FJAT-46737 cultures (10^8 cfu/mL) and the double diluted fermentation supernatant, respectively. The lipopeptide treated group was carried out as following: thirty tomato seedlings were uprooted carefully and the roots were dipped in the crude lipopeptide solution (1mg/mL, pH 6.5) for one hour, then the seedlings were transplanted into pots. Each pot was drenched with 100 mL of the *R. solanacearum* cultures (10^8 cfu/mL) after three days. Two control groups were also included: one was treated only by the *R. solanacearum* cultures and the other was treated only with water. Each treated group was performed three times repeatedly. Survivability of the seedlings was monitored at a regular interval. Above pot experiments were carried out at June to August in 2017 under greenhouse with the following incubated conditions: illumination of 16-h day-8-h night cycle; temperature of 28–32°C; and relative humidity of 60–80%. The disease index (di), disease incidence (DI) and biocontrol efficacy of bacterial wilt was calculated according to the method described by Xiong et al [38].

Antimicrobial spectrum determination of the lipopeptides

The crude lipopeptide extracts from FJAT-46737 were dissolved in methanol to the final concentrations of 2.5 – 30 mg/mL. The antimicrobial activities of the crude lipopeptides were evaluated against eight

indicator strains using the oxford cup method [39]. The indicator strains were grown at 30 ^{o}C in the corresponding liquid media for 48 h. A two layer plate containing fungal spores or bacterial culture suspensions were prepared. Then, four oxford cups were placed onto each plate and 150 μL of the crude lipopeptides or cell-free supernatants were added to each oxford cup. Methanol was served as a negative control. Finally, the plates were cultivated at 30 ^{o}C for 48 h and the inhibition zone diameter was measured. All experiments were repeated by three times.

Effects of fermentation parameters on the antagonistic activities of the lipopeptides

R.solanacearum FJAT-91 was selected as an indicator strain. Culture media of A, B, C, D, E and F in Table S1 and temperatures of 20, 25, 30, 35 and 40°C were selected for cultivating the strain FJAT-46737. The antagonistic activities of the lipopeptides produced under different culture conditions were determined by the aforementioned agar disk diffusion method.

Purification of antibacterial lipopeptides

The lipopeptides were loaded onto a solid phase extraction (SPE) C_{18} cartridge (6g/60mL) and washed with 60 mL of water and different proportions of methanol (10% to 100%), respectively. After evaporation, the fraction was dissolved in water and used for antibacterial activity test.

Statistical analysis

Analysis of variance (ANOVA) of the quantitative data were carried out using statistical software SPSS 19.0. Duncan's multiple range tests were employed to determine the significant differences among the data ($p < 0.05$).

Results

Identification and antagonistic activities of FJAT-46737

The strain FJAT-46737 was a gram-positive and endospore-forming bacterium. Its colonies on the NA plate were flat, slightly rough, nearly circular, light yellow (Figure S2). The phylogenetic analysis of 16*S rRNA* and *gyrB* gene results displayed that the strain FJAT-46737 belongs to the genus *Bacillus*, with a close relationship to the strain *B. velezensis* and *B. amyloliquefaciens,* respectively (Figure S3, S4). The *16 S rRNA* gene of FJAT-46737 exhibits 99.72%, 99.71% and 99.44% similarity to the type strains *Bacillus siamensis* KCTC 13613^{T}, *Bacillus velezensis* CR-502^{T} and *B.amyloliquefaciens* DSM7^{T}. The *gyr B* gene of FJAT-46737 displayed exceeding 99.0% identity to the type species of *B.amyloliquefaciens* and *B. velezensis*. Take into consideration of the high degree of relatedness among *B. amyloliquefaciens*, *B. siamensis* and *B. velezensis*, the ANI method based on the whole genome sequence was used to discriminate the strain FJAT-46737.

The whole genome sequence of strain FJAT-46737 contains 3995340 bp, and the G + C content of the chromosomal DNA was 46.5 mol%. The ANI values between the strain FJAT-46737 and those type strains

B. amyloliquefaciens DSM7^T, *B. siamensis* KCTC 13613^T, and *B. velezensis* KCTC 13012^T were calculated as 94.16%, 94.36% and 98.26%, respectively. The last one displayed ANI values of >98% exceeding the recommended cut-off of 96% for species delineation. This result suggested that the strain FJAT-46737 was a member of *B. velezensis* species.

The gene clusters of bioactive secondary metabolites in strain FJAT-46737 was analyzed using antiSMASH method. Results shown that the strain FJAT-46737 possess 7 gene clusters that responsible for synthesis of surfactin, fengycin, macrolactin, bacillaene, difficidin, bacilysin and bacillibactin (Figure S5), which indicate that the strain FJAT-46737 could display the strong antagonistic activities.

The agar disk diffusion method was used to evaluate antagonistic activities of FJAT-46737 against several animal or plant pathogens. The results showed that this strain exhibited significant activities against the gram-negative bacteria including *R. solanacearum* and *E. coli*, and the filamentous fungi including 3 biotypes of *Fusarium oxysporum* (*F. oxysporum* f. sp. *capsicum*, *F. oxysporum* f. sp. *niveum* and *F. oxysporum* f. sp. *melonis*) (Figure 1).

Biocontrol efficacy of FJAT-46737 against tomato bacterial wilt

The above results showed that the *B. velezensis* FJAT-46737 had *in vitro* antibacterial activity against *R. solanacearum*. Thus, we attempted to investigate its biocontrol efficacy against the tomato bacterial wilt by using the pot experiments in the greenhouse condition. Firstly, we found that DI of the tomato plants in the treatment group with the whole cultures of FJAT-46737 (31.7%) was much lower than that in the control group (93.8%) (Figure 2, Table 1). The biocontrol efficacy of whole cultures of FJAT-46737 against tomato bacterial wilt could reach up to 66.2% in the greenhouse experiments (Table 1).

In order to clarify the biocontrol mechanisms of FJAT-46737, we subsequently evaluated the suppressive effects of its cell-free supernatants on the tomato bacterial wilt. A preliminary experiment indicated the undiluted cell-free supernatant caused seedling injury, but the 2-fold diluted one had no injury effect. Thus, the 2-fold diluted cell-free supernatants were selected to perform the pot experiments. The results showed that an approximately 82.0% biocontrol efficiency could be achieved in the cell-free supernatant treatment group (Figure 2, Table 1), which indicated that the biocontrol ability of FJAT-46737 could be mainly (if not all) attributed to its production of extracellular bioactive substances.

Some previous studies indicated that the lipopeptides secreted by *Bacillus* strains play important roles in biocontrol of tomato bacterial wilt. Thus, we further tested the biocontrol efficiency of the crude lipopeptide extracts from FJAT-46737 against tomato root infection by *R. solanacearum*. A preliminary experiment indicated that high concentration (≥2.5 mg/mL) of lipopeptide led to seedling injury, but 1 mg/ml lipopeptide had no injury effect. Thus, the lipopeptide with concentration of 1 mg/mL was selected to perform the pot experiments. The directly plantlet-soaking treatment with the lipopeptides could significantly reduce mortality of the tomato plants and achieve a biocontrol efficiecy of 96.2% (Figure 2, Table 1). All, these results suggested that one of the antagonistic mechanisms of the strain FJAT-46737 was secretion of lipopeptides.

LC-QTOF-MS/MS analyses of the lipopeptides produced by FJAT-46737

In this study, we used the LC-ESI-MS/MS method to determine the lipopeptide profile of FJAT-46737. Three classes of cyclic lipopeptides iturin (retention time,12.8–22.3 min), fengycin (29.0–36.0 min), and surfactin (48.3–52.0 min) could be detected in present study. The retention times, MS and MS^2 spectral data and identification results of the lipopeptides from *B. velezensis* FJAT-46737 are summarized in Table 2. The results show that the lipopeptides consisted of C_{14}–C_{16} iturin A, C_{13}–C_{15} surfactins, and C_{16} fengycin A/B and C_{16} fengycin A_2/B_2) .

Inhibitory spectra of the antagonistic lipopeptides

We used an agar well diffusion assay to further evaluate antimicrobial activities of the crude lipopeptides from FJAT-46737. The results showed that the crude lipopeptides had significant activities against *R. solanacearum*, *E. coli*, and *F. oxysporum* in a dosage-dependent manner (Table 3 and Table 4). At 48 h, the inhibition zone diameters caused by 10 mg/mL of the crude lipopeptides could reach up to 18.52±0.73 mm, 14.55±0.23 mm, and 14.57±1.85 mm against the pathogens *R. solanacearum* FJAT-91 and FJAT-77, and *E. coli* FJAT-301, respectively (Table 3). It was found that the lipopeptide with the concentration of 0.5 mg/mL displayed antibacterial activity against *R. solanacearum* FJAT-91, but not at 0.25 mg/mL (Figure S6). These results implied that the crude lipopeptides of FJAT-46737 had the strongest antibacterial activity on *R. solanacearum*. Moreover, the inhibition zone diameters caused by 30 mg/mL of the crude lipopeptides on 4 biotypes of *F. oxysporum* (*F. oxysporum* f. sp. *capsicum* FJAT-831, *F. oxysporum* f. sp. *niveum* FJAT-9230 and *F. oxysporum* f. sp. *melonis* FJAT-30265) were all about 20 mm at 48 h.

Effects of medium components and culture conditions on the antibacterial activities and contents of lipopeptides

To further improve antagonistic activities of FJAT-46737, we analyzed effects of the medium components and temperatures on the antibacterial activities of the fermentation supernatants and crude lipopeptides. Given that the crude lipopeptides of FJAT-46737 had strong antibacterial activity on *R. solanacearum*, FJAT-91 was used as the indicator bacterium. Results of the antagonistic activity of the supernatant and crude lipopeptides and lipopeptide contents under different conditions were shown in Table 5 and Table 6.

Using the selected culture media A~F (Table S1), the supernatants and crude lipopeptides of FJAT-46737 exhibited good antibacterial activities against *R. solanacearum* FJAT-91, except the culture supernatant from the medium A (Table 5). Moreover, the antibacterial activities of both the supernatants and crude lipopeptide from the culture media C, D and E were stronger than those from the culture media A, B and F (Table 5). These results suggested that the medium components could significantly affect antibacterial activities of FJAT-46737.

Subsequently, effects of temperature on antibacterial activities of the cell-free supernatants and crude lipopeptides were determined using the culture medium C. The results showed that the culture temperature had significant effects on the antibacterial activities of the cell-free supernatants and crude lipopeptides: i) the cell-free supernatants did not exhibit antibacterial activity when the temperature was higher than 35 ^{o}C; ii) when it was incubated at 25 ^{o}C, both the cell-free supernatants and crude lipopeptides had the strongest antibacterial activities (Table 6).

Contents of the iturins, fengycins and surfactins in different culture conditions were further determined by LC-QTOF-MS and summarized in Table 5 and Table 6. The fengycins were the most abundant lipopeptide family produced by FJAT-46737. Contents of the iturins, fengycins and surfactins in the six culture media varied within the range of 0.31–1.99 mg/L, 9.03–58.66 mg/L and 0.55–1.39 mg/L, respectively. The contents of lipopeptide in the culture medium C and D were significantly higher than those in other four media, indicating the media containing rich organic nitrogen source might be benificail for producing lipopeptide. It was found that adding of yeast extract in culture medium D significantly increased yield of lipopeptide compared with the one without yeast extract adding (in culture medium B). In the culture medium C, production of the lipopeptides (61.04±9.86 mg/L) was the highest compared to those in the other media. Moreover, contents of the iturins, fengycins and surfactins strongly depended on temperature. Content of the fengycins decreased by 96.6% when the culture temperature was increased from 20 ^{o}C to 40^{o}C, on the contrary, that of the surfactins ascended by 59.9%. The temperature ranges of 25–30 ^{o}C, 20–25^{o}C and 35–40 ^{o}C were suitable for the strain FJAT-46737 to produce iturin, fengycin and surfactin, respectively.

Subsequently, a correlation analysis between the cell-free supernatant antibacterial activities and lipopeptide contents in the different culture conditions were carried out (Table 7). It was observed that contents of the fengycins and total lipopeptides were significantly positive correlated with the antibacterial activities of the cell-free supernatants of all the samples ($p < 0.05$), but those of the surfactins and iturins not. These results suggested that the antibacterial activities of the cell-free supernatants were mainly attributed to secretion of the fengycins by FJAT-46737.

Effect of purified lipopeptides on inhibition of *R. solanacearum* growth

The antibacterial activities of the purified lipopeptides against *R. solanacearum* were tested. The results showed that only the fraction obtained by SPE with 70% MeOH (named as SPE70) exhibited antibacterial activity. Therefore, composition of the fraction SPE70 was further determined by LC-QTOF-MS/MS (Figure S7). The results showed that the fraction SPE70 contained only fengycin, indicating that the fengycin plays an important role in the growth inhibition of *R. solanacearum*. This result was consistent with the aboved correlation analysis.

Discussion

Nowadays, it has been recognized that the traditional phenotypic methods and phylogenetic analysis of conserved gene are hardly to distinguish the species including *B. subtilis*, *B. amyloliquefaciens*, *B. velezensis*, *B. siamensis*, *B. licheniformis*, and *B. pumilus* due to their tight relatedness [40]. With the increasing lots of available complete genome sequences, it became easy to discriminate these species. The average nucleotide identity (ANI) based on the complete genome sequences was calculated and cut-off of 96% was proposed for species delineation [30, 40]. In present study, the strain FJAT-46737 displayed 98.26% similarity to the *B. velezensis* type strain KCTC 13012^T, which is well above of the recommended threshold of 96% for species delineation. Thus, the FJAT-46737 was finally identified as *B. velezensis*.

The occurrence of bacterial wilt in crop has brought great economic losses in the word. Chemicals are often used in controlling the disease but lead to environmental pollution and pathogen resistance in plants [3]. Many studies proved that the use of *Bacillus* strains as biological control agent is as a promising and safe strategy for the effective tomato bacterial wilt management. Kwon and Kim reported that *B. subtilis* JW-1 could result in >80% reduction in bacterial wilt disease, which can use as a potential biocontrol agent of bacterial wilt [41]. Xiong *et al*. had isolated *B. amyloliquefaciens* JK6 that could effectively suppress tomato bacterial wilt with the biocontrol efficacies of 58.6% and 52.9% in two greenhouse conditions [38]. *B. subtilis* 916 had been found to exhibit a biocontrol efficiency of 55.6% on the tomato bacterial wilt [42]. In present study, we had obtained a new biocontrol strain *B. velezensis* FJAT-46737 that exhibited broad-spectrum antimicrobial activities against gram-negative bacteria and filamentous fungi. The biocontrol efficacy of the strain FJAT-46737 on controlling the potato bacterial wilt was 66.2%, which was higher than those of many previously reported *Bacillus* spp. strains, such as the strains APF1 (60.3%), JK6 (58.6%) and 916 (55.6%) [38, 42, 43].

The biocontrol ability of the *Bacillus* strains against the plant pathogens has been confirmed to be achieved by induction of host systemic resistance, antibiotic production, secretion of siderophores and lytic enzymes, biofilm formation, or competition for niches within the rhizosphere [44]. Bais *et al* reported that *B. subtilis* 6051 was able to control *Pseudomonas syringae* root infection in *Arabidopsis* because of the secretion of surfactin and the formation of biofilm on the plant roots [15]. Kwon and Kim demonstrated that *B. subtilis* JW-1 induced a significant bacterial wilt disease suppression effect *in vivo* due to the production of cyclic lipopeptide [41]. Xiong *et al*. reported that secretion of the surfactin lipopeptides played important roles in biocontrol of tomato bacterial wilt by the strain JK6 [38]. In the present study, biocontrol efficiency of cell-free supernatant against the tomato bacterial wilt was 82.0%, while that of lipopeptide reached up to 96.2%, inferring that one of the mechanisms of disease suppression by *B. velezensis* FJAT-46737 was lipopeptide secretion.

The *Bacillus* species produces lipopeptides depending on the strains themselves: some strains can co-produce two or three classes of lipopeptides, while others can yield only one class [11-13]. *Bacillus licheniformis* MB01 only produce surfactin [12], *B. subtilis* K1 co-produce surfactins and iturins, while *B. amyloliquefaciens* SYBC H47 yield three types of lipopeptides including bacillomycin, fengycin and surfactin [45]. In present study, the strain *B. velezensis* FJAT-46737 could also co-produce three types of

lipopeptides including C_{14}–C_{16} iturin A, C_{16} fengycin A/B, C_{16} fengycin A_2/ B_2, as well as C_{14}–C_{15} surfactin. Furthermore, yield of the lipopeptides is significantly affected by the medium components (such as carbon and nitrogen sources, trace metals, etc.) and fermentation conditions (such as culture temperature, incubation time, rotary speed, etc.) [46]. In this study, *B. velezensis* FJAT-46737 could yield three types of lipopeptides in the all six common culture media (A~F). Whereas, the antibacterial activities of the crude lipopeptides (10 mg/mL) produced from culture media A, B or F were much weaker than the other three media. Carbonsource had been believed as a primary factor for lipopeptide production, for an example, Li *et al* found that variation of carbonsourse in the culture medium changed the type of lipopeptides produced by *B. licheniformis* HSN221 [47]. However, the types of lipopeptide produced by FJAT-46737 were not changed whether the culture medium containing glucose or not. It was reported that nitrogen sources played an important role in the regulation of biosurfactant synthesis [48]. Our results showed that rich organic nitrogen sources in the media were benificial for producing fengycin and surfactin. Furthermore, influence of yeast extracts on production of fengycin was more significant than that of iturin and surfactin. This might be owing to the fact that response of the enzymatic complex for fengycin biosynthesis to nitrogen sources would be less sensitive than that of the enzymatic complexs for iturin and surfactin [49].

Interestingly, effects of the culture temperatures on contents of the fengycins and surfactins generated by the strain FJAT-46737 displayed a different manner: with the temperature increasing, content of the fengycins decreased, but that of the surfactin rised. These results were not consistent with the report demonstrated by Monteiro *et al*., who found that the low temperature (15 ^{o}C) was suitable temperature for producing surfactins and the amount of fengycin was not affected by temperature changes [46]. Fengycin and surfactin are synthesized nonribosomally by fengycin synthetases and surfactin synthetases, respectively [50]. Usually, the optimum temperature for enzyme activity is 37^{o}C, this can explain why the content of surfactin ascend along with increase of the culture temperature. The high temperature leading to the few fengycin could attribute to the decreasing activity of fengycin syntheases. Generally, the suitable temperatures are 20 ^{o}C–30 ^{o}C for crop growing in the field. Our results indicated that 25 ^{o}C was a favorable temperature for the strain FJAT-46737 to yield lipopeptides with the strongest antibacterial activities, implying that the strain FAJT-46737 and its lipopeptides have good ecosystem adaptability and prospect in future agricultural application. Previous studies demonstrated that the surfactins display antibacterial activities, whereas iturins showed strong antifungal activities with limited antibacterial activity. The fengycin family is specific against filamentous fungi until two cases recently reported by Villegas-Escobar *et al*.[14] and Chen *et al*., [32], who found that the fengycins exhibited strong antibacterial activity against *R. solanacearum in vitro*. In present study, the correlation analysis between the cell-free supernatant antibacterial activities and lipopeptide contents indicated that the antibacterial activities of cell-free supernatant were significantly positive correlated with content of the fengycins. This is agreement with that the report that the significant relationship between the strong antibacterial activity and the production of fengycin and surfactin of *Bacillus* isolates [51]. In addition, only the purified fraction SPE70 exhibited antibacterial activity *in vitro*, which just consisted of fengycins, indicating that the antibacterial activity of lipopeptide mixture could be due to the fengycins. These results further

confirmed antibacterial activity of the fengycins first reported by Villegas-Escobar *et al*. [14] and Chen *et al*., [32].

Conclusion

A new strain *B. velezensis* FJAT-46737 with broad-spectrum antimicrobial activities was confirmed to have strong antibacterial activity against the bacterial wilt pathogen *R. solanacearum* by both the *in vivo* and *in vitro* experiments. Moreover, suppressive effects of FJAT-46737 were associated with to the lipopeptide secretion, especially being attributed to content of the fengycins. Therefore, FJAT-46737 and its lipopeptides would have good application prospects for biocontrol of the bacterial wilt.

Abbreviations

CGMCC: China General Microbiological Culture Collection Center

PDA: potato dextrose agar

LB: Luria–Bertani

NA: nutrient agar

SDS: sodium dodecyl sulfate

gyr B: gyrase subunit B

DNA: deoxyribonucleic acid

RNA: ribonucleic acid

ANI: average nucleotide identity

antiSMASH: antibiotics Secondary Metabolite Analysis Shell

di: disease index

DI: disease incidence

SPE: solid phase extraction

ANOVA: analysis of variance

LC-QTOF-MS/MS: liquid chromatography coupled to quadrupole time-of-flight tandem mass spectrometry.

Declarations

Ethics approval and consent to participate

No animals, human subjects, human material, or human data are used in this study.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files. The *16S rRNA*, *gyrB* and whole genome sequences of this study have been submitted to NCBI GenBank database (accessions MG924092, MH470338 and CP044133).

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

MC, JW, BL and YZ conceived and designed the experiments. MC, JW, YZ, RX and BL were responsible for drafting the article. MC, RX, WY, CG, and ZC were involved in the experiments preformation and data analysis. All authors approved the final version of the manuscript

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References

1. Genin S. Research review: molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. New phytol. 2010; 187: 920–928.
2. Jiang G, Wei Z, Xu J, Chen HL, Zhang Y, She XM, Macho AP, Ding W, Liao DS. Bacterial Wilt in China: history, current status, and future perspectives. Front Plant Sci. 2017; 8: 1549–1559.
3. Yuliar YAN, Koki T. Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. Microbes Environ. 2015; 30: 1–11.
4. Ongena M, Jacques P, Toure Y, Destain J, Jabrane A, Thonart P. Involvement of fengycin-type lipopeptides in the multifaceted biocontrol potential of *Bacillus subtilis*. Appl Microbiol Biotechnol. 2005; 69: 29–38.
5. Maji S, Chakrabartty PK. Biocontrol of bacterial wilt of tomato caused by *Ralstonia solanacearum* by isolates of plant growth promoting rhizobacteria. Australian J Crop Sci. 2014; 8: 208–214.
6. Yendyo S, Ramesh GC, Pandey BR. Evaluation of *Trichoderma* spp., *Pseudomonas fluorescens* and *Bacillus subtilis* for biological control of *Ralstonia* wilt of tomato. F1000 Res. 2018; 6: 2028–2050.
7. Nam HS, Yang HJ, Oh BJ, Anderson AJ, Kim YC. Biological control potential of *Bacillus amyloliquefaciens* KB3 isolated from the feces of *Allomyrina dichotoma* Larvae. Plant Pathol J. 2016; 32: 273-280.
8. Wei Y, Caceres-Moreno C, Jimenez-Gongora T, Wang KK, Sang YY, Lozano-Duran R, Macho AP. The *Ralstonia solanacearum* csp22 peptide, but not flagellin-derived peptides, is perceived by plants from the *Solanaceae* family. Plant Biotech J. 2018; 16:1349–1362.
9. Rocha DJA, Moura AB. Biological control of tomato wilt caused by *Ralstonia solanacearum* and *Fusarium oxysporum* f. sp *lycopersici* by rhizobacteria. Tropical Plant Pathology. 2013; 38: 423–430.
10. Chowdhury SP, Uhl J, Grosch R, Alquéres S, Pittroff S, Dietel K, Schmitt-Kopplin P, Borriss R, Hartmann A. Cyclic lipopeptides of *Bacillus amyloliquefaciens* subsp. *plantarum* colonizing the lettuce rhizosphere enhance plant defense responses toward the bottom rot pathogen *Rhizoctonia solani*. Mol Plant Microbe Interact. 2015; 28: 984–995.
11. Bóka B, Manczinger L, Kecskeméti A, Chandrasekaran M, Kadaikunnan S, Alharbi NS. Ion trap mass spectrometry of surfactins produced by *Bacillus subtilis* SZMC 6179J reveals novel fragmentation features of cyclic lipopeptides. Rapid Commun. Mass Spectrom. 2016; 30: 1581–1590.

12. Chen Y, Liu SA, Mou H, Ma Y, Li M, Hu X. Characterization of lipopeptide biosurfactants produced by *Bacillus licheniformis* MB01 from marine sediments. Front Microbiol. 2017; 8: 871–882.
13. Ma Y, Kong Q, Qin C, Chen YL, Chen YJ, Lv RH, Zhou GH. Identification of lipopeptides in *Bacillus megaterium* by two-step ultrafiltration and LC-ESIMS/MS. AMB Express. 2016; 6: 79–94.
14. Villegas-Escobar V, González-Jaramillo LM, Ramírez M, Moncada RN, Sierrz-Zapata L, Orduz, S. Lipopeptides from *Bacillus* sp. EA-CB0959: active metabolites responsible for *in vitro* and *in vivo* control of *Ralstonia solanacearum*. Bio Control. 2018; 125: 20–28.
15. Bais HP, Fall R, Vivanco JM. Biocontrol of *Bacillus subtilis* against infection of arabidopsis roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. Plant Physiol. 2004; 134: 307–319.
16. Guo Q, Dong W, Li S, Lu X , Wang P , Zhang X , Wang Y , Ma P. Fengycin produced by *Bacillus subtilis* NCD-2 plays a major role in biocontrol of cotton seeding damping-off disease. Microbiol Res. 2014; 169: 533–540.
17. Romero D, de Vicente A, Rakotoaly RH, Dufour SE, Veening JW, Arrebola E, Cazorla F, Kuipers OP, Paquot M, Perez-Garcia P. The iturin and fengycin families of lipopeptides are key factors in antagonism of *Bacillus subtilis* toward Podosphaera fusca. Mol Plant Microbe Interact. 2007; 20: 430–440.
18. Zhao Y, Selvaraj JN, Xing FG, Zhou L, Wang Y, Song HM, Tan XX, Sun LC, Sangare LC, Folly YME, Liu Y. Antagonistic action of *Bacillus subtilis* strain SG6 on *Fusarium graminearum*. PloS One. 2014; 9: e92486.
19. Gong AD, Li HP, Yuan QS, Song XS, Yao W, He WJ, Zhang JB, Liao YC. Antagonistic mechanism of iturin A and plipastatin A from *Bacillus amyloliquefaciens* S76-3 from wheat spikes against *Fusarium graminearum*. PLoS One. 2015; 10: e0116871.
20. Alvarez F, Castro M, Principe A, Borioli G, Fischer S, Mori G, Jofré E. The plant-associated *Bacillus amyloliquefaciens* strains MEP218 and ARP23 capable of producing the cyclic lipopeptides iturin or surfactin and fengycin are effective in biocontrol of sclerotinia stem rot disease. J Appl Microbiol. 2012; 112: 159–174.
21. Zhu Z, Zhang GY, Xu YC, Yang XM, Ran W, Shen QR. Bio-control and growth-promoting effects of bio-manure fermented by lipopeptide-producing bacteria. Acta Pedologica Sinica. 2012; 49: 104–112. (In Chinese)
22. Wu YL. Preparation of microbial lipopeptide-mineral composites and their effects on tomato growth and against bacterial wilt of tomato. Nanjing Agricultural University (In Chinese) 2015.
23. Asaka O, Shoda M. Biocontrol of *Rhizoctonia solani* damping off of tomato with *Bacillus subtilis* RB14. Appl Environ Microbiol. 1996; 62: 4081–4085.
24. Zhou TT, Chen D, Li CY, Liu F, Shen QR, Shen B. Isolation and characterization of *Pseudomonas brassicacearum* J12 as an antagonist against *Ralstonia solanacearum* and identification of its antimicrobial components. Microbiol Res. 2012; 167: 388–394.

25. Cawoy H, Mariutto M, Henry G, Fisher C, Vasilyeva N, Thonart P, Dommes J, Ongena M. Plant defense stimulation by natural isolates of *Bacillus* depends on efficient surfactin production. Mol Plant Microbe Interact. 2014; 27: 87–100.
26. Ohno A, Aao T, Shoda M. Effect of temperature on production of lipopeptide antibiotics, iturin A and surfactin by a dual producer, *Bacillus subtilis* RB14, in Solid-State fermentation. J Biosci Bioeng. 1995; 80: 517–519.
27. Mizumoto S, Shoda M. Medium optimization of antifungal lipopeptide, iturin A, production by *Bacillus subtilis* in solid-state fermentation by response surface methodology. Appl Microb Biotechnol. 2007; 76: 101–108.
28. Nihorimbere V, Cawoy H, Seyer A, Brunelle A, Thonart P, Ongena M. Impact of rhizosphere factors on cyclic lipopeptide signature from the plant beneflcial strain *Bacillus amyloliquefaciens* S499. FEMS Microbiol Ecol. 2012; 79: 176–191.
29. Yoon SH, Ha SM, Lim JM, Kwon SJ, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie van Leeuwenhoek. 2017; 110:1281–1286.
30. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. Proc.Natl.Acad.Sci.U.S.A. 209; 106: 19126–19131.
31. Kim M, Oh HS, Park SC, Chun J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol. 2014; 64(2): 346-351.
32. Chen MC, Wang JP, Zhu YJ, Liu B, Yang WJ, Ruan CQ. Antibacterial activity against *Ralstonia solanacearum* of the lipopeptides secreted from the *Bacillus amyloliquefaciens* strain FJAT-2349. J Appl Microbiol. 2019; 126:1519-1529.
33. Pecci Y, Rivardo F, Martinotti MG, Allegrone G. LC/ESI-MS/MS characterisation of lipopeptide biosurfactants produced by the *Bacillus licheniformis* V9T14 strain. J Mass Spectrom. 2010; 45: 772–778.
34. Pathak KV, Keharia H, Gupta K, Thakur SS, Balaram P. Lipopeptides from the banyan endophyte, *Bacillus subtilis* K1: mass spectrometric characterization of a library of fengycins. J. Am. Soc. Mass Spectrom. 2012; 23: 1716–1728.
35. Pathak KV, Bose A, Keharia H. Identification and characterization of novel surfactins produced by fungal antagonist *Bacillus amyloliquefaciens* 6B. Biotechnol Appl Biochem. 2014; 61: 349–356.
36. Pathak KV, Keharia H. Identification of surfactins and iturins produced by potent fungal antagonist, *Bacillus subtilis* K1 isolated from aerial roots of banyan (*Ficus benghalensis*) tree using mass spectrometry. 3 Biotech. 2014; 4: 283–295.
37. Jemila N, Manresab A, Rabanalc F, Ben Ayed H, Hmidet N, Nasri M. Structural characterization and identification of cyclic lipopeptides produced by *Bacillus methylotrophicus* DCS1 strain. J Chromatogr B. 2017; 1060: 374–386.
38. Xiong HQ, Li YT, Cai YF, Cao Y, Wang Y. Isolation of *Bacillus amyloliquefaciens* JK6 and identification of its lipopeptides surfactin for suppressing tomato bacterial wilt. RSC Advances. 2015; 5: 82042–

82049.

39. Fang ZD. Study methods of plant pathology (3rd ed.), China Agriculture Press, Beijing, 2007, 137-138 (in Chinese).
40. Fan B, Blom J, Klenk HP, Borriss R. *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* form an "Operational group *B. amyloliquefaciens*" within the *B. subtilis* species complex. Frontiers in microbiology, 2017; 8: 00022.
41. Kwon JW, Kim SD. Characterization of an antibiotic produced by *Bacillus subtilis* JW-1 that suppresses *Ralstonia solanacearum*. J Microbiol Biotechnol. 2014; 24: 13–18.
42. Qiao JQ, Chen ZY, Liang XJ, Liu YF, Liu YZ. Biocontrol efficacy on tomato bacterial wilt by *Bacillus subtilis* Bs916. Chinese J Biological Control. 2016; 32: 229-234. (in Chinese).
43. Abdulwareth AA, Ullah KK, Zarqa N, Li B, Alisaand M, Chun-Lan Y, Xie GL. Tomato plant growth promotion and antibacterial related-mechanisms of four rhizobacterial *Bacillus* strains against *Ralstonia solanacearum*. Symbiosis. 2014; 63: 59–70.
44. Tan SY, Jiang Y, Song S, Huang JF, Ling N, Xu YC, Shen QR. Two *Bacillus amyloliquefaciens* strains isolated using the competitive tomato root enrichment method and their effects on suppressing *Ralstonia solanacearum* and promoting tomato plant growth. Crop Prot. 2013; 43: 134–140.
45. Li XH, Zhang YZ, Wei ZW, Guan ZB, Cai YJ, Liao XR. Antifungal activity of isolated *Bacillus amyloliquefaciens* SYBC H47 for the biocontrol of peach gummosis. PloS One. 2016; 11: 1–22.
46. Monteiro FP, de Medeiros FHV, Ongena M, Franzil L, de Souza P E, de Souza JR. Effect of temperature, pH and substrate composition on production of lipopeptides by *Bacillus amyloliquefaciens* 629. Afr J Microbiol Res. 2016; 10: 1506–1512.
47. Li YM, Haddad NIA, Yang SZ, Mu BZ. Variants of lipopeptides produced by *Bacillus licheniformis* HSN221 in different medium components evaluated by a rapid method ESI-MS. Int J Pept Res Ther. 2008; 14: 229–235.
48. Ghribi D, Ellouze-Chaabouni S. Enhancement of *Bacillus subtilis* lipopeptide biosurfactants production through optimization of medium composition and adequate control of aeration. Biotechnol Res Int. 2011; 653654.
49. Akpa E, Jacques P, Wathelet B, Paquot M, Fuchs R, Budzikiewicz H, Thonart P. Influence of culture conditions on lipopeptide production by *Bacillus subtilis*. Appl Biochem Biotech. 2001; 91-93: 551–561.
50. Leães FL, Velho RV, Caldas DGG, Ritter AC, Tsai SM, Brandelli A. Expression of essential genes for biosynthesis of antimicrobial peptides of *Bacillus* is modulated by inactivated cells of target microorganisms. Res Microbiol. 2016; 167: 83–89.
51. Mara I, Cabrefiga J, Montesinos E. Cyclic lipopeptide biosynthetic genes and products, and inhibitory activity of plant-associated *Bacillus* against phytopathogenic bacteria. Plos one. 2015; 10: e0127738.

Tables

Table 1. The biocontrol efficacies and the disease incidence in differently treatment groups of plants.

Treatment group	biocontrol efficiency	Disease incidence (DI)
Control group	0%	93.8%
Whole culture	66.2%	31.7%
2-fold diluted cell-free supernatants	82.0%	17.5%
Lipopeptide (1mg/mL)	96.2%	3.7%

Table 2. Identification of lipopeptides extracted from strain *Bacillus velezensis* FJAT-46737 through LC-QTOF-MS/MS

Retention time (min)	MS m/z $[M+H]^+$ /$[M+Na]^+$	Identified Compounds
12.833	1065.5[a]	C_{14}Iturin A
16.632	1079.7[a]	C_{15}Iturin A
17.536	1079.7[a]	C_{15}Iturin A
21.244	1093.6[a]	C_{16}Iturin A
22.284	1093.6[a]	C_{16}Iturin A
29.067	1449.9[b]	C_{16}FengycinA_2
30.423	1449.9[b]	C_{16}FengycinA_2
30.514	1463.9[b]	C_{16}FengycinA
31.056	1463.9[b]	C_{16}FengycinA
31.735	1449.9[b]	C_{16}FengycinA_2
32.413	1477.9[b]	C_{16}FengycinB_2
33.182	1463.9[b]	C_{16}FengycinA
34.132	1477.9[b]	C_{16}FengycinB_2
34.991	1477.9[b]	C_{16}FengycinB_2
35.262	1491.8[b]	C_{16}FengycinB
35.986	1491.8[b]	C_{16}FengycinB
48.376	1030.8[a]	C_{13}Surfactin
50.26	1044.8[a]	C_{14}Surfactin
50.757	1044.8[a]	C_{14}Surfactin
51.345	1030.8[a]	C_{13}Surfactin
51.978	1058.8[a]	C_{15}Surfactin

Note: a is $[M+Na]^+$ and b is $[M+H]^+$.

Table 3. Antibacterial ability of lipopeptides from strain *Bacillus velezensis* FJAT-46747

		Diameter of inhibition zone (mm)		
indicator strains		*Ralstonia solanacearum* FJAT-91 (tomato pathogens)	*Escherichia coli* FJAT-301	*Ralstonia solanacearum* FJAT-77 (peanut pathogens)
concentration of crude lipopeptides (mg/mL)	10	18.52±0.73^{a}	14.55±0.23^{a}	14.57±1.85^{a}
	5	16.04±0.26^{b}	12.32±1.14^{b}	13.90±0.43^{a}
	2.5	13.87±0.99^{b}	10.02±0.01^{c}	11.19±0.03^{a}
	1	12.51±0.58^{b}	weak	-

Note: Values were expressed as mean ± standard deviation (n = 3).

The difference letter in the same column indicated that the difference between the grades is significantly through Duncan test (p <0.05).

Table 4. Antifungal ability of lipopeptides from strain *Bacillus velezensis* FJAT-46747

		Diameter of inhibition zone (mm)		
indicator strains		*Fusarium oxysporum* f. sp. *capsicum*	*Fusarium oxysporum* f. sp. *melonis*	*Fusarium oxysporum* f. sp. *niveum*
		FJAT-831	FJAT-9230	FJAT-30265
concentration of crude lipopeptides (mg/mL)	30	17.87±0.11^{a}	18.77±0.25^{a}	20.47±3.08^{a}
	20	16.62±0.78^{a}	18.36±0.44^{a}	17.28±0.64^{a}
	10	13.84±0.58^{b}	16.00±0.22^{b}	14.51±1.35^{a}

Note: Values were expressed as mean ± standard deviation (n = 3).

The difference letter in the same column indicated that the difference between the grades is significantly through Duncan test (p <0.05).

Table 5. Diameter of inhibition zone of the culture supernatant and crude lipopeptides (10 mg/mL) produced by FJAT-46737 against *Ralstonia solanacearum* FJAT-91 and the content of lipopeptide under different culture medium.

Diameter of inhibition zone (mm)	Medium	A	B	C	D	E	F
	supernatant	-	-	15.75±0.38^{ab}	14.93±0.77^{b}	16.36±0.60^{a}	12.77±0.88^{c}
	Lipopeptides	13.88±2.54^{b}	-	21.41±1.60^{a}	19.15±0.78^{a}	19.75±0.93^{a}	15.67±1.00^{b}
Yield of lipopeptide in the supernatant (mg/L)	iturin	0.53±0.20^{cd}	0.31±0.09^{d}	0.98±0.02^{b}	0.68±0.14^{c}	1.99±0.18^{a}	0.38±0.02^{d}
	fengycin	22.17±11.94^{b}	9.03±1.69^{b}	58.66±9.81^{a}	50.37±12.46^{a}	24.34±2.75^{b}	9.17±1.21^{b}
	surfactin	0.65±0.42^{a}	0.55±0.23^{a}	1.39±0.06^{a}	1.21±0.35^{a}	0.61±0.06^{a}	0.59±0.13^{a}
	Total lipopeptides	23.34±12.54^{b}	10.07±1.80^{b}	61.04±9.86^{a}	52.27±12.70^{a}	27.54±3.90^{b}	10.22±1.29^{b}

Note: Values were expressed as mean ± standard deviation (n = 3).

The difference letter in the same row indicated that the difference between the grades is significantly through Duncan test (p <0.05). The effects of medium components on the antibacterial activities and contents of lipopeptides were carried out at 25°C

Table 6. Diameter of inhibition zone of the culture supernatant and crude lipopeptides (10 mg/mL) produced by FJAT-46737 against *Ralstonia solanacearum* FJAT-91 and the content of lipopeptide under different culture temperature.

Diameter of inhibition zone (mm)	Temperature	20°C	25°C	30°C	35°C	40°C
	supernatant	12.36±0.46^{b}	14.47±1.06^{a}	12.06±0.26^{b}	-	-
	Lipopeptides	14.34±0.90^{b}	17.16±0.46^{a}	15.53±1.83^{ab}	11.00±0.49^{c}	-
Yield of lipopeptide in the supernatant (mg/L)	iturin	0.65±0.03^{b}	0.78±0.07^{a}	0.86±0.10^{a}	0.61±0.07^{b}	0.45±0.01^{c}
	fengycin	55.11±4.74^{a}	56.47±6.59^{a}	23.62±6.34^{b}	9.55±0.27^{c}	1.88±0.28^{c}
	surfactin	0.59±0.06^{b}	0.83±0.19^{b}	0.78±0.13^{b}	1.22±0.21^{a}	1.47±0.15^{a}
	Total lipopeptides	56.30±4.76^{a}	57.94±4.76^{a}	25.41±6.37^{b}	11.39±0.53^{c}	3.72±0.36^{c}

Note: Values were expressed as mean ± standard deviation (n = 3).

The difference letter in the same row indicated that the difference between the grades is significantly through Duncan test (p <0.05). The effects of temperature on the antibacterial activities and contents of lipopeptides were carried out using the culture media C.

Table 7. Correlation coefficients between antibacterial activities of the fermentation supernatant and the lipopeptide contents.

	Pearson correlation coefficient	Kendall correlation coefficient	Spearman correlation coefficient
Iturin	0.570	0.597*	0.740*
Fengycin	0.695*	0.559*	0.726*
Surfactin	-0.100	0.078	0.033
Total lipopeptide	0.705*	0.559*	0.726*

Test of significance by two-tailed

*p < 0.05;

Figures

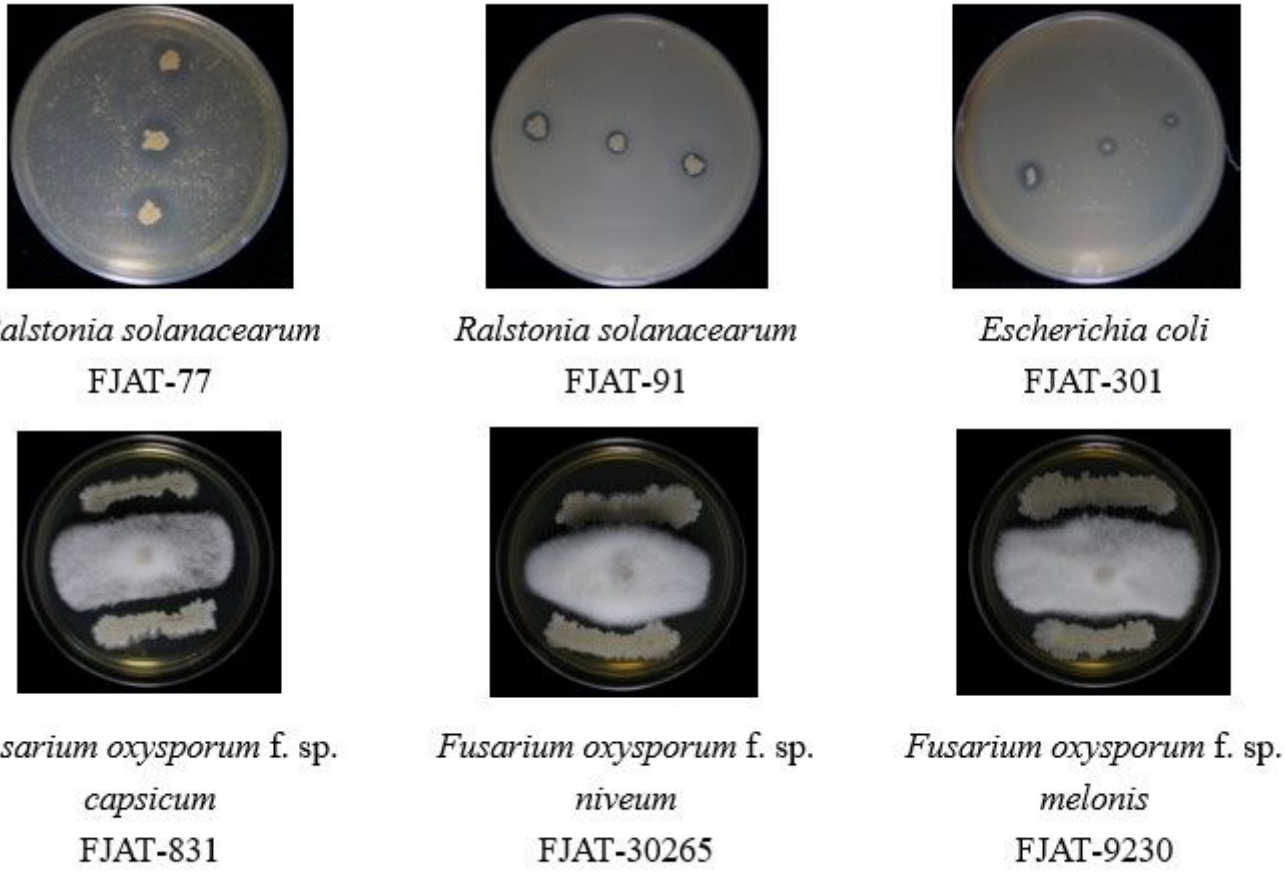

Figure 1

The spectrum of antagonism activity of FJAT-46737 against bacteria and filamentous fungi.

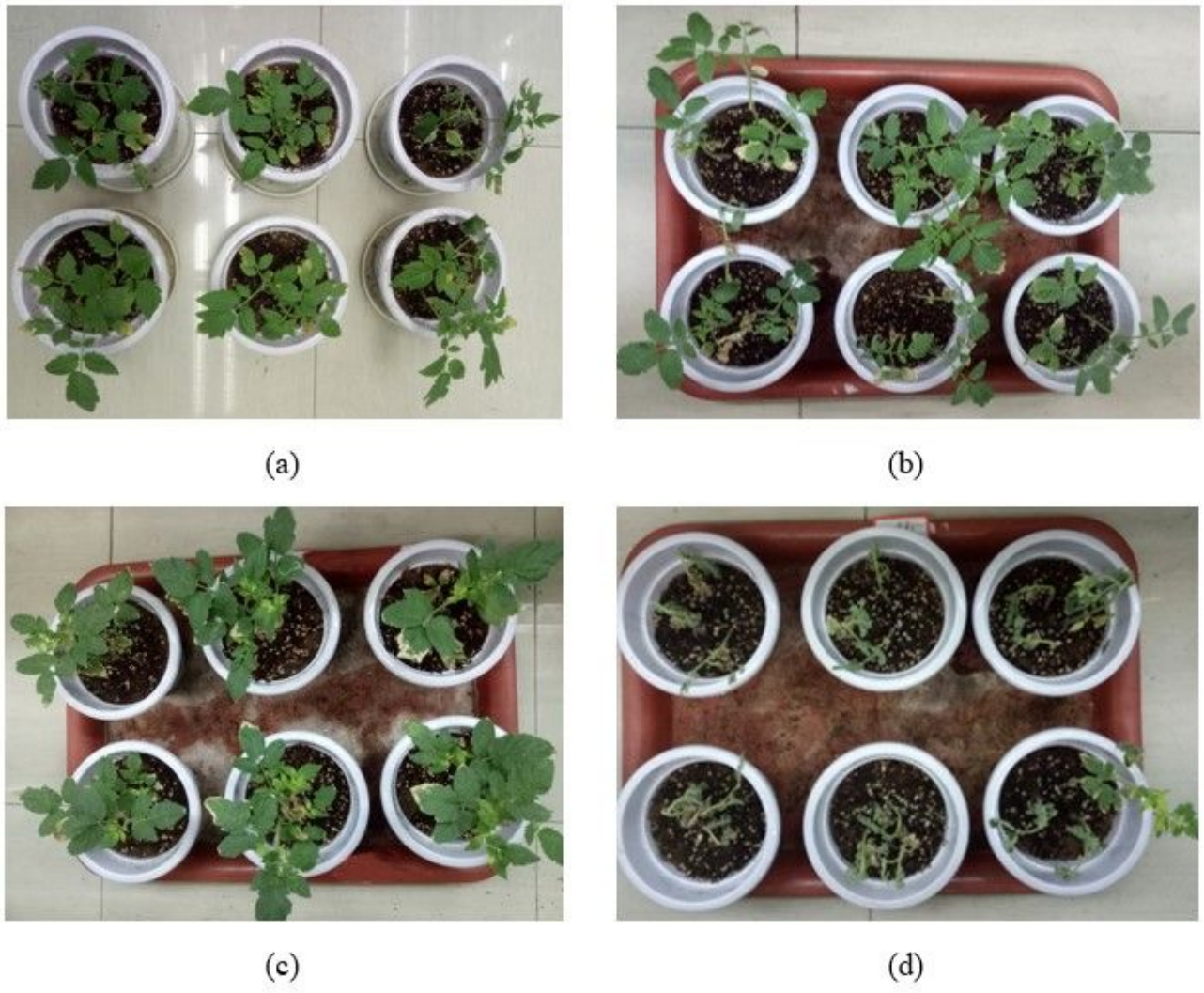

Figure 2

Biocontrol effect of the whole cultures of FJAT-46737 (a), the fermentation supernatant (b), 1mg/mL crude lipopeptides (c) , and control (d) against bacterial wilt disease.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SI46737.docx