Biology and Fertility of Soils (2022) 58:553–563
https://doi.org/10.1007/s00374-022-01634-z

ORIGINAL PAPER

Potassium phosphite enhanced the suppressive capacity of the soil microbiome against the tomato pathogen *Ralstonia solanacearum*

Lv Su[1,2] · Haichao Feng[1] · Xingxia Mo[1] · Juan Sun[1] · Pengfei Qiu[1] · Yunpeng Liu[2] · Ruifu Zhang[1,2] · Eiko E. Kuramae[3,4] · Biao Shen[1] · Qirong Shen[1]

Received: 19 August 2021 / Revised: 7 March 2022 / Accepted: 8 March 2022 / Published online: 26 April 2022

Abstract

High-throughput sequencing, culture-dependent workflows, and microbiome transfer experiments reveal whether potassium phosphite (KP), an environmentally acceptable agricultural chemical, could specifically enrich the antagonistic bacterial community that inhibited the growth of the pathogen *Ralstonia solanacearum*. The application of KP enriched the potential antagonistic bacteria *Paenibacillus* and *Streptomyces* in soil, but depleted most dominant genera belonging to gram negative bacteria, such as *Pseudomonas*, *Massilia*, and *Flavobacterium* on day 7. Moreover, the KP-modulated soil microbiome suppressed *R. solanacearum* growth in soil. The predicted functions related to the synthesis of antagonistic substances, such as streptomycin, and the predicted functions related to tellurite resistance and nickel transport system were significantly enriched, but the synthesis of lipopolysaccharide (distinct component lipopolysaccharide in gram negative bacteria) were significantly depleted in the KP-treated soils. In addition, the copy numbers of specific sequences for *Streptomyces coelicoflavus* and *Paenibacillus favisporus* were significantly increased in the soil amended with KP, inhibited the growth of *R. solanacearum*, and had a higher tolerance of KP than *R. solanacearum*. Our study linked the application of fertilizers to the enrichment of antagonistic bacteria, which could support future work that aims to precisely regulate the soil microbiome to protect the host from infection by soil-borne pathogens.

Keywords Agrochemical · Antagonistic strains · Soil bacterial community · Soil-borne pathogen

Introduction

Bacterial wilt is a serious disease caused by *Ralstonia solanacearum*. This vascular pathogen causes large economic losses annually and leads to imbalanced bacterial communities. Once *R. solanacearum* successfully infects hosts, it is difficult to control bacterial wilt, and prevention is still the major strategy. Moreover, after harvest, the root residuals in the soil provide the niche and nutrition for *R. solanacearum* to facilitate the next seasonal invasion (Yadeta and Thomma 2013), and *R. solanacearum* abundances are essential for successful infection (Singh et al. 2018). Thus, decreasing the initial abundance of *R. solanacearum* may contribute to the prevention of bacterial wilt disease outbreaks, for example, through the use of fumigant chloropicrin (Zhang et al. 2017).

Agricultural chemicals are widely used in the control of soil-borne disease. The application of agricultural chemicals significantly affects the soil microbial community that may have important roles in plant growth and soil quality. (Meena et al. 2020). The application of inorganic germicide

The authors Lv Su and Haichao Feng contributed equally to this paper.

✉ Biao Shen
shenbiao@njau.edu.cn

1 Jiangsu Provincial Key Lab of Solid Organic Waste Utilization, Jiangsu Collaborative Innovation Center of Solid Organic Wastes, Educational Ministry Engineering Center of Resource-Saving Fertilizers, Nanjing Agricultural University, Nanjing 210095, Jiangsu, People's Republic of China

2 Key Laboratory of Agricultural Microbial Resources Collection and Preservation, Ministry of Agriculture and Rural Affairs, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, People's Republic of China

3 Microbial Ecology Department, Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands

4 Ecology and Biodiversity, Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

copper depleted populations of bacteria, cellulolytic fungal species, and *Streptomyces* in sandy soil (Kostov and Van Cleemput 2001). Moreover, the application of certain agricultural chemicals may enrich potential beneficial microbes in soil (Han et al. 2020; Sederholm et al. 2018; Wang et al. 2019). The relative abundances of the beneficial bacteria *Rhizobiales*, *Nitrosomonadaceae*, and *Bryobacter* and functional genes related to N metabolism, carbohydrate metabolism, and cell processes were enriched in soil amended with selenium (Liu et al. 2019). The application of chloropicrin fumigation increased the relative abundance of the potential antagonistic microbe Actinobacteria, which may result in an enhanced soil suppressive capacity against *R. solanacearum* (Zhang et al. 2017). However, it is generally unknown whether the enriched beneficial bacteria could inhibit the growth of pathogens and enhance the suppressive potential of the soil microbiome.

Phosphite is environmentally safe and inexpensive, has very low toxicity to animals, and is attracting increasing attention (Garbelotto et al. 2007). Several studies have shown that the application of phosphite stimulated citrus yields (Albrigo 1999) and increased the dry weights of cucumber plants (Mofidnakhaei et al. 2016). However, phosphite cannot provide available P for plants. It is likely that phosphite could be converted to phosphate in soil as a result of soil microbial activity (Metcalf and Wolfe 1998). Moreover, phosphite can trigger plant host defence to tolerate plant pathogen invasion (Costa et al. 2018; Felipini et al. 2016), directly inhibit the growth of pathogens, and pose a low risk of resistance development in pathogens, such as *Phytophthora* (Dempsey et al. 2018; Niere et al. 1994) and *R. solanacearum* (Norman et al. 2006). However, how phosphite reshapes the soil microbiome and the role of the phosphite-modulated soil microbiome in affecting soil-borne pathogens are not clear.

Here, we determined the effects of potassium phosphite (KP) on the composition of the soil bacterial community. Moreover, culture-dependent and microbiome transfer experiments were performed, with the aims of identifying the agricultural chemicals that could contribute to selective enrichment of antagonistic bacteria and uncovering the mechanism of *R. solanacearum* inhibition by the KP-modulated microbiome. We found that KP enriched the antagonistic bacteria, such as *Streptomyces coelicoflavus* and *Paenibacillus favisporus*, and enhanced the soil suppressive potential towards *R. solanacearum*.

Materials and methods

Ralstonia solanacearum (race 1, biovar 3, phylotype I, and sequevar 14) was isolated from bacterial wilt-diseased tomato plants and kindly provided by Dr. Wang (College of Resources and Environment, Nanjing Agricultural University); the KP solution was adjusted to a pH of 6.5 with potassium hydroxide; Luvisol soil (FAO) was collected from the upper soil layer (5–30 cm) of an open field covered with grass at the Experimental Base of Nanjing Agricultural University (32.01´N, 118.85´E). The soil properties are listed in the Supplementary methods (Additional file 1 Table S1).

Two other soils, Luvisol soil (FAO), from a grape plantation of Hebei Province (36.96′N, 115.39′E) were collected from the upper soil layer (5–30 cm) of an open field covered with grass (HN) and planted grape for two years (HG). All soils were air-dried and sieved (20 mesh). The Hebei soil was only used to count the numbers of culturable Actinobacteria on gauze number 1.

Effects of KP on the soil bacterial community

The experiment included one treatment (soil amended with KP) and a control (CK). Approximately 75 ml of sterilized water was added to 1.5 kg of dry soil from Nanjing to wet the soil. Next, 150 ml of an *R. solanacearum* cell suspension (cell density: 8×10^6 cfu ml^{-1}) was added to the wet soil and mixed (final cell concentration: 8×10^5 cfu g^{-1} soil). *R. solanacearum*, as a soil-borne pathogen, can use root residuals to multiply; thus, tomato root tissues from healthy plants were added to the soils to simulate field conditions. Approximately 15 g of dry root tissues cut into approximately 1-mm lengths was added to 1.5 kg of soil at a rate of 1.0% (w/w) (Lewis 1977). Then, the above soil was evenly divided into two parts. The soil treated with or without KP were the KP treatment and control, respectively. KP was applied at a concentration of 0.5% in soil (w/w) (Norman et al. 2006) (KP treatment). The soil moisture of both the KP and the control treatments was adjusted to 45% of the soil water holding capacity. Then, 750 g of soil was divided into 25 replicates consisting of 30 g of soil in a 50-ml centrifuge tube and incubated at 30 °C. The soils from the three replicates were randomly taken from KP treatment and control at days 7, 14, and 30 for soil DNA extraction and high-throughput sequencing (2 treatments × 3 replicates × 3 sample times = 18 samples).

The effects of KP on the numbers of culturable Actinobacteria were determined in two other soils (HN and HG). The same processes for the effects of KP on the microbial community of soil from Nanjing were performed. Three replicates from the two soils were randomly taken on day 7. One gram of the sampled soils was diluted and then spread on gauze No. 1 agar plates (5 g K_2HPO_4, 20 g soluble starch, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 1 g KNO_3, 0.5 g NaCl, 18 g agar). To inhibit the growth of fungi and other bacteria in No. 1 agar medium, 3.3 ml 3% $K_2Cr_2O_7$ was added. The plates were incubated for 5 days at 28 °C, and the numbers of all culturable Actinobacteria were determined.

DNA isolation and Illumina HiSeq of the soil DNA

Total soil DNA was extracted using the PowerSoil® DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The V4 hypervariable regions of the 16S rRNA gene were amplified using primers 515F (5-GTGCCAGCMGCCGCGGTAA-3) and 907R (5-CCGTCAATTCCTTTGAGTTT-3) (Biddle et al. 2008). Subsequently, 0.4 µl of the primers and approximately 10 ng of template DNA were analyzed via PCR with the following thermal cycling conditions: initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and extension at 72 °C for 60 s, with a final extension step at 72 °C for 5 min after the cycling was complete. The PCR products were detected by electrophoresis in a 1% (w/v) agarose gel and then purified with the GeneJET Gel Extraction Kit (Thermo Scientific). The purified PCR amplicons were sequenced using the Illumina HiSeq (250-bp paired-end reads) platform at Novogene Bioinformatics Technology Co., Ltd. (Beijing, China).

The sequencing data were mainly processed on the USEARCH platform (Edgar 2013). Briefly, sequences with a quality score lower than 0.5 or a length shorter than 200 bp and singletons were discarded. Noisy sequences were filtered, chimerism was inspected, and an OTU cutoff was assigned at the 97% identity level. Representative sequences for each OTU were selected and classified according to the RDP database for bacteria (cutoff = 80%). To correct for differences in the sequencing depth, the bacterial read counts were rarefied to the lowest number (28,606) of sequences present in each sample set.

Inhibition of *R. solanacearum* by the KP-modulated soil microbiome

A soil microbiome transfer experiment was performed to determine the function of the KP-modulated soil microbiome. The soil microbiome transfer experiment was performed based on a previous study (Li et al. 2019) with slight modifications. The same processes for determining the effects of KP on the soil microbial community were performed but without the addition of *R. solanacearum* to the soil from Nanjing. On day 7, 5 g of KP-treated soil and control soil were added separately to 45 ml of sterile water in a flask on a shaker at 200 rpm for 30 min followed by sonification for 1 min at 47 kHz twice with shaking for another 30 min. Next, the soil suspension of each treatment was filtered with sterile filter paper (15 µm pore size) to remove soil particles. To remove water-soluble nutrients/chemicals, such as KP, the filtrate was centrifuged at 3000 × g for 30 min, and the supernatant was discarded. The pelleted microorganisms were resuspended in 5 ml of sterile water.

Approximately 20 ml of sterilized water was added to 360 g of dry sterilized soil (2 × 99 min at 121 °C) to wet the soil; this soil was mixed with 9 ml of the *R. solanacearum* cell suspension (cell density at 8×10^7 cfu ml^{-1}) and 3.6 g of dry sterilized tomato root tissues (approximately 1-mm lengths) and then divided evenly into 2 parts. Then, 3 ml of the cell suspension extracted from the KP treatment and control soils was applied separately to 180 g of sterilized soil containing root tissues. Each 180-g sample of the above soil was divided into 6 replicates of 30 g each in 50-ml sterilized centrifuge tubes, referred to as the MK microbial community from the KP-treated soil (MK) or the microbial community from the control soil (MC) treatments, and then incubated at 30 °C. The soils from three replicates were randomly sampled on day 20 (Howard et al. 2017) for DNA extraction.

Bacterial isolation and Sanger sequencing of 16S rRNA genes

The sampled Nanjing soil at 7 days from the KP treatment was serially diluted and then spread on nutrient agar (NA) and gauze No. 1 agar plates. The plates were incubated for 3–5 days at 28 °C. Because our target microbes were dominant taxa in the results of high-throughput sequencing, 33 dominant colonies (numbers of similar morphologies > 5 in each plate) were selected. A loop of bacterial cells was added to 500 µl of water and incubated for 15 min at 95 °C. Next, the cells were cooled on ice for 1 min and centrifuged at 10,000 × *g* for 1 min to remove the cell debris. The supernatant of the cell lysate was used as a DNA template for the amplification of the 16S rRNA genes. Information on the detailed primers F27 and R1492 and the PCR steps are listed in Additional file 1 Table S2 and Table S3, respectively. Sanger sequencing was performed by Qin Ke Company (Nanjing, China). The sequences of these bacteria were classified against the 16S ribosomal RNA database using NCBI BLAST. The 16S rRNA gene sequences of the isolates were clustered to the OTUs at 100% sequence similarity in USEARCH.

Inhibition of *R. solanacearum* growth by *P. favisporus* and *S. coelicoflavus in vitro*

Approximately 2 µl of the *P. favisporus* Y7 cell suspension (cell density: 10^8 cfu ml^{-1}) and 5-mm-diameter agar plugs of *S. coelicoflavus* F13 grown in gauze No. 1 agar plates for 5 days were spotted onto the center of NA medium agar plates and incubated at 30 °C (strain Y7) and 28 °C (strain F13) for 3 days. Then, 200 µl of *R. solanacearum* (cell density: 10^7 cfu ml^{-1}) was sprayed onto the plates. After 48 h of incubation at 30 °C, the inhibition diameters of the two strains were measured with a ruler. Each treatment had three replicates.

Effect of KP on the growth of *R. solanacearum*, *P. favisporus* and *S. coelicoflavus in vitro*

Two hundred microliters of *R. solanacearum* (approximately 10^8 cfu ml^{-1}) and *P. favisporus* Y7 (approximately 10^8 cfu ml^{-1}) was separately inoculated in 3 ml of LB (Luria Broth) liquid containing KP at a concentration of 0.5%. Two hundred microliters of *S. coelicoflavus* F13 spores (approximately 10^8 cfu ml^{-1}) was inoculated in 20 ml of LB liquid containing KP at a concentration of 0.5%. The LB liquid without KP was used as the control. Each treatment had four replicates. After 48 h of incubation at 30 °C (*R. solanacearum* and *P. favisporus* Y7) and 28 °C (*S. coelicoflavus* F13) at 170 rpm, the cell densities (OD_{600}) of *R. solanacearum* and strain Y7 and the dry biomass of strain F13 were measured.

Draft genomes of two strains of bacteria identified as *Paenibacillus favisporus* and *Streptomyces coelicoflavus*

The genomes of the two isolates *Paenibacillus favisporus* and *Streptomyces coelicoflavus*, which were highly abundant in the microbial community of the KP-treated soil, were sequenced. The sequences were analyzed according to the method described in a previous study (Desta et al. 2019). Briefly, the low-quality sequences were removed by adapter removal (version 2.1.7). After filtering, a total of 9,141,712 (98.65%) and 16,401,350 (97.68%) high-quality paired-end reads were obtained for strains *P. favisporus* and *S. coelicoflavus*, respectively. All reads were quality corrected by SOAPec (version 2.0) based on the k-mer frequency, with the k-mer used for correction set to 17. The genome was assembled de novo using A5-miseq (version 20,150,522). The draft genome sequences of *P. favisporus* and *S. coelicoflavus* contained 17 and 59 contigs (a sequence length greater than 1 kb), respectively. The coding DNA sequences (CDs) in the draft genomes were predicted by GeneMarkS (version 4.32). The predicted CDs were searched against the NCBI NR protein database and the Kyoto Encyclopedia of Genes and Genomes (KEGG). The whole-genome shotgun project has been deposited in GenBank under the accession numbers PRJNA579492 (*P. favisporus*) and PRJNA577208 (*S. coelicoflavus*).

Quantitative PCR to measure the copies of functional genes

The copy numbers of *fliC* (*R. solanacearum*), 16S rRNA gene (total bacteria), con31_49 (*S. coelicoflavus* F13), and con2_67 (*P. favisporus* Y7) were quantified by quantitative PCR. After sequencing the genomes of *S. coelicoflavus* and strain *P. favisporus*, unknown functional genes from contig31_6449 (strain *S. coelicoflavus*) and contig2_3067 (strain *P. favisporus*) were found without homologous genes in the NCBI database. Thus, the gene fragments were used to design primers (con31_49 for *S. coelicoflavus* and con2_67 for *P. favisporus*) in Premier 5. The designed primers were tested by primer blast (www.ncbi.nlm.nih.gov/tools/primer-blast/), and no microbes were obtained.

Quantitative PCR (qPCR) assays were performed using the SYBR Premix Ex Taq™ (Perfect Real-Time) Kit (Takara Biotechnology Co., Dalian, China) with the ABI StepOne™ Real-Time PCR System (Applied Biosystems, USA). Each reaction was performed in a 20-µl volume. Detailed primer information and PCR steps are listed in the supporting information (Additional file 1 Table S2 and Table S3). Standard curves were developed by serially diluting the plasmids with known positive inserts to final concentrations of 10^2 to 10^7 gene copies μl^{-1}. The QPCR efficiencies ranged from 90 to 105%, and the R^2 values for all four assays were greater than 0.99.

Data analysis

The OTU tables were converted into a suitable input file for bacterial diversity analysis using Mothur (Schloss et al. 2009). Principal coordinate analysis (PCoA) of the bacterial community composition was performed by calculating the Bray–Curtis dissimilarity in R. Differences between groups were tested for significance by a permutation-based analysis of variance by using the adonis function of the vegan package in R. The significant phyla and genera (top 50) between the control and the KP treatment were identified with DESeq2 in R, and the significant genera were shown in a circular treemap using the ggraph package in R. The associations among the bacterial community and the abundances of *S. coelicoflavus P. favisporus* and *R. solanacearum* were determined by the cor.test function in R.

Changes in soil bacterial functions were predicted by PICRUSt2 software (https://github.com/picrust/picrust2/wiki). The significant predicted pathways and Kegg orthologs (KOs) between the control and the KP treatment were identified with DESeq2 in R. The significant predicted pathways that had great differences (top 15) are shown in a hotmap using the pheatmap package in R.

The data followed the normal distribution according to the Shapiro–Wilk test (Shapiro and Wilk 1965). The bacterial community diversity indices, the number of culturable Actinobacteria, the number of functional gene copies, the cell density (OD600) of strain *P. favisporus*, and the biomass of strain *S. coelicoflavus* in vitro were assessed using Student's *t* test.

Results

KP depleted *R. solanacearum* but enriched potential antagonistic bacteria in soil

The copy numbers of the *fliC* (representing *R. solanacearum*) and 16S rRNA genes (representing total bacteria) were significantly reduced by approximately 1.91-fold to 2.44-fold and 1.61-fold to 4.81-fold, respectively, at all sampling times in the KP treatment (Fig. 1a and b).

The rarefaction curve showed that the soil microbiome from both KP treatment and control reached saturation stage with increasing sequencing depth (Additional file 1 Fig. S1). The Shannon diversity of the soil bacterial community was slightly decreased in the soil amended with KP at days 7 and 14 (Additional file 1 Fig. S2a); however, the diversity recovered at day 30. No significant differences in Chao diversity was found between KP treatment and control at days 7, 14, and 30 (Additional file 1 Fig. S2b).

The microbial community composition of the control at different sampling times was significantly different from that of the KP treatment ($P=0.001$, $R=0.45$) (Fig. 1c). The difference in bacterial community composition between the control and KP treatments at day 7 was greater than that at days 14 and 30. Moreover, the bacterial community composition of the control at day 7 was different from that at days 14 and 30, suggesting that the time factor of tomato root residuals significantly affected the soil bacterial community.

Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes were the dominant phyla in the bacterial communities (Additional file 1 Fig. S3). Actinobacteria was significantly enriched, but Proteobacteria and Bacteroidetes were significantly depleted in the KP treatment at days 7 and 14. However, the relative abundance of Proteobacteria and Firmicutes in the KP treatment was significantly higher than that in the control at day 30. To increase the robustness of our results, we determined the number of culturable Actinobacteria in two other soils supplemented with KP. The numbers of culturable Actinobacteria in the HN and HG soils amended with 0.5% KP were 1.5-fold and 1.4-fold

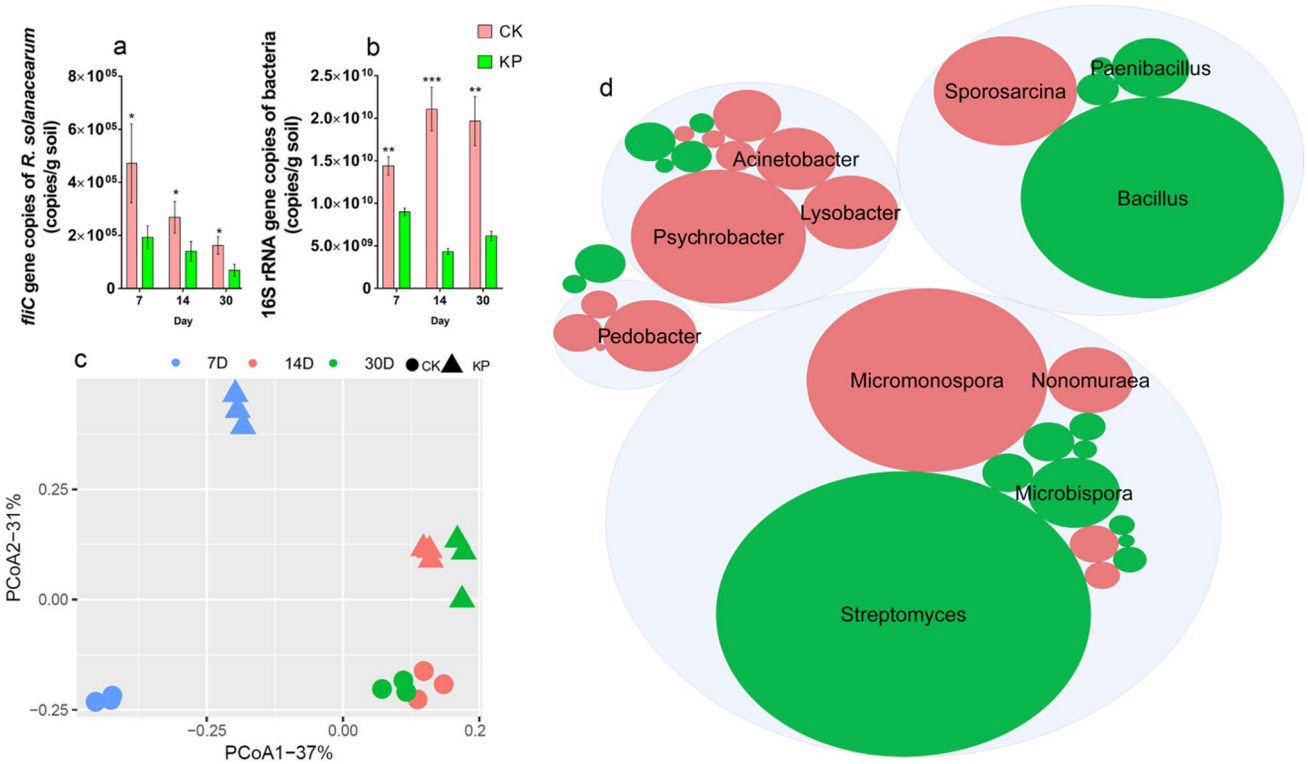

Fig. 1 Effects of KP on the soil bacterial communities. Copy numbers of *fliC* (**a**) and 16S rRNA (**b**) between the KP treatment and the control. Statistical significance was determined based on Student's *t* test. $*P<0.05$, $**P<0.01$, $***P<0.001$. Unless otherwise noted, "CK" and "KP" refer hereafter to the control and KP treatments, respectively. **c** Principal coordinate analysis of the bacterial communities in the KP-treated soil and the control soil at different time points. **d** Significant differences in the relative abundance levels of bacterial genera between the KP treatment and the control at 7 days. The largest circles represent the phylum level. The inner circles represent the genus level. The colors of the circles indicate genera enriched in the KP treatment (green) or the control (red). The size of the circle represents the relative abundance of that genus

higher than those in the corresponding controls, respectively (Additional file 1 Fig. S4).

At the genus level, the relative abundance of the dominant bacterium *Streptomyces* (25.29%) was significantly increased in the KP treatment on all sampling days (Fig. 1d; Fig. S5; Fig. S6; Table S4; Table S5; Table S6). *Paenibacillus* was significantly enriched in the KP treatment at days 7 and 14 (Fig. 1d; Fig. S5). Specifically, in addition to *Micromonospora*, *Arthrobacter*, *Agromyces* and *Sporosarcina*, the other significantly altered genera (top 50) belonging to Actinobacteria and Firmicutes, such as *Actinoallomurus* and *Actinocatenispora*, were significantly enriched in the KP treatment at day 7 (Fig. 1d). In addition to *Sphingomonas*, the other significantly altered genera (top 50) belonging to Bacteroidetes and Proteobacteria, such as *Pseudomonas*, *Massilia*, and *Flavobacterium*, were significantly depleted in the KP treatment at day 7 (Fig. 1d). However, *Bacillus* and *Flavobacterium* were significantly depleted and enriched in the KP treatment at days 14 and 30, respectively (Additional file 1 Fig. S5 and Fig. S6). Moreover, *Sphingobium*, *Chitinophaga*, and *Ensifer* were significantly depleted in the soil of the KP treatment at all sampling times. Overall, the application of KP significantly altered the composition of the soil bacterial community and enriched certain potential antagonistic bacteria against *R. solanacearum*.

KP-modulated soil microbiome inhibited the growth of *R. solanacearum*

As the potential antagonistic bacteria were highly enriched in the soil amended with KP at 7 days, we speculated that the KP-modulated microbiome from 7 days may inhibit the growth of *R. solanacearum*. To confirm whether the KP-modulated microbiome could inhibit *R. solanacearum*, a soil microbiome transfer experiment was performed. The results showed that the KP-modulated microbiome could significantly inhibit the growth of *R. solanacearum*, and the copy number of *fliC* (*R. solanacearum*) was significantly lower by approximately 1.38-fold in the MK treatment (the soil amended with the microbiome from the KP treatment) than in the MC treatment (the soil amended with the microbiome from the control) ($P = 0.004$) (Fig. 2a), suggesting that the KP-modulated soil microbiome could inhibit the growth of *R. solanacearum*.

Functional inferences (PICRUSt2) in soil microbiome

To determine the reasons that the KP-modulated soil microbiome inhibited the growth of *R. solanacearum*, microbiome functions of soil sampled at 7 day were predicted by PICRUSt2. The results showed that most synthesis of antagonistic pathways (great differences (top 15) between the control and KP treatments), such as streptomycin, ansamycin, and polyketide, were significantly enriched in the KP treatment, suggesting that the enriched bacteria in the KP treatment may contain certain genes related to the synthesis of these antagonistic substances (Fig. 3). Moreover, the predicted pathway related to lipopolysaccharide (a distinct component in the cell wall of gram-negative bacteria) was significantly depleted in the KP treatment, suggesting that KP may mainly depress the growth of gram-negative bacteria. We further identified the regularity differences between the control and KP treatments at the gene level of PICRUSt2 results. We found that the predicted genes related to tellurite resistance

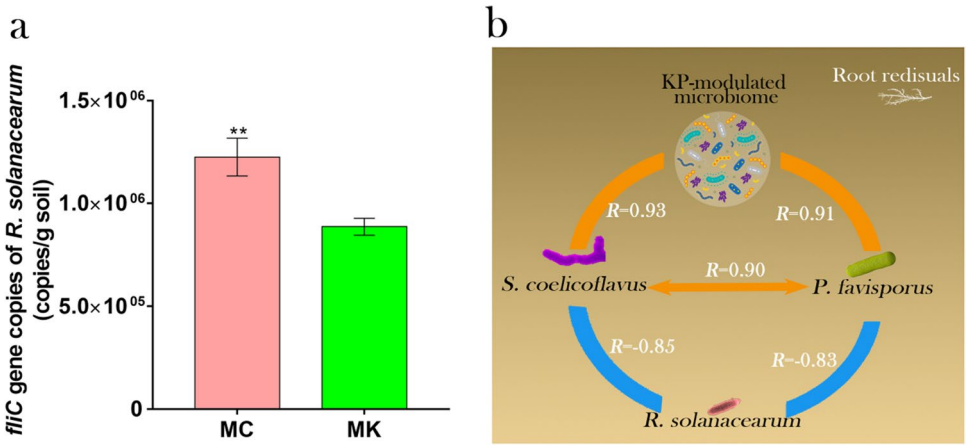

Fig. 2 Inhibition of *R. solanacearum* by the KP-modulated soil microbiome. **a** Inhibition of *R. solanacearum* by the KP-modulated microbiome. MK and MC represent the soil amended with the microbiome from the KP treatment (MK) and the soil amended with the microbiome from the control (MC), respectively. Statistical significance was determined based on Student's *t* test. $^{**}P < 0.01$. **b** Associations among the KP-modulated microbiome, the abundances of antagonistic bacteria *S. coelicoflavus* and *P. favisporus* and pathogen *R. solanacearum*. The associations were determined based on Pearson correlation distance, and all of the *p*-values of these associations were less than 0.05

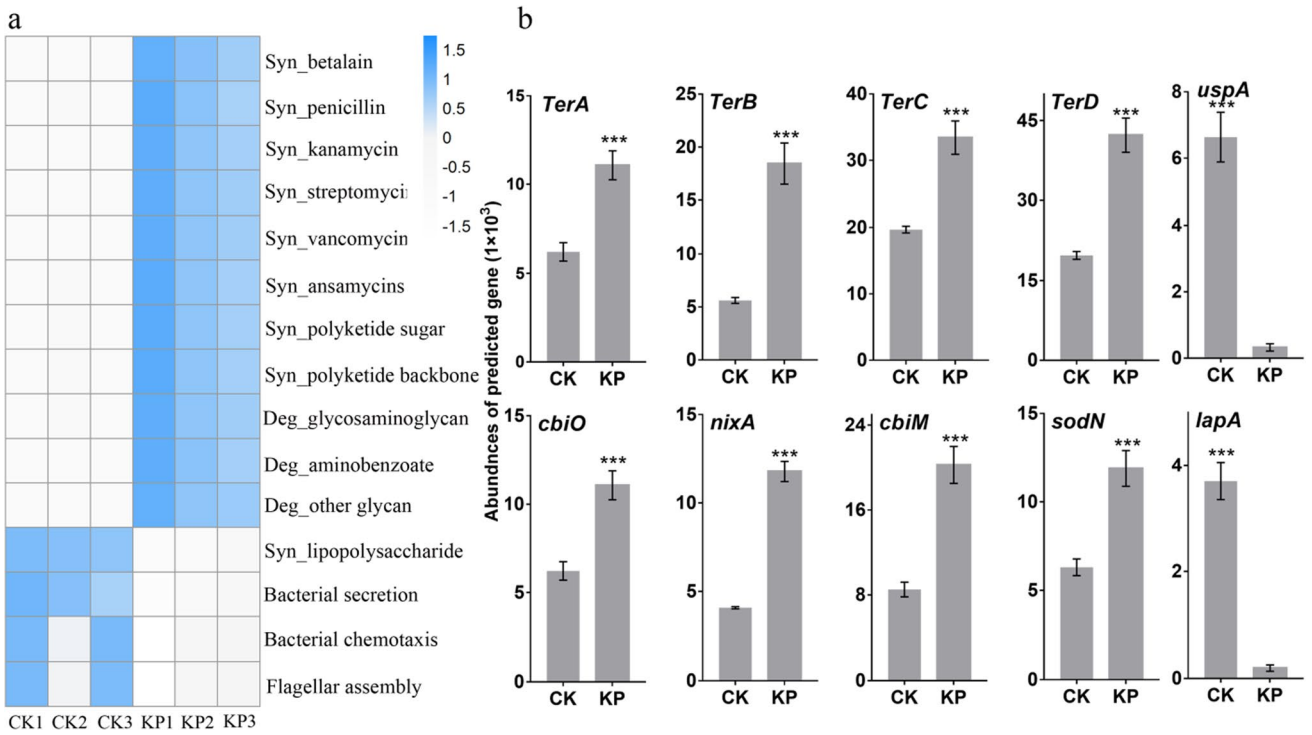

Fig. 3 Changes in the predicted pathways (**a**) and KOs (Kegg orthologs) (**b**) between the control and KP treatments. Statistical significance was determined by DESeq2. The *P* values were adjusted by FDH method. $*P<0.05$, $**P<0.01$, $***P<0.001$. The *terA*, *terB*, *terC*, and *terD* genes represent tellurite resistance proteins. The *cbiO*, *nixA*, *cbiM*, and *sodN* genes represent the nickel transport system. The genes enes *uspA* and *lapA* represent universal stress proteins and universal stress proteins, respectively. Syn and deg represent synthesis and degradation

proteins (*terA*, *terB*, *terC*, and *terD*) and nickel transport systems (*cbiO*, *nixA*, *cbiM*, and *sodN*) were significantly enriched in the KP treatment. However, the predicted genes related to universal stress protein (*uspA*) and lipopolysaccharide assembly protein (*lapA*) were significantly depleted in the KP treatment.

S. coelicoflavus and *P. favisporus* contribute to the antagonism of the KP-modulated microbiome

We hypothesized that the potential antagonistic bacteria enriched in the KP-modulated soil microbiome may be the main reason for the inhibition of *R. solanacearum*. Thus, we isolated 33 dominant strains from the KP-treated Nanjing soil and found OTU1 and OTU39, for which the sequences were clustered to the 16S rRNA sequences of strains F13 (*Streptomyces coelicoflavus*) and Y7 (*Paenibacillus favisporus*) at 100% sequence similarity (Fig. 4a). Quantitative real-time PCR results showed that the copy numbers of the specific gene fragments con31_49 of *S. coelicoflavus* and con2_67 of *P. favisporus* were significantly higher in the KP treatments than in the control ($P=0.01$) at three sampling times (Fig. 4b). This result indicated that *S. coelicoflavus* and *P. favisporus* were enriched in the soil amended with KP. Supporting the above results, the copy numbers of the specific gene fragments con31_49 of *S. coelicoflavus* and con2_67 of *P. favisporus* were also significantly higher in the MK treatment than in the MC treatment ($P=0.01$) (Additional file 1 Fig. S7).

The associations among the KP-modulated microbiome and the abundances of strains *S. coelicoflavus*, *P. favisporus*, and *R. solanacearum* were determined. The results showed that the KP-modulated microbiome was significantly and positively associated with the abundances of strains *S. coelicoflavus* and *P. favisporus*, and the abundance of *R. solanacearum* was significantly and negatively associated with the potential antagonistic bacteria (Fig. 2b), suggesting that the enriched *S. coelicoflavus* and *P. favisporus* may contribute to inhibiting the growth of *R. solanacearum* in the KP-modulated soil microbiome.

To support the above result, we tested whether *S. coelicoflavus* and *P. favisporus* inhibited the growth of *R. solanacearum*. The results showed that obvious inhibition zones of *P. favisporus* and *S. coelicoflavus* to *R. solanacearum* were found on NA (nutrient agar medium) plates (Fig. 4c).

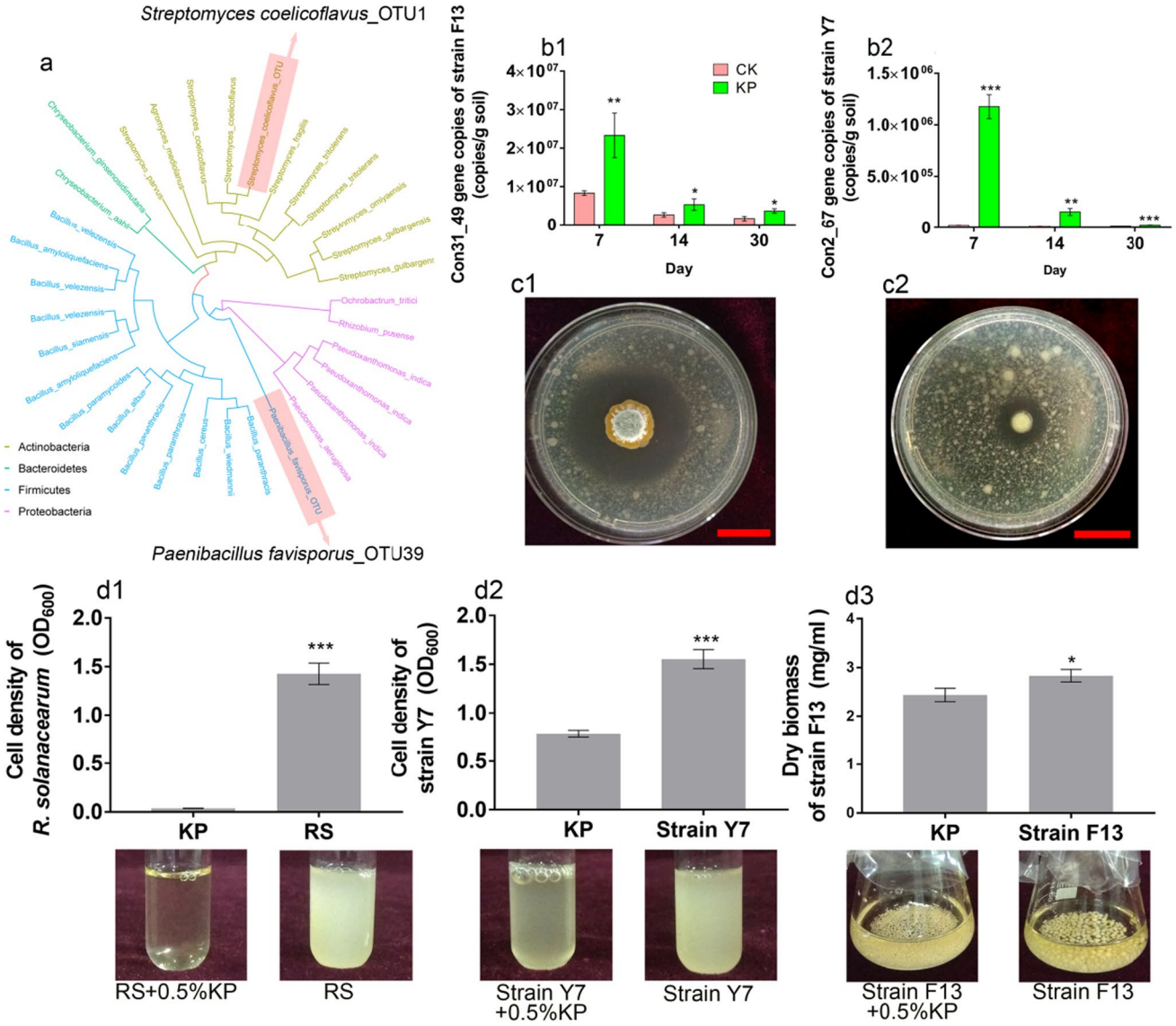

Fig. 4 Characteristics of isolated bacteria in soil and determination of their abundances by q-PCR. **a** The phylogenetic diversity of the isolates from the soil amended with KP at the species level. The isolates for which the 16S rRNA sequences were clustered to sequences of target OTUs (*Streptomyces* and *Paenibacillus*) at 100% sequence similarity are marked with a light red background. **b** Copy numbers of con31_49 of strain *Streptomyces coelicoflavus* F13 (b1) and con2_67 of strain *Paenibacillus favisporus* Y7 (b2) in the soil amended with KP. **c** Inhibition of *R. solanacearum* by antagonistic bacteria *S. coelicoflavus* F13 (c1) and *P. favisporus* Y7 (c2). **d** Inhibition level of *R. solanacearum* and strains *P. favisporus* Y7 and *S. coelicoflavus* F13 by KP. Statistical significance was determined based on Student's *t* test. $*P<0.05$, $**P<0.01$, $***P<0.001$. RS represents *R. solanacearum*

S. coelicoflavus and *P. favisporus* have higher tolerance of KP than *R. solanacearum*

Based on the predicated functions in PICRUSt2 results, we speculated that *S. coelicoflavus* and *P. favisporus* (gram-positive bacteria) have a higher tolerance to KP than *R. solanacearum* (gram-negative bacteria). Indeed, *R. solanacearum* barely grew in the LB liquid medium amended with KP at a concentration of 0.5% (Fig. 4d). KP slightly inhibited the growth of *S. coelicoflavus*. Although KP significantly inhibited the growth of *P. favisporus*, the inhibition level of *R. solanacearum* was much higher than that of *P. favisporus*.

Functional analysis based on the genomes of *P. favisporus* and *S. coelicoflavus*

We further determined the functions of the isolates from their genomes to support the above results. The results showed that both strains contained the metabolic pathways related to the biosynthesis of streptomycin, prodigiosin,

monobactam, phenylphopaniod, and validamycin (Fig. S8). Moreover, the genes related to nickel transport systems, such as *nixA* (high-affinity nickel-transport protein) were found in both strains (Additional file 2). The genes related to tellurite resistance proteins, such as *terD* (tellurium resistance protein) were found in strain *S. coelicoflavus* (Additional file 2).

Discussion

Previous studies have shown that potential antagonistic bacteria were enriched in soil amended with agricultural chemicals by high-throughput sequencing (Liu et al. 2019; Wang et al. 2019). However, there is still a lack of experimental evidence about whether the soil microbiome could inhibit the growth of soil-borne pathogens after being modulated by the addition of exogenous chemicals. In the current study, by isolating strains and performing soil microbiome transfer experiments, we showed for the first time that the agricultural chemically modulated soil microbiome could inhibit the growth of *R. solanacearum* in soil and explored the potential mechanism.

Bacterial wilt caused by *Ralstonia solanacearum* is a destructive and widespread soil-borne disease. Once the pathogen is introduced into a field, it is difficult to eradicate it (Salanoubat et al. 2002). At present, there are no effective methods to stably control bacterial wilt diseases (de Pedro Jové et al. 2021). Steering the soil microbiome to enhance resistance provides a new opportunity to manage soil-borne diseases. Previous studies have shown that plant pathogen infection could induce the host to steer the soil microbiome to enhance host resistance (Berendsen et al. 2018; Yuan et al. 2018). However, the pathogen infection-mediated soil microbiome could not be used in application. Here, we found that an environmentally acceptable agricultural chemical potassium phosphite could enhance the soil microbiome suppressive capacity against the tomato pathogen *R. solanacearum* (Fig. 2). Antagonistic bacteria play important roles in inhibiting the growth of soil-borne pathogens. In the present study, we found that the copy numbers of specific sequences from antagonistic bacteria *S. coelicoflavus and P. favisporus* were enriched in the KP-treated soil (Fig. 3). Our results suggested that KP enriched antagonistic bacteria to enhance the antagonistic ability of the soil microbiome. In agreement with our study, the chloropicrin fumigation-enriched potential antagonistic bacteria Actinobacteria may enhance the soil suppressive capacity against *R. solanacearum* (Zhang et al. 2017). Similarly, the potential antagonistic bacteria *Streptomyces* were enriched in soil amended with glucose and fructose (Dundore-Arias et al. 2019). This information sheds light on the potential for using exogenous chemical amendments to strategically steer soil microbial community assembly and contributes to the establishment of pathogen-suppressive soils in agricultural systems, providing a new potential strategy for the control of tomato bacterial wilt disease.

It is known that the species that adapted to certain environments could occupy the main niches in such environments. For example, after soil heat disturbance, bacteria that are heat tolerant and have high growth rates significantly increase in relative abundance (van der Voort et al. 2016) that may explain the enrichment of the antagonistic bacteria *S. coelicoflavus and P. favisporus* in the current study. Moreover, the dominant species decrease the abundances of other species to maintain the occupied niches that is termed positive frequency-dependent selection (FDS). The FDS ecological pattern of certain microbes, such as *Streptomyces* (Wright and Vetsigian 2016) and *Myxobacteria* (Greig and Goddard 2015) was found. In agreement with the previous studies, we found that the dominant *Streptomyces coelicoflavus* F13 inhibited the growth of another species *R. solanacearum*. However, a great abundance of certain beneficial bacteria, such as *Streptomyces*, was applied in soil; the beneficial bacteria still could not be the dominant species and were inhibited by indigenous microbes that seem to violate the FDS theory (Mitter et al. 2019; Wei et al. 2017). Likely, the applied bacteria may not adapt to the complex soil environment that limits the application of beneficial bacteria. Thus, how to create the environment that contributed to the enrichment of the beneficial bacteria is still a great challenge. Here, we found that many potential antagonistic bacteria, such as *Streptomyces* and *Paenibacillus*, were enriched in the soil amended with KP suggesting that the application of KP created the suitable environments for these antagonistic bacteria. Overall, our results provided a novel strategy to enrich the antagonistic bacteria in soil and extended the understanding of FDS ecological pattern in soil bacterial community.

In the present study, KP mainly inhibited gram-negative bacteria (Bacteroidetes and Proteobacteria) but not gram-positive bacteria (Actinobacteria and Firmicutes). To support this result, we found that the inhibition level of *R. solanacearum* was greatly higher than that of *S. coelicoflavus* and *P. favisporus*. Moreover, few bacteria, such as *Pseudomonas stutzeri*, that contain the *ptxD* gene (phosphite oxidation) could convert phosphite to phosphate in soil (Figueroa and Coates 2017). However, the *ptxD* gene was not found in the genomes of *S. coelicoflavus* and *P. favisporus*. Likely, the cell structures between gram-positive bacteria and gram-negative bacteria may explain this result. It is known that because of the differences in cell structures between gram-positive bacteria and gram-negative bacteria, certain antibiotics, such as penicillin, selectively inhibit gram-positive bacteria or gram-negative bacteria (Kapoor et al. 2017). Specifically, the distinct component phosphoteic acid in gram-positive bacteria is negatively charged and can adsorb

metal cations. Phosphite (PO_3^{3-}) is also negatively charged. The mutual repulsion between negative charges may lead to a decrease in the concentrations of phosphite around gram-positive bacteria. Moreover, the thicker cell wall in gram-positive bacteria may also prevent phosphite from entering the cells. In addition, the antibiotic 1,6-dideoxy-1,6-diphosphoramidate mannitol, in which phosphite is involved in the main synthetic reactions, can depress the synthesis of lipopolysaccharide (distinct component lipopolysaccharide in gram-negative bacteria) (Johnsson 2016). In the current study, the relative abundances of predicted pathways related to the synthesis of lipopolysaccharide were significantly decreased in the KP treatment (Fig. 3). Lipopolysaccharide plays a fundamental role in the protection of gram-negative bacteria from environmental stress factors. It is likely that KP may depress the ability to resist environmental stresses, resulting in a low competitiveness of gram-negative bacteria in soil. In addition, the relative abundances of predicted genes related to tellurite resistance proteins and nickel (reducibility) transport systems were significantly increased in the KP treatment. Because of the reducibility characteristics of phosphite, the resistance reaction mechanism of phosphite may be similar to that of nickel, which may transport phosphite or nickel out of cells. Moreover, a previous study showed that the tellurite resistance protein was related to the structural constituent of the cell wall (Pontieri et al. 2018), suggesting that changes in certain structural constituents (such as enrichment of phosphoteic acid or peptidoglycan) of the cell wall in the enriched bacteria in KP treatment may enhance the resistance ability of phosphite. Revealing the molecular mechanisms underlying phosphite resistance has great potential in alleviating bacterial resistance. However, more biomolecular experiments are required that are beyond the aims of the present study.

Conclusions

Our results showed that the application of potassium phosphite (KP) enhanced the antagonistic ability of the soil microbiome to inhibit *R. solanacearum*. Based on this finding, we proposed a promising strategy to steer the soil microbiome to enhance its resistance to soil diseases. Moreover, our results enhanced the mechanistic understanding of positive frequency-dependent selection in microbial community. The application of an environmentally acceptable KP bridge to the enrichment of antagonistic bacteria could enable future work that aims to precisely steer the soil microbiome to protect the host from infection by soil-borne pathogens. We suggest future studies on the effect of KP in different soil types since different soils harbor different microbiomes, which consequently can have different responses to microbial inoculation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00374-022-01634-z.

Acknowledgements The authors thank Ms. Chao Zhou and Ms. Zhenghua Wu for technical assistance. Publication number 7417 of the Netherlands Institute of Ecology (NIOO-KNAW).

Author contribution Lv Su composed the main text, conceived the project, and performed almost all experiments except for the following. Haichao Feng analyzed the data of high throughput sequencing and provided suggestions of ecology concepts. Xingxia Mo and Juan Sun isolated strains from soils. Pengfei Qiu extracted soil DNA. Yunpeng Liu, Eiko E. Kuramae, and Ruifu Zhang provided suggestions for the manuscript. Biao Shen and Qirong Shen organized and supervised the project, respectively.

Funding This work was supported by the Chinese Natural Science Fund Program (41571242).

Availability of data and material The raw sequences were submitted to the NCBI Sequence Read Archive (SRA) under BioProject accession PRJNA577427. The whole-genome shotgun project has been deposited in GenBank under the accession numbers PRJNA579492 (strain Y7) and PRJNA577208 (strain F13).

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

Albrigo L (1999) Effects of foliar applications of urea or nutriphite on flowering and yields of Valencia orange trees. Proceedings of the Florida State Horticultural Society. Florida State Horticultural Society, Florida State, pp 1–4

Berendsen RL, Vismans G, Yu K, Song Y, de Jonge R, Burgman WP, Burmølle M, Herschend J, Bakker PA, Pieterse CM (2018) Disease-induced assemblage of a plant-beneficial bacterial consortium. ISME J 12:1496–1507

Biddle JF, Fitz-Gibbon S, Schuster SC, Brenchley JE, House CH (2008) Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. Proc Natl Acad Sci 105:10583–10588. https://doi.org/10.1073/pnas.0709942105

Costa BHG, de Resende MLV, Monteiro ACA, Ribeiro Júnior PM, Botelho DMdS, Silva BMd (2018) Potassium phosphites in the protection of common bean plants against anthracnose and biochemical defence responses. J Phytopathol 166:95–102

de Pedro Jové R, Sebastià P, Valls M (2021) Identification of type III secretion inhibitors for plant disease management. Methods Mol Biol 2213:39–48. https://doi.org/10.1007/978-1-0716-0954-5_4

Dempsey JJ, Wilson I, Spencer-Phillips PTN, Arnold D (2018) Suppression of the in vitro growth and development of *Microdochium nivale* by phosphite. Plant Pathol 67:1296–1306

Desta M, Wang W, Zhang L, Xu P, Tang H (2019) Isolation, characterization, and genomic analysis of Pseudomonas sp. strain SMT-1, an efficient fluorene-degrading bacterium. Evol Bioinform 15:1176934319843518

Dundore-Arias JP, Felice L, Dill-Macky R, Kinkel LL (2019) Carbon amendments induce shifts in nutrient use, inhibitory, and resistance phenotypes among soilborne *Streptomyces*. Front Microbiol 10:498. https://doi.org/10.3389/fmicb.2019.00498

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10:996

Felipini RB, Boneti JI, Katsurayama Y, Neto ACR, Veleirinho B, Maraschin M, Di Piero RM (2016) Apple scab control and activation of plant defence responses using potassium phosphite and chitosan. Eur J Plant Pathol 145:929–939. https://doi.org/10.1007/s10658-016-0881-2

Figueroa IA, Coates JD (2017) Microbial phosphite oxidation and its potential role in the global phosphorus and carbon cycles. Adv Appl Microbiol 98:93–117. https://doi.org/10.1016/bs.aambs.2016.09.004

Garbelotto M, Schmidt D, Harnik T (2007) Phosphite injections and bark application of phosphite+ Pentrabark™ control sudden oak death in coast live oak. Arboric Urban for 33:309

Greig D, Goddard M (2015) Ecology: tribal warfare maintains microbial diversity. Curr Biol 25:R618–R620. https://doi.org/10.1016/j.cub.2015.05.044

Han L, Liu Y, Fang K, Zhang X, Liu T, Wang F, Wang X (2020) Azoxystrobin dissipation and its effect on soil microbial community structure and function in the presence of chlorothalonil, chlortetracycline and ciprofloxacin. Environ Pollut 257:113578

Howard MM, Bell TH, Kao-Kniffin J (2017) Soil microbiome transfer method affects microbiome composition, including dominant microorganisms, in a novel environment. FEMS Microbiol Lett 364:fnx092. https://doi.org/10.1093/femsle/fnx092

Johnsson RE (2016) Synthesis and evaluation of mannitol-based inhibitors for lipopolysaccharide biosynthesis. Int J Med Chem 2016:3475235–3475235. https://doi.org/10.1155/2016/3475235

Kapoor G, Saigal S, Elongavan A (2017) Action and resistance mechanisms of antibiotics: a guide for clinicians. J Anaesthesiol Clin Pharmacol 33:300–305

Kostov O, Van Cleemput O (2001) Microbial activity of Cu contaminated soils and effect of lime and compost on soil resiliency. Compost Sci Util 9:336–351

Lewis JA (1977) Effect of plant residues on chlamydospore germination of *Fusarium solani* f. sp. phaseoli and on *Fusarium* Root Rot of Beans. Phytopathology 77:925–929

Li X, Jousset A, de Boer W, Carrión VJ, Zhang T, Wang X, Kuramae EE (2019) Legacy of land use history determines reprogramming of plant physiology by soil microbiome. ISME J 13:738–751. https://doi.org/10.1038/s41396-018-0300-0

Liu K, Cai M, Hu C, Sun X, Cheng Q, Jia W, Yang T, Nie M, Zhao X (2019) Selenium (Se) reduces Sclerotinia stem rot disease incidence of oilseed rape by increasing plant Se concentration and shifting soil microbial community and functional profiles. Environ Pollut 254:113051. https://doi.org/10.1016/j.envpol.2019.113051

Meena RS, Kumar S, Datta R, Lal R, Vijayakumar V, Brtnicky M, Sharma MP, Yadav GS, Jhariya MK, Jangir CK (2020) Impact of agrochemicals on soil microbiota and management: a review. Land 9:34

Metcalf WW, Wolfe RS (1998) Molecular genetic analysis of phosphite and hypophosphite oxidation by *Pseudomonas stutzeri* WM88. J Bacteriol 180:5547–5558

Mitter B, Brader G, Pfaffenbichler N, Sessitsch A (2019) Next generation microbiome applications for crop production-limitations and the need of knowledge-based solutions. Curr Opin Microbiol 49:59–65

Mofidnakhaei M, Abdossi V, Dehestani A, Pirdashti H, Babaeizad V (2016) Potassium phosphite affects growth, antioxidant enzymes activity and alleviates disease damage in cucumber plants inoculated with *Pythium ultimum*. Arch Phytopathol 49:207–221. https://doi.org/10.1080/03235408.2016.1180924

Niere JO, Deangelis G, Grant BR (1994) The effect of phosphonate on the acid-soluble phosphorus components in the genus *Phytophthora*. Microbiology 140:1661–1670

Norman DJ, Chen J, Yuen JMF, Mangravita-Novo A, Byrne D, Walsh L (2006) Control of Bacterial Wilt of Geranium with Phosphorous Acid. Plant Dis 90:798–802

Pontieri P, Hartings H, Di Salvo M, Massardo DR, De Stefano M, Pizzolante G, Romano R, Troisi J, Del Giudice A, Alifano P, Del Giudice L (2018) Mitochondrial ribosomal proteins involved in tellurite resistance in yeast Saccharomyces cerevisiae. Sci Rep 8:12022. https://doi.org/10.1038/s41598-018-30479-6

Salanoubat M, Genin S, Artiguenave F, Gouzy J, Mangenot S, Arlat M, Billault A, Brottier P, Camus JC, Cattolico L (2002) Genome sequence of the plant pathogen *Ralstonia solanacearum*. Nature Nature 415:497–502

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537

Sederholm MR, Schmitz BW, Barberán A, Pepper IL (2018) Effects of metam sodium fumigation on the abundance, activity, and diversity of soil bacterial communities. Appl Soil Ecol 124:27–33

Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). Biometrika 52:591–611

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

van der Voort M, Kempenaar M, van Driel M, Raaijmakers JM, Mendes R (2016) Impact of soil heat on reassembly of bacterial communities in the rhizosphere microbiome and plant disease suppression. Ecol Lett 19:375–382. https://doi.org/10.1111/ele.12567

Wang F, Zhou T, Zhu L, Wang X, Wang J, Wang J, Du Z, Li B (2019) Effects of successive metalaxyl application on soil microorganisms and the residue dynamics. Ecol Indic 103:194–201. https://doi.org/10.1016/j.ecolind.2019.04.018

Wei Z, Huang J, Yang T, Jousset A, Xu Y, Shen Q, Friman V-P (2017) Seasonal variation in the biocontrol efficiency of bacterial wilt is driven by temperature-mediated changes in bacterial competitive interactions. J Appl Ecol 54:1440–1448

Wright ES, Vetsigian KH (2016) Inhibitory interactions promote frequent bistability among competing bacteria. Nat Commun 7:11274. https://doi.org/10.1038/ncomms11274

Yadeta K, Thomma B (2013) The xylem as battleground for plant hosts and vascular wilt pathogens. FRONT PLANT SCI 4:97. https://doi.org/10.3389/fpls.2013.00097

Yuan J, Zhao J, Wen T, Zhao M, Li R, Goossens P, Huang Q, Bai Y, Vivanco JM, Kowalchuk GA, Berendsen RL, Shen Q (2018) Root exudates drive the soil-borne legacy of aboveground pathogen infection. Microbiome 6:156. https://doi.org/10.1186/s40168-018-0537-x

Zhang S, Liu X, Jiang Q, Shen G, Ding W (2017) Legacy effects of continuous chloropicrin-fumigation for 3-years on soil microbial community composition and metabolic activity. AMB Express 7:178. https://doi.org/10.1186/s13568-017-0475-1