Bacillus amyloliquefaciens WS-10 as a potential plant growth-promoter and biocontrol agent for bacterial wilt disease of flue-cured tobacco

Waqar Ahmed1,2,3†, Guisu Zhou4†, Jun Yang1,3,5, Shahzad Munir1, Ayesha Ahmed1, Qi Liu1,3, Zhengxiong Zhao2* and Guanghai Ji1,3*

Abstract

Background: Bacterial wilt disease caused by the soilborne bacterium Ralstonia solanacearum is a serious threat to flue-cured tobacco production. In this study, an indigenous disease suppressive Bacillus strain was isolated from the rhizosphere soil of healthy tobacco plants, and its biocontrol and plant growth promoting (PGP) potential were evaluated in in-vivo and in-vitro assays.

Results: Through isolation and screening of 250 isolates, WS-10 was found to be the best candidate antagonistic strain against R. solanacearum (WS-001). In-vitro assays revealed that the isolated strain WS-10 (Bacillus amyloliquefaciens) showed an effective antagonistic activity against R. solanacearum WS-001 and several plant-pathogenic fungi. As promising PGP rhizobacteria, WS-10 had the ability of nitrogen fixation, solubilization of inorganic potassium and phosphate, and biosynthesis of indole-3-acetic. In a co-culture assay, it significantly inhibits the growth of WS-001. Our greenhouse experiments showed that the soil physicochemical properties and accumulation of dry matter contents in different plant parts (roots, stems, and leaves) were significantly increased in the presence of B. amyloliquefaciens WS-10. The soil treated with B. amyloliquefaciens WS-10 displayed significantly higher values of the average well color development index, the utilization ability of 6 types of carbon sources by rhizosphere microorganisms, and the diversity indices of the rhizosphere microbial communities. In planta assay, B. amyloliquefaciens WS-10 significantly reduced tobacco bacterial wilt disease incidence by up to 73.36, 43.82, and 86.82% under three different treatments by improving the functional diversity and biological activity of the soil microbial community.

Conclusions: Obtained findings suggested that B. amyloliquefaciens WS-10 had an excellent potential as a growth-promoting and biocontrol agent of tobacco bacterial wilt disease due to its multiple beneficial traits of nutrient solubilization and disease suppression. Thus, we conclude that B. amyloliquefaciens WS-10 was a high potential PGP and biocontrol strain for healthy production of tobacco crop.

Keywords: Bacillus amyloliquefaciens, Flue-cured tobacco, Biological control, Ralstonia solanacearum, Plant growth-promoter

Background

Ralstonia solanacearum is a soilborne phytopathogenic bacterium that causes bacterial wilt disease in many economic field crops, including tomato, potato, tobacco, eggplant, ginger, and banana (Wu et al. 2014). The bacterium...
has a broad host range and is widely distributed in the temperate, tropical, and subtropical regions of the world (Paret et al. 2008). It infects more than 250 plant species of 54 different families and is ranked as the world’s second most important phytopathogenic bacterium (Paudel et al. 2020). Due to broad host range and wide geographical distribution, *R. solanacearum* forms a highly diverse species complex that is classified into 4 phylotypes, 5 races, and 6 biovars (García et al. 2019). The worldwide yield losses caused by *R. solanacearum* range from 15 to 55% (Kim et al. 2016).

*Ralstonia solanacearum* survives in the soil and infected plant tissues, wild host, and freshwater for a long time (up to 40 years), even without a host plant (Genin and Denny 2012). Infected soil acts as a primary source of inoculum, and the bacterium invades the plant through spots of primary and secondary roots development, and wounds formed on roots due to mechanical operation (Singh et al. 2018). After entering a susceptible host, it multiplied systematically to disrupt xylem tissue. Infected plants generally show symptoms of yellowing and unilateral wilting of leaves leading to marginal necrosis. The color of parenchyma cells changes from light yellow to dark brown, and dark water-soaked lesions develop on the stem surface (García et al. 2019).

Tobacco (*Nicotiana tabacum* L.) is an economically important industrial crop worldwide, including China (Ma et al. 2018). Tobacco production is adversely affected by bacterial wilt disease (Wu et al. 2020). The incidence of tobacco bacterial wilt is recorded at around 15–35% but can extend up to 75% when it prevails with other soil-borne root rot diseases (Black Shank) caused by *Phytophthora nicotianae*. In the regions of mono-cropping and high yield losses, range between 50 and 60%, and in the case of a severe outbreak, they can reach 100% (Cai et al. 2021). Long-term continuous mono-cropping has resulted in an epidemic form of tobacco bacterial wilt disease, and makes it difficult to control its incidence (Chen et al. 2020).

In recent years, many integrated disease management practices in the form of cultural control, crop rotation, resistant cultivars, bioorganic fertilizers, and chemical control have been adopted to control bacterial wilt disease (Qi et al. 2020). Crop rotation, the use of cover crops, soil amendments with biochar and organic matter were found to be practical approaches to successfully manage many soilborne diseases through breaking the pathogen life cycle (Fan et al. 2020). The amendment of biochar in the soil improves soil health, functional diversity of rhizosphere microorganisms, mitigates the pathogen load, and reduces the incidence of tobacco bacterial wilt disease (Li et al. 2021).

Nowadays, biocontrol via disease suppressive potential strains, i.e., *Bacillus* sp., *Pseudomonas* sp., and *Lysobacter* sp., are considered to be an efficient management strategies against soilborne diseases. These biocontrol agents produce special antimicrobial compounds (Wei et al. 2021) and are used as microbial consortia to alter the soil microbial diversity (Zhang et al. 2020). Previous studies suggested that rhizobacteria and endophytic fungi such as *Bacillus* sp. (Wu et al. 2020), *Pseudomonas* sp. (Zhuo et al. 2019), and *Trichoderma harzianum* (Jogaiah et al. 2013) have strong plant growth promotion and biocontrol activity against many phytopathogens. Similarly, *P. aeruginosa* NXHG29 efficiently control the incidence of tobacco bacterial wilt and black shank disease through mechanisms of direct antagonism and niche exclusion (Ma et al. 2018). The present study aimed to develop safe and potential microbial biocontrol strategies by screening *Bacillus* sp. strains from the rhizosphere soil of healthy tobacco plants to manage pathogen causes flue-cured tobacco bacterial wilt disease. Additionally, the plant-beneficial traits of candidate strains related to disease suppression and plant growth promotion were characterized in *in-vitro* and *in-vivo* assays.

Methods

**Bacterial strains, culture media, and growth conditions**

In this study, *Ralstonia solanacearum* WS-001 (Accession No. MW730714) was isolated from the tissues of tobacco plants infected with bacterial wilt disease and *Bacillus* strains were isolated from the rhizosphere soil samples of healthy tobacco plants collected from Wenshan (23° 36’, 104° 24’ E), Yunnan Province, China. A colony showing the typical morphology (Additional file 1: Fig. S1) of *R. solanacearum* on Kelman’s tetrazolium chloride (TTC) agar medium was picked and grown on Casamino acid-Peptone-Glucose (CPG) medium (Casein hydrolysate 1 g/L; Peptone 10 g/L; Glucose 5 g/L; and pH 7.0) and incubated at 28 °C for 48 h (Kelman 1954). Antagonistic *Bacillus* strains were isolated on Luria–Bertani (LB) medium (Bacto tryptone 10 g/L; Yeast extract 5 g/L; NaCl 10 g/L; Agar 18 g/L and pH 7.0) based on the colony morphology as described by De Vos et al. (2009). Pure cultures of bacterial strains were stored at −80 °C in 50% glycerol (v/v) for future use.

**Pathogenicity test**

A pathogenicity assay was performed to confirm the virulence of isolated strain WS-001 and to fulfill Koch’s postulates by adopting the methodology of Yuan et al. (2014). For the pathogenicity assay, 30 days old seedlings of flue-cured tobacco cultivar Hongda (highly susceptible) and K326 (resistant) were uprooted and washed with sterilized distilled water to remove the
soil and slightly injured with a needle. The seedlings were placed in a 100 mL suspension of WS-001 (1 × 10^7 CFU/mL) incubated at 160 rpm and 28 °C for 30 min and then transplanted into pots containing a mixture of double sterilized peat and soil (1:3). In comparison, the control plants were treated with the same volume of CPG broth, and symptoms were observed as described by Yuan et al. (2014).

**In-vitro screening test for antimicrobial activity**

The antibacterial activity of isolated strains against *R. solanacearum* WS-001 was determined on LB medium plates using the disc-diffusion technique (Li et al. 2014). For the screening test, *R. solanacearum* WS-001 and isolated bacterial strains were cultured overnight at 28 °C and 160 rpm in CPG broth and LB broth, respectively; and adjusted to an optical density of OD_{600 nm} ≈ 0.5 (1 × 10^7 CFU/mL) using a spectrophotometer (GE Uitrospec 2100 pro) (Zhang et al. 2020). Briefly, ≈150 μL aliquot culture of the pathogenic bacterium was spread on LB medium plates and dried for 2–3 min. A sterilized filter paper disc (0.5 cm) was taken and placed in the middle of the LB medium plates. Subsequently, 10 μL aliquot culture from each isolated strain (1 × 10^7 CFU/mL) and sterilized distilled water (control) were punched on the filter paper disc and incubated at 28 °C for 48 h to observe the growth inhibition. The isolated strains with an inhibition zone diameter (≥ 1.5 cm) were selected as biocontrol strains for further study. The antibacterial activity was expressed as a growth inhibition ratio (GIR) using the following formula (Li et al. 2014): GIR (%) = [(Inhibition zone diameter − Colony diameter)/Inhibition zone diameter] × 100. Antifungal activity of isolated strain WS-10 was determined by a dual culture technique on potato dextrose agar medium plates as previously described by Cui et al. (2019).

**Raising of nursery**

Seeds of flue-cured tobacco (*Nicotiana tabacum* L.) cultivar Yun87 were provided by the College of Tobacco Sciences, Yunnan Agricultural University, Kunming, China. The nursery was grown in floating foam polystyrene trays (162 wells) in the greenhouse 45–60 days before use according to the method of Dai et al. (2009). The seeds were sown in a mixed nursery medium composed of perlite, vermiculite, and turf at a ratio of 3:3:4. One to two seeds of the flue-cured tobacco cultivar Yun87 were placed in each well and slightly covered with medium.

**In-vivo assay for biocontrol activity of candidate Bacillus strains**

A pot experiment was conducted in the greenhouse of Yunnan Agricultural University, Kunming, China, to evaluate the biocontrol effect of three selected *Bacillus* strains against tobacco bacterial wilt disease as described by Yuan et al. (2014). Seedlings (35 days old) of tobacco cultivar Yun87 were transplanted into pots (40 × 35 cm) containing 10 kg of disease-free red soil. In addition, fertilizer was applied to overcome the nutrient deficiency (Additional file 1: Table S1). Tobacco seedlings were inoculated with pathogenic strain WS-001 (1 × 10^7 CFU/mL) and biocontrol *Bacillus* strains (1 × 10^7 CFU/mL) 50 mL/pot respectively, through the root drenching method by slightly injuring the roots one day after transplantation. The experiment was carried out in a completely randomized design with ten plants in each treatment and each treatment was repeated thrice. The greenhouse conditions were maintained as a day/night temperature (28/20 °C) with a 14 h light and 10 h dark photosynthesis period. The incidence of tobacco bacterial wilt disease was graded according to GB/T 23222-2008 (National Standardization Management Committee, China. 2009). Bacterial wilt symptoms were observed with the interval of 5 days after post-inoculation and evaluated using a disease rating scale as described by Cai et al. (2021). The disease index (DI) and protective value (PV) were calculated using the following formula: DI (%) = (Σ (Disease index × Number of diseased plants in this index))/(Highest disease index × Total number of plants investigated) × 100 and PV = [(DI_{ck} − DI)/DI_{ck}] × 100. Here: DI_{ck}; disease index of the control group, DI_{ck}; disease index of treatment.

**Molecular characterization of potential biocontrol and pathogen strains**

Total genomic DNA of isolated strains WS-001 and WS-10 were extracted using a TIANamp Bacteria DNA isolation kit (TIANGEN®) according to the manufacturer's instructions. Molecular identification of isolated strains was made by the PCR amplification of the 16S rRNA gene (Zhang et al. 2020). The PCR amplification conditions for the 16S rRNA gene were as follow: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 60 s, and a final extension at 72 °C for 7 min.

Multilocus sequence analysis (MLSA) was performed for the high-resolution phylogenetic relationship of species within a genus (Huang et al. 2017). For sequence alignment, MLSA for 2 housekeeping genes rpoB and gyrB of *Bacillus* strain WS-10; egl and mutS of WS-001.
were performed using ClustalW. The primers used for 16S rRNA, rpoB, gryB, egl, and mutS genes are shown in Table 1. The PCR amplification conditions for rpoB, gryB, egl, and mutS genes were similar with 16S rRNA except for annealing (Table 1). The PCR amplification products were then sent to the company (TSINGKE Co. Ltd Beijing, China) for sequencing, and the obtained sequences were analyzed online using the BlastN program (http://www.ncbi.nlm.nih.gov/BLAST). A phylogenetic tree was constructed with the neighbor-joining method using MEGAX software (Kumar et al. 2016).

In-vitro assay for plant growth-promoting traits of the biocontrol agent

Phosphate solubilization

Pikovskaya (PVK) medium (Glucose 10 g/L, Ca₃(PO₄)₂ 5 g/L, NaCl 0.2 g/L, (NH₄)₂SO₄ 0.5 g/L, MgSO₄·7H₂O 0.1 g/L, KCl 0.2 g/L, Yeast extract 0.5 g/L, MnSO₄ 2 mg/L, FeSO₄·7H₂O 2 mg/L, Bromphenol blue 25 mg/L, Agar 20 g/L, and pH 7.2) was used to determine the phosphate solubilizing activity of Bacillus strain WS-10 (Jeon et al. 2003). Briefly, Bacillus strain WS-10 was cultured on PVK medium plates and incubated at 28 °C for 7 days. The growth was associated with using inorganic phosphate in Ca₃(PO₄)₂ as a sole phosphate source which was determined as a clear zone around the bacterial colony.

Potassium solubilization

Potassium feldspar (PF) solid medium (Sucrose 10 g/L, MgSO₄·7H₂O 0.5 g/L, (NH₄)₂SO₄ 0.2 g/L, NaCl 0.1 g/L, CaCO₃ 0.1 g/L, Potassium feldspar 5.0 g/L, Bromophenol blue 25 mg/L, Agar 20 g/L, and pH 7.3) (Wang et al. 2018), incubated at 28 °C for 7 days and a circle was observed around the bacterial colonies.

Nitrogen fixation

To determine the nitrogen fixation ability, Bacillus strain WS-10 was grown on nitrogen-free (NF) Ashby medium (Glucose 5 g/L, Mannitol 5 g/L, CaCl₂·2H₂O 0.1 g/L, MgSO₄·7H₂O 0.1 g/L, Na₂MoO₄·2H₂O 5 mg/L, K₂HPO₄ 0.9 g/L, KH₄PO₄ 0.1 g/L, FeSO₄·7H₂O 0.01 g/L, CaCO₃ 5 g/L, Bromophenol blue 25 mg/L, Agar 20 g/L, and pH 7.2) (Zhang and Kong 2014). Bacillus strain WS-10 was grown on PF medium plates, incubated at 28 °C for 7 days, and a clear zone around the bacterial colonies was observed.

Indole-3-acetic acid production

Salkowsk reagent was used to determine the indole-3-acetic acid (IAA) production ability of Bacillus strain WS-10 (Abdallah et al. 2018). Briefly, the isolated strain was grown in a 200 mL Landy medium without L-tryptophan, incubated at 28 °C and 160 rpm for 60 h. Cell-free supernatants were collected by centrifugation at 10,000 rpm for 10 min, and 1.5 mL supernatants were added with 1.5 mL of Salkowski reagent and incubated for 30 min in the dark at room temperature to observe color variation. In contrast, the same volume of Landy medium was added in the Salkowski reagent as a control.

In-vitro co-culture assay

In-vitro co-culture assay was performed according to the method of Cui et al. (2019) to confirm the antagonistic activity of Bacillus strain WS-10 against the pathogenic bacterium R. solanacearum WS-001. For the co-culture assay, overnight cultures of Bacillus strain WS-10 and R. solanacearum WS-001 were prepared in LB (broth) and CPG (broth), respectively, and adjusted to an optical density of OD₆₀₀ nm ≈ 0.5 (1 × 10⁷ CFU/mL). In brief, WS-10 (25 mL) culture was used to initiate the co-culture with WS-001 (25 mL) in CPG liquid medium, incubated at 160 rpm and 28 °C for 12 h, while the same volume (50 mL) of WS-001 cultured in CPG broth was used as a control. Serial dilutions (up to 10 folds) of co-culture were made, and CFU/mL of co-cultured strains were recorded separately based on their distinct colony morphologies on the LB and TTC medium.

Greenhouse assay

A greenhouse experiment was conducted during the growing season in 2020 Bofeng County, Jinning
(24° 40’ N, 102° 35’ E), Yunnan Province, China, to understand the biocontrol effect of candidate *Bacillus* strain WS-10 on tobacco bacterial wilt disease. Infected free red soil was collected from the mountains, and tobacco was grown for the first time in the soil (no other crop had been grown before). Tobacco seedlings of cultivar Yun87 (45 days old) were transplanted in the pot (40 × 35 cm) containing 13 kg of soil and 1 kg peat. In addition, fertilizer was also applied in each pot as a base (before plantation) and top fertilizer (20 days after transplantation) to overcome the nutrient deficiency (Additional file 1: Table S1), and greenhouse conditions were maintained as mentioned above.

One month after transplantation, when tobacco plants reached 5–6 leaves stage, *R. solanacearum* WS-001 (1 × 10^7 CFU/mL) and biocontrol agent WS-10 (1 × 10^7 CFU/mL) culture were prepared and inoculated via root drenching method. The experiment was performed under 4 conditions: CK; [control; application of *R. solanacearum* WS-001 (100 mL/pot)], T1; [combined application of *R. solanacearum* WS-001 (100 mL/pot) and biocontrol strain WS-10 (100 mL/pot)], T2; [first application of WS-001 (100 mL/pot), then 3 days later application of WS-10 (100 mL/pot)], T3; [first application of WS-10 (100 mL/pot), then 3 days later application of WS-001 (100 mL/pot)]. Bac- terial wilt symptoms were observed once a week after post-inoculation to the end of the experiment in each treatment using a disease rating scale to evaluate the disease index and protective value as described above in “In-vivo assay for biocontrol activity of candidate *Bacillus* strains” section (Cai et al. 2021). The experiment was conducted under a completely randomized design with 15 plants in each treatment and each treatment was repeated thrice.

**Analysis of soil physicochemical properties**

Soil samples were collected in replicates from each treatment at the end of the experiment using the root shaking method as described by Cai et al. (2021). Bulk soil samples collected for physicochemical analysis were stored at room temperature, while rhizosphere soil samples collected for functional diversity and diversity indices analysis of rhizosphere microorganisms were stored at 4 °C. The alkali-hydrolysable nitrogen method was used to measure the available nitrogen (mg/kg). Soil organic matter (g/kg), available phosphorus (mg/kg), and available potassium (mg/kg) were determined by acidified potassium dichromate (K₂Cr₂O₇—H₂SO₄) heating method, 0.5 mol/L NaHCO₃ solutions (pH 8.5), and CH₃COONH₄ extraction method as described by Cai et al. (2021), and pH was determined using pH meter.

**Determination of functional diversity and diversity indices of soil microbial community**

The functional diversity of a rhizospheric microbial community was analyzed using the Biolog EcoPlate™ method following the manufactures instructions. The metabolic activity of the rhizospheric microbial community on Biolog EcoPlate™ was recorded in the form of average well color development (AWCD) and the utilization ability of 6 types of carbon sources (carbohydrates, amino acids, polymers, amines, carboxylic acid, and phenolic acids) as described by Cai et al. (2021). The following formulas were used to calculate the AWCD and diversity indices [Shannon index (H) and McIntosh index (U)]:

\[
AWCD = \frac{\sum (C_i - R)}{31};
\]

\[
H = - \sum P_i \ln P_i; \quad \text{McIntosh};
\]

\[
U = \sqrt{\sum n_i^2}
\]

Here Ci represents the absorbance value of the i-th carbon source, R represents the absorbance value with water holes, Pi represents the comparison between ni and the sum of the relative absorbance values of the entire plate, and ni represents the relative absorbance value of the i-th hole.

**Assessment of tobacco plant dry matter**

The dry matter content of tobacco plant parts (roots, stem, and leaves) was determined in each treatment at the end of the experiment. Briefly, 5 tobacco plants per treatment were uprooted, washed to remove extra soil, and air-dried naturally in the shade. The roots, stems, and leaves were separated and incubated at 105 °C for 30 min and then dried to constant weight at 80 °C for 48 h to measure the contents of the dry matter.

**Statistical analysis**

Data were statistically analyzed using analysis of variances in Microsoft ExcelTM (2013) and SPSS version 22.0 (SPSS Inc., Chicago, IL, USA); the means were subjected to Duncan’s multiple range tests at \( P \leq 0.05 \). All figures were processed and analyzed using Adobe Illustrator CS5 (Adobe Systems Inc., San Francisco, CA, USA) and GraphPad Prism (8.0.2).

**Results**

**Isolation of pathogenic bacterial strain**

The pathogen was successfully isolated from the diseased tobacco plant parts. A differentiation test was performed on the TTC medium to identify virulent and avirulent strains of *R. solanacearum*. Virulent strains produce fluid-like white color colonies with red or pink center,
while colonies produced by avirulent type were smaller, dry, and non-fluidal after 48 h of incubation (Additional file 1: Fig. S1).

Pathogenicity analysis
Based on the molecular identification of the isolated strain WS-001, a pathogenicity test was performed according to Koch’s postulates using the flue-cured tobacco cultivar Hongda and K326 (Additional file 1: Fig. S2). Plants of flue-cured tobacco cultivar (Hongda) treated with R. solanacearum WS-001 generally showed symptoms of yellowing and unilateral wilting of leaves with marginal necrosis, dark water-soaked lesions on the stem (Additional file 1: Fig. S2-i), and death of the whole plant, while control plants remained healthy. Whereas, no visible symptoms were observed on the plants of flue-cured tobacco cultivar (K326), except stunting plant growth (Additional file 1: Fig. S2-ii). The stem streaming test proved that R. solanacearum WS-001 was successfully colonized in the vascular tissue and produced high cell densities. A large number of milky-white bacteria oozed out from the infected stem when placed in the water, while no bacteria oozed from the healthy stem (Additional file 1: Fig. S3).

Isolation, identification, and functional traits of antagonistic bacteria
A total of 250 bacterial strains were isolated from soil collected from the rhizosphere of healthy tobacco plants. They produced rounded white-colored mucus colonies on LB medium with a rough to dry appearance (Additional file 1: Fig. S4). Among these isolated strains, 23 isolates (9.2%) showed their antibacterial activity against the R. solanacearum WS-001 bacterial strain at 1 × 10^7 cfu/mL on LB agar plates (data not shown). In the second round, the antibacterial activity of these 23 isolates was screened against R. solanacearum WS-001 at a higher concentration of OD_{600 nm} ≈ 0.6 (3 × 10^8 CFU/mL). Fourteen (60.86%) of these 23 isolates showed strong antibacterial activity with an inhibition zone diameter of about 1.5 to 2.86 cm (Table 2). From the 14 isolates, a subset of 3 Bacillus strains WS-05, WS-10, and WS-25 were found to be the most superior with effective reduction of pathogen growth in the in-vitro antibacterial assay and were retained for further study (Fig. 1).

Assessment of biocontrol effect of candidate Bacillus strains on tobacco bacterial wilt
A pot experiment was conducted to evaluate the efficacy of biocontrol agents (WS-05, WS-10, and WS-25) against R. solanacearum WS-001 (Fig. 2). Bacterial wilt symptoms were observed 3 days after the inoculation of pathogenic strain WS-001, and the whole plant died after 4 weeks of inoculation (Fig. 2-i; CK). However, a significant difference was observed in the biocontrol efficacy of each isolated strain WS-05, WS-10, and WS-25 compared with control (Fig. 2-i; A–C). Furthermore, plants inoculated with WS-10 showed minimum DI and maximum PV compared with plants inoculated with WS-05 and WS-25 (Fig. 2-ii). Therefore, WS-10 was selected as a potential biocontrol agent against tobacco bacterial wilt disease for further study.

Antifungal activity
Results of the in-vivo assay revealed WS-10 as a potential biocontrol agent compared with other isolated strains WS-05 and WS-25 (“Assessment of biocontrol effect of candidate Bacillus strains on tobacco bacterial wilt” section). Then the antifungal activity of selected strain WS-10 was checked against many pathogenic fungi such as; Fusarium oxysporum caused root rot of Panax notoginseng, F. graminearum caused Fusarium head blight in wheat and barley, E. oxysporum caused Fusarium wilt of Dendrobium chrysotoxum, and Colletotrichum capsici caused pepper anthracnose (Fig. 3).

| Isolated strains | Inhibition zone diameter (cm) | Colonies diameter (cm) | Growth inhibition ratio (%) |
|------------------|------------------------------|------------------------|-----------------------------|
| WS-10            | 2.86 ± 0.04                  | 0.7 ± 0.057            | 75.57 ± 0.644               |
| WS-25            | 2.26 ± 0.03                  | 0.9 ± 0.068            | 57.54 ± 1.164               |
| WS-05            | 2.89 ± 0.05                  | 1.33 ± 0.044           | 51.30 ± 1.380               |
| WS-18            | 2.54 ± 0.06                  | 1.23 ± 0.057           | 55.20 ± 0.557               |
| WS-61            | 2.82 ± 0.03                  | 1.42 ± 0.068           | 49.63 ± 1.170               |
| WS-115           | 2.16 ± 0.04                  | 1.14 ± 0.093           | 47.10 ± 2.364               |
| WS-47            | 2.56 ± 0.04                  | 1.28 ± 0.068           | 50.00 ± 0.771               |
| WS-133           | 1.84 ± 0.05                  | 1.00 ± 0.100           | 45.64 ± 1.883               |
| WS-96            | 2.68 ± 0.11                  | 1.44 ± 0.093           | 45.62 ± 3.102               |
| WS-117           | 2.32 ± 0.09                  | 1.28 ± 0.106           | 44.77 ± 0.542               |
| WS-87            | 1.8 ± 0.06                   | 1.06 ± 0.073           | 40.81 ± 2.151               |
| WS-151           | 2.32 ± 0.08                  | 1.44 ± 0.093           | 37.67 ± 1.996               |
| WS-196           | 1.6 ± 0.02                   | 0.98 ± 0.089           | 38.82 ± 2.262               |
| 06               | 2.56 ± 0.07                  | 1.64 ± 0.093           | 35.36 ± 2.435               |

Significance difference (P < 0.05) between growth inhibitions ratios (%) of different isolated strains are indicated by different small letters within a column according to Duncan’s multiple range test at P < 0.05 of five replicates (± SEM)

A Growth inhibition ratio (%) = (Inhibition zone diameter – Colony diameter)/Inhibition zone diameter × 100
Molecular characterization and identification of pathogenic and biocontrol bacterial strains

Multilocus sequence analysis was performed for 3 housekeeping genes 16S rRNA (1465 bp), egl (850 bp), and mutS (750 bp) of pathogenic strain WS-001 and 16S rRNA (1453 bp), rpoB (1089 bp), and gyrB (1173 bp) of biocontrol strain WS-10 for the high-resolution phylogenetic relationship of species within a genus. MLSA for 3 housekeeping genes revealed that isolated strains WS-001 and WS-10 were identified as *R. solanacearum* (Accession No. MW730714) and *B. amyloliquefaciens* (Accession No. MW730713) with 99% similarity (Fig. 4 and Additional file 1: Fig. S5).

Characterization of plant-beneficial traits of WS-10 *in-vitro* assay

Several traits of *B. amyloliquefaciens* WS-10, as plant growth promoters, were tested in the *in-vitro* assay (Fig. 5). After 7 days of incubation at 28 °C, visible dissolusion and clear halos were formed around WS-10 colonies grown on NF (Fig. 5A), PVK (Fig. 5B), and PF (Fig. 5C) solid medium, which indicated that WS-10 had the ability to utilize air-nitrogen and decompose rock phosphate and potassium. Salkowski reagent produced the orange-red color, which indicated the biosynthesis of Indole-3-acetic acid (Additional file 1: Fig. S6).

In-vitro co-culture assay

*In-vitro* co-culture test was performed for isolated strain WS-10 to evaluate the growth suppression ability against *R. solanacearum* WS-001 in LB and TTC medium, respectively. The colony count method was adopted based on their distinct colony morphologies WS-10 (rounded white-colored mucus) and *R. solanacearum* WS-001 (white-colored colonies with a red center) to quantify the growth of each isolate. This study revealed that the population dynamic of strain WS-10 was recorded 10^8 CFU/mL in co-culture assay compared with *R. solanacearum* WS-001 (10^3 CFU/mL). In contrast, strain WS-001 showed 10^10 CFU/mL when cultured alone (Additional file 1: Fig. S7).

Soil physicochemical characteristics

This study revealed that compared with control (application of WS-001), the application of biocontrol *Bacillus*
strain WS-10 significantly affects the soil physicochemical properties associated with tobacco plants (Table 3).

A significant difference was observed in the availability of soil pH, organic matter, total nitrogen, and available...
phosphorus and potassium contents in the soil treated with WS-10 compared with control.

**Figure 5** In-vitro plant growth-promoting traits of biocontrol agent WS-10. Nitrogen fixation (A), Phosphorus solubilization (B), Potassium solubilization (C)

**Table 3** Analysis of soil physicochemical properties

| Items     | pH       | OM (g/kg)    | T.N (mg/kg) | A.P (mg/kg) | A.K (mg/kg) |
|-----------|----------|--------------|-------------|-------------|-------------|
| CK        | 6.63±0.06c | 15.3±0.26c   | 2.47±0.30c  | 16.3±0.88d  | 115.35±1.24c|
| T1        | 6.89±0.02b | 17.2±0.07b   | 3.14±0.15a  | 26.9±1.90a  | 128.15±4.65a|
| T2        | 6.82±0.06bc| 16.9±0.41bc  | 2.95±0.27b  | 19.7±0.43c  | 121.86±3.84b|
| T3        | 7.31±0.12a | 19.9±0.14a   | 3.21±0.19a  | 24.1±1.75b  | 129.18±4.21a|

Here OM organic matter, TN total nitrogen, AP available phosphorus, AK available potassium

Different letters in the same column meant significant differences among treatments according to Duncan’s multiple range test at P<0.05

**Fig. 6** Average well color development (index) of soil microbial diversity under different treatments after particular hours of incubation. Significant difference among the treatments is shown by Duncan’s multiple range test at P<0.05

**Average well color development of rhizospheric microbial community**

This study showed that the AWCD of the rhizospheric microbial community increased with the application of *Bacillus* strain WS-10. A significant difference was observed in the AWCD of the rhizospheric microbial community treated with WS-10 compared with control (Fig. 6). The AWCD of T3 was found to be the highest than T1, T2, and CK; however, a non-significant difference was observed among the AWCD values of T1 and T3.

**Utilization ability of six types of carbon sources by rhizospheric microbial community**

The metabolic activity of the rhizospheric microbial community was determined according to differences in the utilization ability of 6 types of carbon sources in Biolog EcoPlate™. This study showed that the utilization ability of six types of carbon sources (Carbohydrates, Amino acids, Polymers, Amines, Carboxylic acid, and Phenolic acids) increased in all treatments treated with biocontrol *Bacillus* strain WS-10 than control (Table 4). However, the utilization ability of T1 and T2 remained almost the same, and a non-significant difference was observed between them.
The functional diversity of the rhizospheric microbial community significantly increased in all treatments treated with biocontrol Bacillus strain WS-10 compared with control (Table 5). The species evenness index (McIntosh index) and species richness index (Shannon index) were increased in all treatments after the application of WS-10 than control. However, a non-significant difference was observed between T1 and T2.

Biocontrol efficacy of Bacillus strain WS-10 against tobacco bacterial wilt disease

The result revealed that Bacillus strain WS-10 effectively reduce the tobacco bacterial wilt disease (Fig. 7). Tobacco bacterial wilt symptoms were first observed after 49 days (at the vigorous growing stage) of transplantation in control, whereas they emerged in treatments T1, T2, and T3 after 70 days, 56 days, and 77 days of transplantation, respectively. These results indicated that the biocontrol strain WS-10 delayed the development of tobacco bacterial wilt disease by 7 to 28 days. Significant differences (P < 0.05) in the occurrence of tobacco bacterial wilt disease between the treatments treated with Bacillus strain WS-10 and control were observed after 49, 56, 63, 70, 77, 84, and 91 days of transplantation. At the end of the experiment, it was observed that the application of antibacterial strain WS-10 successfully reduced the incidence.
of tobacco bacterial wilt disease by 73.36, 43.82, and 86.82% in T1, T2, and T3, respectively (Table 6).

Accumulation of dry matter contents
This study revealed that the accumulation of dry matter contents in different parts (roots, stems, and leaves) of tobacco plants increased in all treatments after the application of Bacillus strain WS-10 than the control (Fig. 8). These results indicated that WS-10 had a strong plant growth promoter ability and increased the dry matter contents of tobacco plants.

Discussion
Tobacco bacterial wilt caused by R. solanacearum is one of the most devastating tobacco diseases resulting in unprecedented loss worldwide. The notoriously damaging pathogen persists in soil for a longer time, making disease management nearly an unachievable goal (Yuan et al. 2016). Currently, antimicrobial compounds derived from bacteria and chemical pesticides (e.g., zinc thiazole, bismuthazol, and saisentong) are the only measures used to control the disease, but they are ineffective (Chen et al. 2020). Because the improper use of agrochemicals posed a significant risk to the environment, public health, and evolved pathogen resistance. Thus, it is necessary to shift from chemical-based treatments to nature-based cures, where biocontrol offers an environmentally friendly alternative for more promising disease management (Ahmed et al. 2022).

Plant growth-promoting rhizobacteria improve plant health and represent a great source of biocontrol against various phytopathogens. Pseudomonas sp., Bacillus sp., Streptomyces sp., and other bacterial species have shown efficient biocontrol against bacterial wilt (Zhuo et al. 2019). In the present study, a total of 250 strains were isolated from the rhizosphere of healthy tobacco plants, out of which 14 isolates showed significant antibacterial activity against R. solanacearum (WS-001) in a dual culture assay. Among the 14 isolates, 3 isolates WS-05, WS-10, and WS-25 were found to be superior strains as they significantly inhibited the growth of pathogenic strain WS-001 in the in-vitro antibacterial assay and were selected as candidate strains for initial evaluation of biocontrol efficiency. The percentage representing 1.2% of the 250 isolated strains is consistent with the reported percentage (0.1–1%) of antibiotic-producing Bacillus spp. in nature (He et al. 2021). Among the 3 isolated strains, only WS-10 was selected and retained as a potential biocontrol agent because of its efficient protective value (>80%) against tobacco bacterial wilt disease in the pot experiment. Further, antifungal activity results revealed that WS-10 inhibited the growth of different pathogenic fungi in in-vitro conditions, which makes it a strong antagonistic agent with dual antimicrobial characteristics. The molecular identification based on 16S rRNA, rpoB, and gyrB revealed that WS-10 belongs to Bacillus amyloliquefaciens.

Many previous studies have reported that the application of biocontrol agents (PGPR) in the soil could reduce the incidence of soilborne diseases, improve yields, and biomass in many plants (Liu et al. 2013). Obtained results are similar to the above findings; biocontrol agents WS-10 promote the tobacco plant’s growth and dry matter contents in different parts (roots, stem, and leaves) of tobacco plants. Growth promotion in tobacco plants relates to the in-vitro production of IAA (μg/mL) (one of the pivotal phytohormones involved in cell expansion, division, and differentiation), nitrogen fixation, and potassium and phosphate solubilization. Similarly, the application of a biocontrol agent (WS-10) improved the soil physicochemical properties and functional diversity of rhizosphere microorganisms, which directly promotes plant growth and enhances plant resistance to disease; and obtained results are similar to the findings of Tang et al. (2020).

The biocontrol aspect of plant growth-promoting rhizobacteria (PGPR) has recently gained attention for sustainable agriculture. In the present study, WS-10 performed dual roles as a PGPR and a biocontrol agent against the bacterial wilt pathogen. It is evident from the experiment conducted under greenhouse conditions that WS-10 remarkably reduced the disease incidence of WS-001 in tobacco plants. Corroborating the in-vitro antagonism between WS-10 and WS-001 as shown in co-culture assays, WS-10 could directly antagonize pathogens by producing antimicrobial compounds. Bacillus sp.
is known to produce a plethora of bioactive compounds that directly inhibit plant pathogens. Especially, a large part of their genome encodes several bioactive compounds such as antibiotics, enzymes, and volatile compounds (Müller et al. 2020). It is reported that, like other phytopathogenic bacteria, R. solanacearum also required an entry site such as wounds to develop a systemic infection (Denny 2007). Obtained results of 2 different greenhouse assays demonstrated that when R. solanacearum was inoculated with root injury technique, the development of systemic infection was quick compared with the root drenching method (without causing an injury).

In addition to directly inhibiting pathogens, rhizobacteria also activate the plant’s immune system, a process termed induced systemic resistance or priming (Vannier et al. 2019). In the present study, when WS-10 treatment was applied to a plant before pathogen inoculation, the disease incidence was lowest (86.82%) than when both were applied at the same time or when the pathogen was applied before WS-10. It can be speculated that WS-10 induced resistance in tobacco when applied before the pathogen and activated plant defense responses so that the plant could limit the disease infection; thus, the disease incidence was lowest. On the other hand, when both WS-10 and pathogen were applied simultaneously (T1), the disease incidence was lower than when the pathogen was applied before WS-10 (T2). The disease incidence was higher in T2 because the pathogen had probably already developed an infection in tobacco plants. However, still, WS-10 was able to reduce the disease incidence by up to 43.82%. Thus, it was concluded that the application of WS-10 before pathogen attack proved more beneficial for plant growth and developed resistance in plants against the pathogen. The present results are in accordance with previous studies, that advanced application B. amyloliquefaciens YN201732 exhibited a better protective effect than therapeutic effect against tobacco powdery mildew (Jiao et al. 2020).

Soil microbial diversity plays an important role in the long-term sustainability of the soil ecosystem. Rich soil biodiversity can stabilize the soil ecosystem and enhance the function and biological activity of the soil (Chaer et al. 2009). In this study, analysis of diversity indices, AWCD, and utilization ability of carbon sources proved that the application of biocontrol agent WS-10 significantly improved the functional diversity and biological activity of the rhizosphere microbial community. Obtained results are similar to the findings of Tang et al. (2020), who proved that the application of YH-07 effectively suppresses the incidence of tomato Fusarium wilt by improving the soil functional of soil microorganisms.

Conclusions
Bacillus amyloliquefaciens WS-10 isolated from the rhizosphere of healthy tobacco plants showed remarkable PGP and biocontrol properties. Improve the soil physico-chemical properties and functional diversity of the soil microbial community, makes it a valuable tool for flue-cured tobacco cultivation. Furthermore, an in-planta study validated the biofertilizer and biocontrol potential of WS-10, whereas in-vitro antagonism showed a positive correlation with in-vivo disease suppression. Also, WS-10 performed more efficiently when applied earlier than pathogen attack, as prevention is better than cure. Thus, the study builds a foundation to develop WS-10 based bio-product with dual functions as both growth promoters and biocontrol agents for the tobacco plant. However, the actual mechanism underlying this intertwined interaction among plants, rhizobacteria, and pathogen warrants in-depth research. Further studies will focus on enhancing the control effect of B. amyloliquefaciens WS-10 on tobacco bacterial wilt disease through the extraction of antimicrobial compounds and by exploring the effect of B. amyloliquefaciens WS-10 on microbial diversity through high-throughput sequencing to understand the underlying biocontrol mechanism better.

Abbreviations
R. solanacearum: Ralstonia solanacearum; AWCD: Average well color development; TTC: Kelman’s tetrazolium chloride; CPG: Casamino acid-peptone-glucose; LB: Luria–Bertani; CFU: Colony forming units; CK: Control; IAA: Indole-3-acetic acid; MLSA: Multilocus sequence analysis; NF: Nitrogen-free Ashby medium; DI: Disease incidence; PV: Protective value; PGPR: Plant growth-promoting rhizobacteria; PGP: Plant growth-promoter; PF: Potassium feldspar; PV/K: Pikovskaya medium.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s41938-022-00527-5.

Additional file 1. Table S1 Amount of fertilizer applied per plant. Fig. S1 Colony morphology of isolated bacterial strain Ralstonia solanacearum WS-001 on TTC medium. Fig. S2 Symptoms produced by WS-001 on tobacco plant. Fig. S3 Stem streaming test. Fig. S4 Colony morphology of WS-10 on LB medium plates. Fig. S5 Phylogenetic tree constructed for molecular identification of WS-001 and WS-10. Fig. S6 Indole-3-acetic acid production ability of biocontrol agent WS-10. Fig. S7 Co-culture assay.

Acknowledgements
Not applicable.

Authors’ contributions
ZZ and CJ conceived and designed the experiments. WA, GH, JY, and QL performed the experiments. WA, JY, and QL analyzed the data. WA, SM, and AA wrote the manuscript. All authors contributed to the final draft of the manuscript. All authors read and approved the final manuscript.

Funding
This study was financially supported by the Yunnan Agricultural University Scientific Research Foundation (KKX900187), the Science & Technology Platform
Plan of Yunnan Province (2019K005), the National Key R&D Program of China (2019YFD1002000), and the Yunnan Ten Thousand Talents Plan Leading Talents of Industrial Technology Project of China (YNWR-CYYS-2019-046).

Available data and materials
This material is the author's own original work, which has not been previously published elsewhere and has no conflict of interest.

Declarations

Ethical approval and consent to participate
The paper reflects the author’s own research and analysis in a truthful and complete manner. All authors have been personally and actively involved in substantial work leading to the paper and contributed to preparing the final draft of the manuscript and will take public responsibility for its content.

Consent for publication
The manuscript has not been published in whole or in part elsewhere and is not currently being considered for publication in another journal. All the authors have seen the final version of the manuscript.

Competing interests
The authors declared that they have no conflict of interest.

Author details
1State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan Agricultural University, Kunming 650201, China. 2College of Resources and Environment, Yunnan Agricultural University, Kunming 650201, China. 3Key Laboratory of Agro-Biodiversity and Pest Management of Ministry of Education, Yunnan Agricultural University, Kunming 650201, China. 4College of Tobacco Science, Yunnan Agricultural University, Kunming 50201, China. 5College of Resources, Environment and Chemistry, Chuxiong Normal University, Chuxiong 675000, China.

Received: 11 August 2021 Accepted: 8 March 2022
Published online: 19 March 2022

References
Abdallah DB, Frikha-Gargouri O, Tounsi S (2018) Rhizospheric competence, plant growth promotion and biocontrol efficacy of Bacillus amyloliquefaciens subsp. plantarum strain 32a. Biol Control 124:61–67. https://doi.org/10.1016/j.biocontrol.2018.01.013
Ahmed W, Yang J, Tian Y, Munir S, Liu Q, Zhang J, Ji G, Zhao Z (2022) Ralstonia solanacearum, a deadly pathogen: revisiting the bacterial wilt biocontrol potential of the endophytic Bacillus amyloliquefaciens YN201732 against tobacco powdery mildew and its growth promotion. Biol Control 143:104160. https://doi.org/10.1016/j.biocontrol.2019.104160
Jogisha S, Abdelrahman M, Tran LP, Shin-ichi I (2013) Characterization of rhizosphere fungi that mediate resistance in tomato against bacterial wilt disease. J Exp Bot 64(12):3829–3842. https://doi.org/10.1093/jxb/ert212
Kelma A (1954) The relationship of pathogenicity of Pseudomonas solanacearum to colony appearance in a tetrazolium medium. Phytopathology 44(12):693–695
Kim BS, French E, Caldwell D, Harrington EJ, Iyer-Pascuaz AS (2016) Bacterial wilt disease: host resistance and pathogen virulence mechanisms. Physiol Mol Plant Pathol 95:37–43. https://doi.org/10.1016/j.pmpp.2016.02.007
Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7):1870–1874. https://doi.org/10.1093/molbev/msw054
Li L, Feng X, Tang M, Hao W, Han Y, Zhang G, Wan S (2014) Antibacterial activity of Lansiumamide B to tobacco bacterial wilt (Ralstonia solanacearum). Microbiol Res 169(7–8):522–526. https://doi.org/10.1016/j.micres.2013.12.003
Li C, Ahmed W, Li D, Yu L, Xu L, Xu T, Zhao Z (2021) Biochar suppresses bacterial wilt disease of flue-cured tobacco crops by improving soil health and functional diversity of rhizosphere microorganisms. Appl Soil Ecol 171:104314. https://doi.org/10.1016/j.apsoil.2021.104314
Liu Y, Shi J, Feng Y, Yang X, Li X, Shen Q (2013) Tobacco bacterial wilt can be biologically controlled by the application of antagonistic strains in combination with organic fertilizer. Biol Fertil Soils 49(4):447–464. https://doi.org/10.1007/s00374-012-0740-z
Ma L, Zhang HY, Zhou XK, Yang CG, Zheng SC, Duoj LO, Mo MH (2018) Biological control tobacco bacterial wilt and black shank and root colonization by bio-organic fertilizer containing bacterium Pseudomonas denitrificans NXHG29. Appl Soil Ecol 129:136–144. https://doi.org/10.1016/j.apsoil.2018.05.011
Müller P, Schwarz E, Dietel K, Junge H, Herfort S, Weydmann M, Lasch P, Cernea T, Berg G, Vater J (2020) Profiling for bioactive peptides and volatiles of plant growth promoting strains of the bacillus subtilis complex. Front Microbiol 11:1432. https://doi.org/10.3389/fmicb.2020.01452
Paret ML, de Silva AS, Cileay RA, Alvarez AM (2008) Ralstonia solanacearum race 4: risk assessment for edible ginger and floricultural ginger industries in Hawaii. HortTechnology 18(1):90–96
Paudel SD, Dobhal SD, Alvarez AM, Aref M (2020) Taxonomy and phylogenetic research on Ralstonia solanacearum species complex: a complex pathogen with extraordinary economic consequences. Pathogens 9(11):886. https://doi.org/10.3390/pathogens9110886
Qi G, Chen S, Ke L, Ma G, Zhao X (2020) Cover crops restore declining soil properties and suppress bacterial wilt by regulating rhizosphere bacterial
communities and improving soil nutrient contents. Microbiol Res 238:126505. https://doi.org/10.1016/j.micres.2020.126505

Singh N, Phukan T, Sharma PL, Kabyasheek K, Barman A, Kumar R, Sonti RV, Genni S, Ray SK (2018) An innovative root inoculation method to study Ralstonia solanacearum pathogenicity in tomato seedlings. Phytopathology 108(4):436–442. https://doi.org/10.1094/PHYTO-08-17-0291-R

Tang T, Sun X, Liu Q, Dong Y, Xiang Y (2020) Different effects of soil bacterial communities affected by biocontrol agent YH-07 on tomato Fusarium wilt inhibition. RSC Adv 10(58):34977–34985

Vannier N, Agler M, Hacquard S (2019) Microbiota-mediated disease resistance in plants. PLoS Pathogens 15(8):e1007740. https://doi.org/10.1371/journal.ppat.1007740

Wang W, Wu Z, He Y, Huang Y, Li X, Ye BC (2018) Plant growth promotion and alleviation of salinity stress in Capsicum annuum L. by Bacillus isolated from saline soil in Xinjiang. Ecotoxicol Environ Saf 164:520–529. https://doi.org/10.1016/j.ecoenv.2018.08.070

Wei L, Yang J, Ahmed W, Xiong X, Liu Q, Huang Q, Ji G (2021) Unraveling the association between metabolic changes in inter-genus and intra-genus bacteria to mitigate clubroot disease of Chinese cabbage. Agronomy 11(12):2424. https://doi.org/10.3390/agronomy11122424

Wu K, Yuan S, Wang L, Shi J, Zhao J, Shen B, Shen Q (2014) Effects of bioorganic fertilizer plus soil amendment on the control of tobacco bacterial wilt and composition of soil bacterial communities. Biol Fertil Soils 50(6):961–971. https://doi.org/10.1007/s00124-011-0301-3

Wu X, Li H, Wang Y, Zhang X (2020) Effects of bio-organic fertilizer fortified by Bacillus cereus QJ-1 on tobacco bacterial wilt control and soil quality improvement. Biocontrol Sci Technol 30(4):351–369. https://doi.org/10.1080/09583157.2020.1711870

Yuan S, Wang L, Wu K, Shi J, Wang M, Yang X, Shen Q, Shen B (2014) Evaluation of Bacillus-fortified organic fertilizer for controlling tobacco bacterial wilt in greenhouse and field experiments. Appl Soil Ecol 75:86–94. https://doi.org/10.1016/j.apsoil.2013.11.004

Yuan S, Li M, Fang Z, Liu Y, Shi W, Pan B, Wu K, Shi J, Shen B, Shen Q (2016) Biological control of tobacco bacterial wilt using Trichoderma harzianum amended bioorganic fertilizer and the arbuscular mycorrhizal fungi Glomus mosseae. Biol Control 92:164–171. https://doi.org/10.1016/j.biocntrol.2015.10.013

Zhang C, Kong F (2014) Isolation and identification of potassium-solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants. Appl Soil Ecol 82:18–25. https://doi.org/10.1016/j.apsoil.2014.05.002

Zhang J, Wei L, Yang J, Ahmed W, Wang Y, Fu L, Ji G (2020) Probiotic consortia: reshaping the rhizospheric microbiome and its role in suppressing root-rot disease of Panax notoginseng. Front Microbiol 11:701. https://doi.org/10.3389/fmicb.2020.00701

Zhuo T, Chen S, Fan X, Hu X, Zou H (2019) An improved control efficacy against tobacco bacterial wilt by an engineered Pseudomonas mosselii expressing the npAA gene from phytopathogenic Ralstonia solanacearum. bioRxiv. https://doi.org/10.1101/570628

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.