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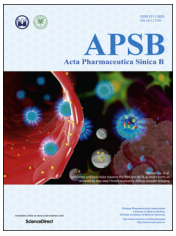

ORIGINAL ARTICLE

Rhizospheric microbial communities are driven by *Panax ginseng* at different growth stages and biocontrol bacteria alleviates replanting mortality

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Abstract The cultivation of *Panax* plants is hindered by replanting problems, which may be caused by plant-driven changes in the soil microbial community. Inoculation with microbial antagonists may efficiently alleviate replanting issues. Through high-throughput sequencing, this study revealed that bacterial diversity decreased, whereas fungal diversity increased, in the rhizosphere soils of adult ginseng plants at the root growth stage under different ages. Few microbial community, such as *Luteolibacter*, Cytophagaceae, *Luteibacter*, *Sphingomonas*, Sphingomonadaceae, and Zygomycota, were observed; the relative abundance of microorganisms, namely, *Brevundimonas*, Enterobacteriaceae, *Pandoraea*, Cantharellales, *Dendryphion*, *Fusarium*, and Chytridiomycota, increased in the soils of adult ginseng plants compared with those in the soils of 2-year-old seedlings. *Bacillus subtilis* 50-1, a microbial antagonist against the pathogenic *Fusarium oxysporum*, was isolated through a dual culture technique. These bacteria acted with a biocontrol efficacy of 67.8%. The ginseng death rate and *Fusarium* abundance decreased by 63.3% and 46.1%, respectively, after inoculation with *B. subtilis* 50-1. Data revealed that microecological degradation could result from ginseng-driven changes in rhizospheric microbial communities; these changes are associated with the different ages and developmental stages of ginseng plants. Biocontrol using microbial antagonists alleviated the replanting problem.

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1. Introduction

Panax ginseng C.A. Meyer demonstrates neuroprotective effects against ischemic stroke[1]. Ginseng plants are mainly distributed in Asia, particularly in China and South Korea[2]. The current annual global market value of this species is approximately 3.5 billion dollars[3]. Wild ginseng resources have dwindled because of excessive and predatory exploitation; thus, wild ginseng has been gradually substituted with cultivated ginseng in the mainstream market[4,5]. Ginseng is continuously cultivated in fixed plots for 4–5 years; however, subsequent replanting commonly fails because of obstacles to continuous cropping[6]. Decades of crop rotation are needed for successful replanting. The replanting issue is a severe drawback that hinders the development of the ginseng industry and thus requires urgent resolution.

The replanting problem is caused by the deterioration of soil physicochemical properties, allelopathy/autotoxicity, outbreak of soil-borne diseases, and changes in soil microbial communities[7–9]. The change in soil microbial community is a major factor that hinders crop replantation[10,11]. Imbalances in rhizospheric microbial communities disrupt ginseng cultivation[12]. Microbial communities change during ginseng cultivation[13], and the increased abundance of pathogenic microorganisms is related to the occurrence of soil-borne disease[14]. Collective changes in the rhizospheric microbial community may cause replanting issues.

Plants of different ages can alter microbial community[15]. The continuous cropping of *Panax quinquefolius* L. changes the microbial community in arable soil[16]. Ginseng plants of different ages drive changes in microbial community. Specifically, rhizospheric and nonrhizospheric soil microbial communities in a particular site become drastically different with ginseng growth[4]. The diversity and relative activity of soil microbial communities change throughout plant development[17]. However, the mechanism through which *Panax* plants of different ages and developmental stages mediate microbial community is unclear.

Root rot is a severe disease that hinders the replantation of *Panax* plants[18]. *Fusarium oxysporum* is the main pathogenic fungus of root rot in *Panax* plants[14,19]. The relative abundance of *F. oxysporum* increases with notoginseng growth and is significantly related with the death rate of ginseng seedlings[14]. The application of biocontrol bacteria could effectively alleviate the occurrence of root rot. Biological control using microbial antagonists has attracted interest as an effective method to decrease the abundance of plant pathogens due to its nontoxic nature[20]. Biocontrol bacteria have important roles in plant defense, and many isolates have shown antagonistic activity against phytopathogenic fungi[21]. In tomato, *Bacillus amyloliquefaciens* RWL-1 inhibits the growth of *F. oxysporum*[22]. Nevertheless, microbial antagonists against ginseng root rot are rare.

Herbgenomics has been utilized in recent investigations on medicinal plants. It involves the use of genomic tools, including metagenomic sequencing technology, to facilitate the analysis of rhizospheric microecology[23]. In the present study, 16S and 18S rRNA genes were analyzed through high-throughput sequencing to illustrate the changes in microbial diversity and composition in the rhizosphere soil of ginseng seedlings at different ages and developmental stages. Furthermore, biocontrol bacteria against *F. oxysporum* were isolated through a dual culture technique, and their inhibitory activity against the target pathogen in replanting soil was confirmed. The results of this study provide insight into the reasons that underlie the replanting issues caused by rhizospheric microbial communities. These data may provide an effective soil bioremediation method to replanting issues associated with Chinese medicinal plants.

2. Materials and methods

2.1. Field experiment and soil extraction

The field experiment was performed in a ginseng plantation in Jingyu, Jilin Province (42°20′N, 126°50′E, 775 m a.s.l.), the main ginseng-producing region in China. This region has a northern temperate continental climate and receives an annual precipitation of approximately 767 mm. The plough layer in the plantation consists of gray-brown soil.

Disease occurrence and mortality rates of ginseng seedlings generally increase after 2 years of consecutive growth. Thus, we analyzed the influence of 2-, 3-, and 4-year-old transplanted seedlings on rhizospheric microbial communities. 2-, 3-, and 4-year-old ginseng seedlings were transplanted in each plot in our plantation and denoted as 2-y, 3-y, and 4-y, respectively. Field plots were arranged following a completely randomized block design, with 3 replicate plots (1.7 m × 8.0 m) per plant age. Ginseng was cultivated strictly in accordance with the standard operating procedures of good agricultural practice[24,25]. The distinct stages of ginseng development are as follows: vegetative, flowering, fruiting, root growth, and annual dormancy (Supplementary Information Table S1). During dormancy, the aboveground parts of ginseng wither and underground root activities weaken. Thus, soil samples obtained during this stage were excluded from analyses.

This experiment included 36 soil samples that were obtained from 2-, 3-, and 4-year-old ginseng seedlings at 4 developmental stages, namely, vegetative (2-Ve, 3-Ve, and 4-Ve), flowering (2-Fl, 3-Fl, and 4-Fl), fruiting (2-Fr, 3-Fr, and 4-Fr), and root growth (2-Ro, 3-Ro, and 4-Ro). Six ginseng seedlings were randomly collected from each plot (1.7 m × 8.0 m). Roots were shaken free of soil, and rhizosphere soil fractions were brushed and pooled into one sample. Soil samples were obtained from 3 replicates per treatment and were homogenized by passing through a 2 mm sieve prior to further processing. The soil characteristics are described in Supplementary Information Table S2.

2.2. DNA extraction and PCR amplification

Total soil DNA was extracted from 0.1 g of freeze-dried soil using a MoBio Powersoil Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) in accordance with the manufacturer's instructions. Then, 16S and 18S rRNA gene fragments from each sample were amplified using the conserved primers 27F/338R[26] and 817F/1196R[27], respectively. The forward and reverse primers contained an eight-base pair barcode (Supplementary Information Table S3). Amplification and purification were performed as previously described[28]. Purified PCR products were quantified with Qubit®3.0 (Life Invitrogen, Germany). The amplicons were pooled in equimolar ratios for sequencing.

2.3. High-throughput sequencing

The pooled DNA product was paired-end sequenced (2 × 250) on an Illumina HiSeq platform (Shanghai Biozeron Co., Ltd., China) following standard protocols. Raw FASTQ files were demultiplexed and quality filtered using QIIME with the following

pipeline[29]. UPARSE (version 7.1 http://drive5.com/uparse/) was used to cluster operational taxonomic units with 97% similarity cutoff. Chimeric sequences were identified and removed using UCHIME. The phylogenetic affiliation of each 16S and 18S rRNA gene sequence was analyzed using Ribosomal Database Project[30] and Silva schemes[31]. Rarefaction analysis based on Mothur v.1.21.1 was performed to identify diversity indexes, including Chao 1 and Shannon diversity (H') indexes[32]. Taxa were identified using RDP Classifier through complete linkage hierarchical clustering using *R* package HCLUST (http://sekhon.berkeley.edu/stats/html/hclust.html). PCoA was used to compare groups of samples based on unweighted UniFrac distance metrics in QIIME[29]. Features for the differentiation of soil microbial communities were characterized with linear discriminant analysis effect size (LEfSe) (http://huttenhower.sph.harvard.edu/lefse/)[33]. All metagenomic data were submitted to the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The accession numbers of 16S rRNA and 18S rRNA genes are SRP103168 and SRP103176, respectively.

2.4. *Quantitative PCR (qPCR) of Fusarium*

To compare the changes in the relative abundance of *Fusarium* in rhizosphere soils of ginseng seedlings, its copy numbers were calculated using the ITS-Fu-F/ITS-Fu-R[34]. qPCR was performed as previously described[27] with minor modifications. Standard curves for the estimation of *Fusarium* copy numbers were generated using a 10-fold serial dilution of a plasmid containing a full-length copy of *F. oxysporum* 18S rRNA gene. qPCR reactions (25 μL) were carried out using an SYBR Green PCR Master mix (Takara, Toyobo, Japan). *Fusarium* copy numbers were calculated using a regression equation for each assay; this equation related the cycle threshold value to the known number of copies in the standards.

2.5. *Isolation and selection of antagonistic bacteria*

A dual culture assay was used to screen microbial antagonists against *F. oxysporum* from the fresh rhizosphere soils of 3-year ginseng seedlings in the root growth stage. Pathogenic *F. oxysporum* was isolated and confirmed following the procedures in our previous study[35]. Soil (10 g) was homogenized in 100 mL of sterile distilled water. Bacteria were isolated through serial dilution technique. The soil suspension was subjected to 10-fold serial dilution (from 10^{-2} to 10^{-5}). Approximately 100 μL of all diluents were transferred to Petri dishes with Luria–Bertani [LB, yeast extract (5 g), peptone (10 g), NaCl (10 g), and agar (10 g) in 1 L of water] medium. The plates were incubated at 28 °C, and colonies were selected and purified. The isolated single strain was screened on the basis of its antagonistic activity against *F. oxysporum* in a dual culture[22]. The zone of inhibition was measured following the method described in a previous study[36] to screen the antagonistic bacterium and examine the antagonistic activity of the candidates.

2.6. *Identification of antagonistic bacterial strain*

The antagonistic bacterial strain was identified as *Bacillus subtilis* 50-1 through morphological and molecular methods. The morphology of *B. subtilis* 50-1 strain was recorded after 24 h of incubation on LB medium. Strain 50-1 was molecularly identified through 16S rRNA amplification[37]. The amplified PCR product was analyzed on a 3730 XL sequencer (Applied Biosystems, Foster City, CA, USA), and the generated sequence was submitted to GenBank (accession number KY962803). Neighbor-joining trees were constructed in MEGA v6.0 to generate Kimura 2-parameter distance matrixes for each sequence following standard parameters. The numbers at the branched knots were the bootstrap values based on 1000 resamplings for the maximum likelihood.

For the further identification of strain 50-1, total genomic DNA was extracted (Tiangen, Beijing, China) and purified using RNase-free DNase I (Takara, Kyoto, Japan). The complete genome sequence was assembled using a hybrid sequencing strategy that combines the PacBio RS II and Illumina HiSeq sequencing platforms. Genome sequencing was performed with Illumina HiSeq. 2500 using the PE250 strategy following the manufacturer's protocol. Scaffolds were generated by subjecting the reads obtained with the Illumina PCR adapter and the filtered low-quality reads to *de novo* assembly[38,39]. Gene prediction from the genome assembly was conducted using Glimmer, and gene functions were annotated through BLASTP against the NR, COG, and KEGG databases. GeneMarkS with an integrated model was used to combine GeneMarkS-generated parameters and Heuristic model parameters[40]. An annotated genome overview was created using Circos[41]. The whole genome was deposited in NCBI (BioProject ID PRJNA383782).

2.7. *Evaluation of the biocontrol efficacy of antagonists in replanting soils*

A pot experiment was performed to assess the biocontrol efficacy of bacterium 50-1 in replanting soils. Each pot contained 1 kg of soil, which had been previously used to cultivate ginseng seedlings for 3 years. Root rot also occurred in these soils. 2-year-old ginseng seedlings (3 plants) were transplanted to each pot. The pots were placed in the phytotron under the following conditions: 26 ± 2 °C, 60% humidity, and 14 h of light alternated with 10 h of darkness. After one week of cultivation, the rhizospheres of the seedlings were inoculated with 1 mL (10^6 cfu/mL) of cultures containing biocontrol strains. Controls were established by inoculating pots with cultures containing inactivated strains. Five pots served as one replicate, and 3 replicates were prepared. At 2 months following inoculation, the death rate of the seedlings was calculated as follows: number of dead seedlings divided by the total number of transplanted seedlings in each treatment. The rhizosphere soils of 3 randomly selected seedlings were collected and served as one sample for the analysis of the relative abundance of *Fusarium.* The experiment was replicated 3 times.

2.8. *Statistical analyses*

SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Variables were considered for all treatment replicates and subjected to ANOVA. Mean values were compared by calculating the least significant difference at the 5% level.

3. Results

3.1. *Taxonomic diversity of bacterial and fungal communities*

A total of 2,296,684 classifiable 16S rRNA sequence reads were obtained from 36 soil samples (Supplementary Information Table S3).

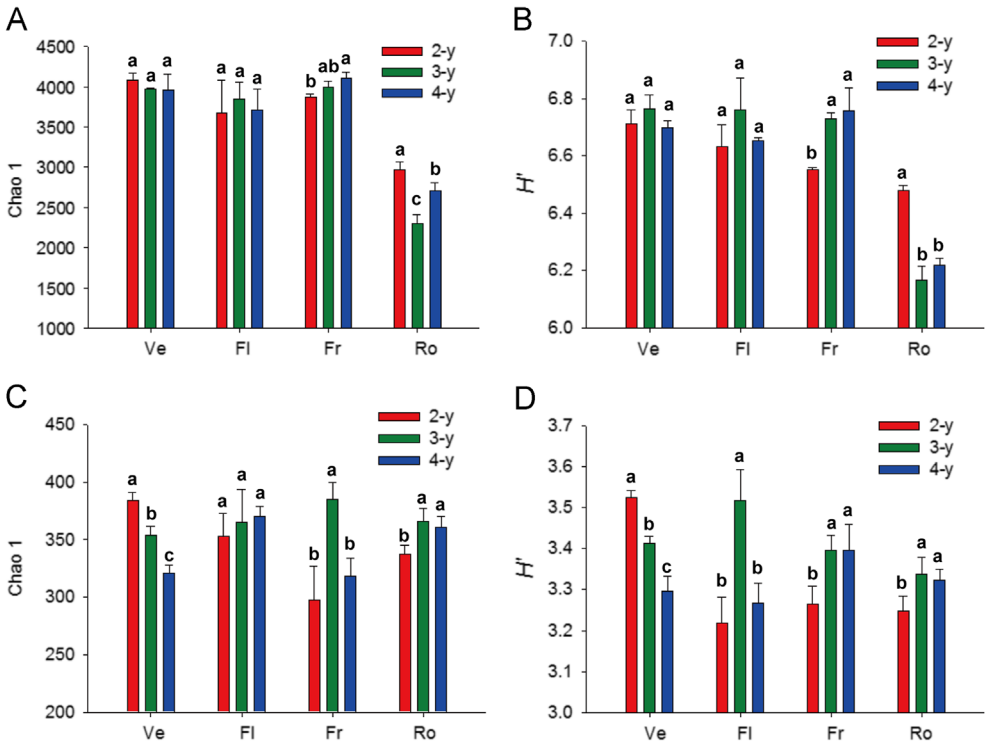

Figure 1 Bacterial and fungal diversities in the rhizosphere of ginseng seedlings of different ages and developmental stages. (A) and (B) show the Chao 1 and Shannon diversity (H') of bacterial community. (C) and (D) show the Chao 1 and H' of fungal community. Ve, Fl, Fr and Ro represent vegetative, flowering, fruiting, and root growth stages, respectively. Data are presented as mean ± SD ($n = 3$). Non-identical letters denote significant difference in the same developmental stage of ginseng plant with different ages at 0.05 significance level.

The mean number of classifiable sequences per sample was 63,796 (dominant length: 283–293 bp). The bacterial diversity indexes H' and Chao 1 were significantly higher in the rhizosphere of 4-y seedlings than those in the rhizosphere of 2-y seedlings at the fruiting stage (Fig. 1A and B). During root growth, H' and Chao 1 values drastically decreased in the rhizospheres of 3- and 4-y seedlings relative to those in the rhizospheres of 2-y seedlings.

A total of 1,321,521 classifiable fungi sequences with a mean of 36,709 sequences were obtained from each soil sample (dominant length: 411–414 bp; Supplementary Information Table S3). H' and Chao 1 values markedly decreased in the rhizosphere with increasing ginseng age at the vegetative stage (Fig. 1C and D). During root growth, H' and Chao 1 values were evidently higher in the soils of 3- and 4-y seedlings than those in the soil of 2-y seedlings.

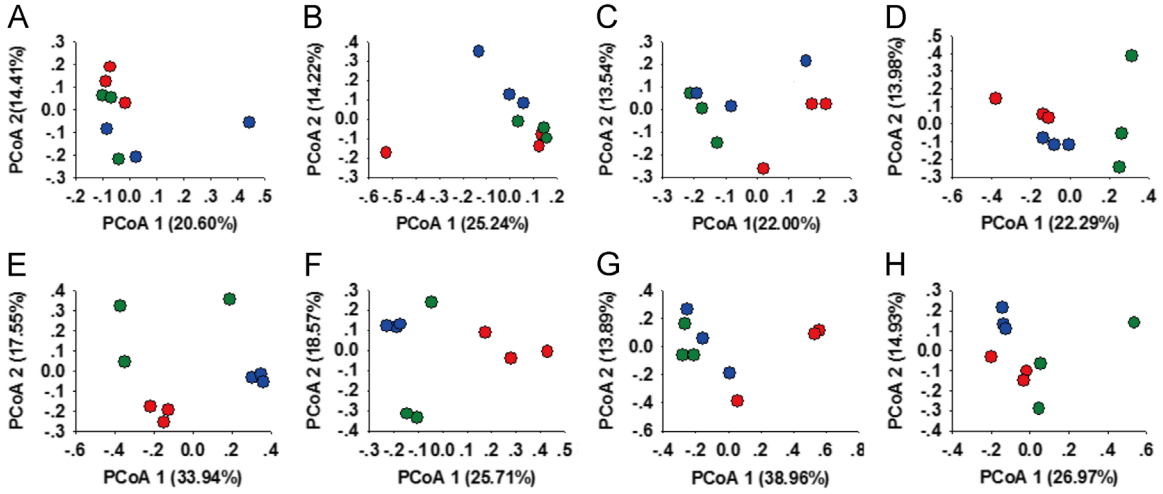

Figure 2 Changes in bacterial and fungal communities in the rhizosphere of ginseng seedlings of different ages and developmental stages. (A)–(D) PCoA ordination plots display the relatedness of samples separated using Unweighted UniFrac distance of classified 16 S rRNA gene sequences at vegetative, flowering, fruiting, and root growth stages, respectively. (E)–(H) PCoA ordination plots show the relatedness of samples separated using Unweighted UniFrac distance of classified 18S rRNA gene sequences at the Ve, Fl, Fr, and Ro stages, respectively. Red, green, and blue represent the samples in the rhizosphere of 2-, 3- and 4-y transplanted ginseng seedlings, respectively.

3.2. *Variation in bacterial and fungal community compositions*

PCoA ordination and Bray–Curtis distance matrix revealed differences in the bacterial communities in the soils of ginseng plants of different ages and developmental stages (Fig. 2 and Supplementary Information Fig. S1). The second principal components (14.41% and 14.22% contributions, respectively) demonstrated that at the vegetative and flowering stages, the bacterial communities in the soils of 2-y seedlings differed from those in the soils of 4-y seedlings (Fig. 2A and B). The first principal component axis (22.00% contributions) indicated that at the fruiting stage, the bacterial communities in the soils of 2-y seedlings significantly differed from those in the soils of 3-y seedlings (Fig. 2C). Additionally, the first principal component axis (22.29% contribution) suggested that the bacterial communities in the soils of 3-y seedlings significantly differed from those in the soils of 2- and 4-y seedlings; the second principal component (13.98% contribution) demonstrated that the bacterial communities in the soils of 2-y seedlings differed from those in the soils of 4-y seedlings (Fig. 2D). LEfSe revealed differences in the rhizospheric bacterial communities of seedlings of different ages and developmental stages (Supplementary Information Fig. S2).

PCoA ordination and Bray–Curtis distance matrix revealed that fungal communities differed in the soils of ginseng seedlings under different ages and developmental stages (Fig. 2 and Supplementary Information Fig. S1). In the vegetative stage, the first principal component axis (33.94% contribution) of the fungal communities in the soils of 4-y seedlings markedly differed from those in the soils of 2-y seedlings. Moreover, the second principal component axis (17.55% contribution) of the fungal communities in the soils of 2-y seedlings considerably differed from those in the soils of 3-y seedlings (Fig. 2E). At the flowering and fruiting stages, the first principal components (25.71% and 38.96% contribution, respectively) in the fungal communities in the soils of 2-y ginseng seedlings significantly differed from those in the soils of 3- and 4-y seedlings (Fig. 2F and G). During root growth, the first principal component (26.97% contribution) of fungal communities in the soils of 3-y seedlings considerably differed from those in the soils of 2- and 4-y seedlings, and the second principal component (14.93% contribution) in fungal communities in the soils of 2-y

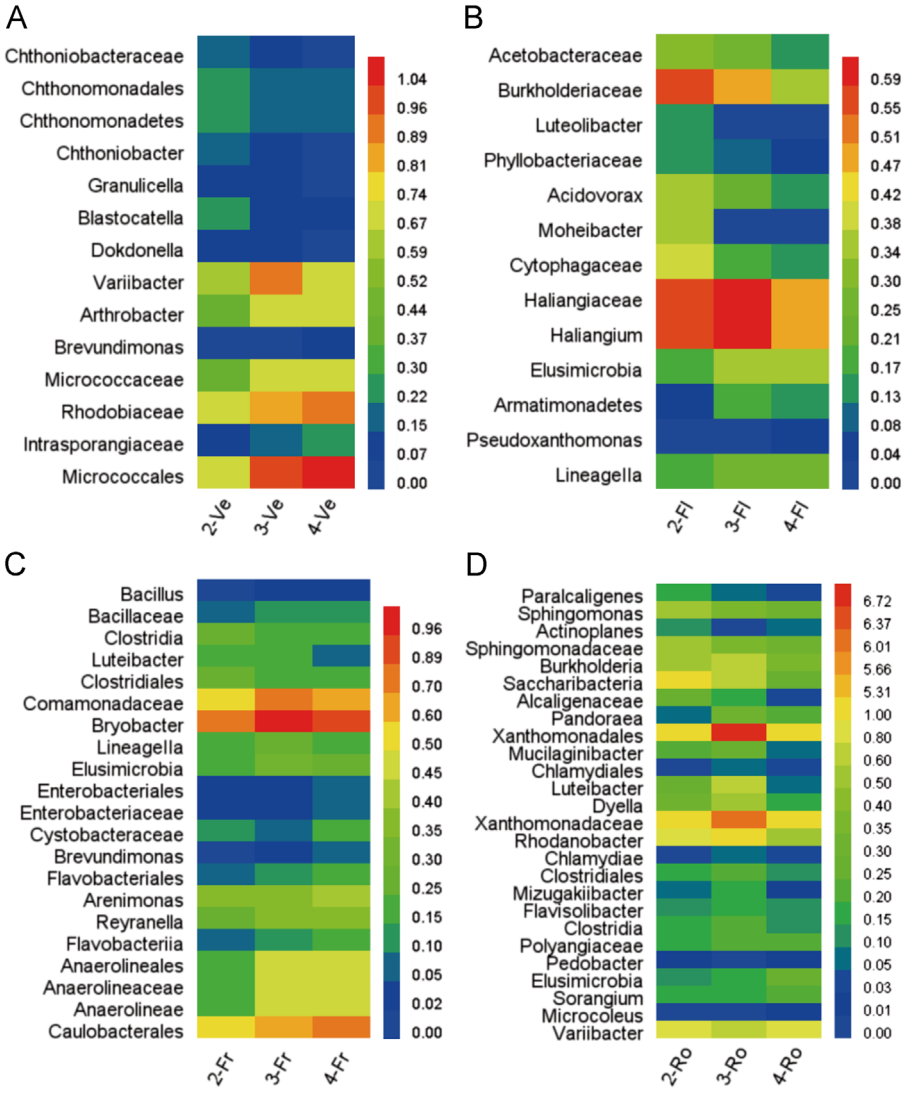

Figure 3 Relative abundance of the bacterial taxa detected by the linear discriminant analysis effect size (LEfSe) as biomarker. (A)–(D) represent the vegetative, flowering, fruiting, and root growth stages, respectively. 2-, 3- and 4- represent the samples in the rhizosphere of 2-, 3-, and 4-y transplanted ginseng seedlings. Data represent the mean values of $n=3$.

seedlings differed from those in the soils of 4-y seedlings (Fig. 2H). LEfSe analysis revealed that fungal composition differed in the soils of seedlings at different ages and developmental stages (Supplementary Information Fig. S3).

3.3. Changes in the relative abundance of bacterial taxa

The relative abundance of bacterial groups changed in the soils of ginseng plants of different ages and developmental stages (Fig. 3). The relative abundance of Chthoniobacteraceae, Chthonomonadales, Chthonomonadetes, *Chthoniobacter*, *Granulicella*, and *Blastocatella* decreased with plant age during the vegetative stage (Fig. 3A). The relative abundance of *Arthrobacter*, *Brevundimonas*, Micrococcaceae, Rhodobiaceae, Intrasporangiaceae, and Micrococcales was significantly higher in the soils of 3- and 4-y seedlings than that in the soils of 2-y seedlings. The relative abundance of *Luteolibacter*, Phyllobacteriaceae, *Acidovorax*, *Moheibacter*, and Cytophagaceae in the soils of 3- and 4-y seedlings decreased, and the abundance of Elusimicrobia and Armatimonadetes drastically increased during the flowering stage (Fig. 3B). The relative abundance of *Bacillus*, Enterobacteriales, Enterobacteriaceae, *Brevundimonas*, and Anaerolineae was significantly higher in the soils of 3- and 4-y seedlings than that in the soils of 2-y seedlings; additionally, the abundance of *Luteibacter*, Clostridia, and Clostridiales decreased in the soils of 3- and 4-y seedlings in the fruiting stage (Fig. 3C). The relative abundance of *Paralcaligenes*, Sphingomonadaceae, Saccharibacteria, *Sphingomonas*, and Alcaligenaceae significantly decreased with plant age, whereas that of *Pandoraea*, Chlamydiales, and Chlamydiae was higher in the soils of 3- and 4-y seedlings than that in the soils of 2-y seedlings at the root growth stage (Fig. 3D).

3.4. Changes in the relative abundance of fungal taxa

The relative abundance of fungal taxa changed in the soils of ginseng plants of different ages and developmental stages (Fig. 4). The relative abundance of Cystofilobasidiales, Ophiostomataceae, *Ophiostoma*, Ophiostomatales, and Cystofilobasidiaceae significantly decreased with seedling age, and the abundance of Pezizales, Cantharellales, *Dendryphion*, Pezizomycetes, and Tubeufiaceae was higher in the soils of 4-y seedlings than that in the soils of 2- and 3-y seedlings at the vegetative stage

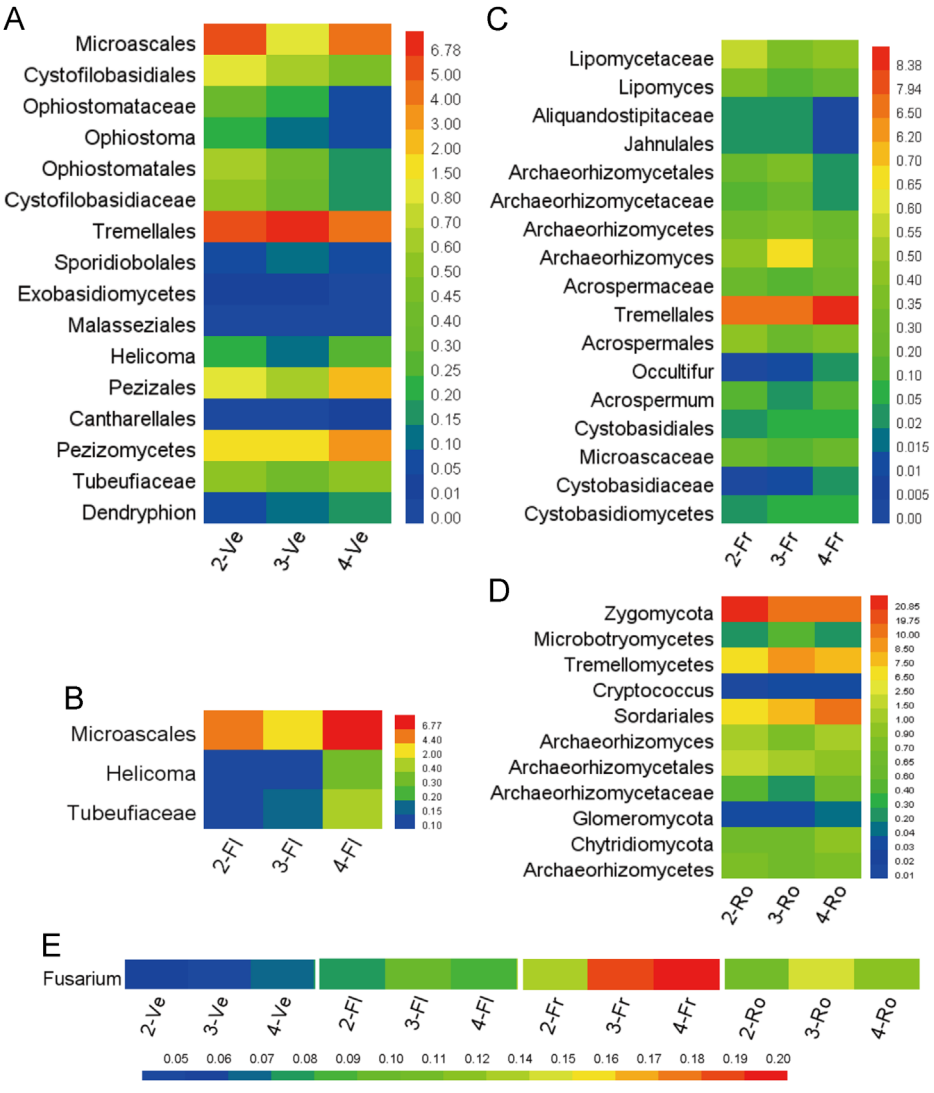

Figure 4 Relative abundance of the fungal taxa detected by LEfSe as biomarker and *Fusarium*. (A)–(D) represent the vegetative, flowering, fruiting, and root growth stages, respectively. (E) Relative abundance of *Fusarium*. Data are presented as mean ± SD ($n = 3$).

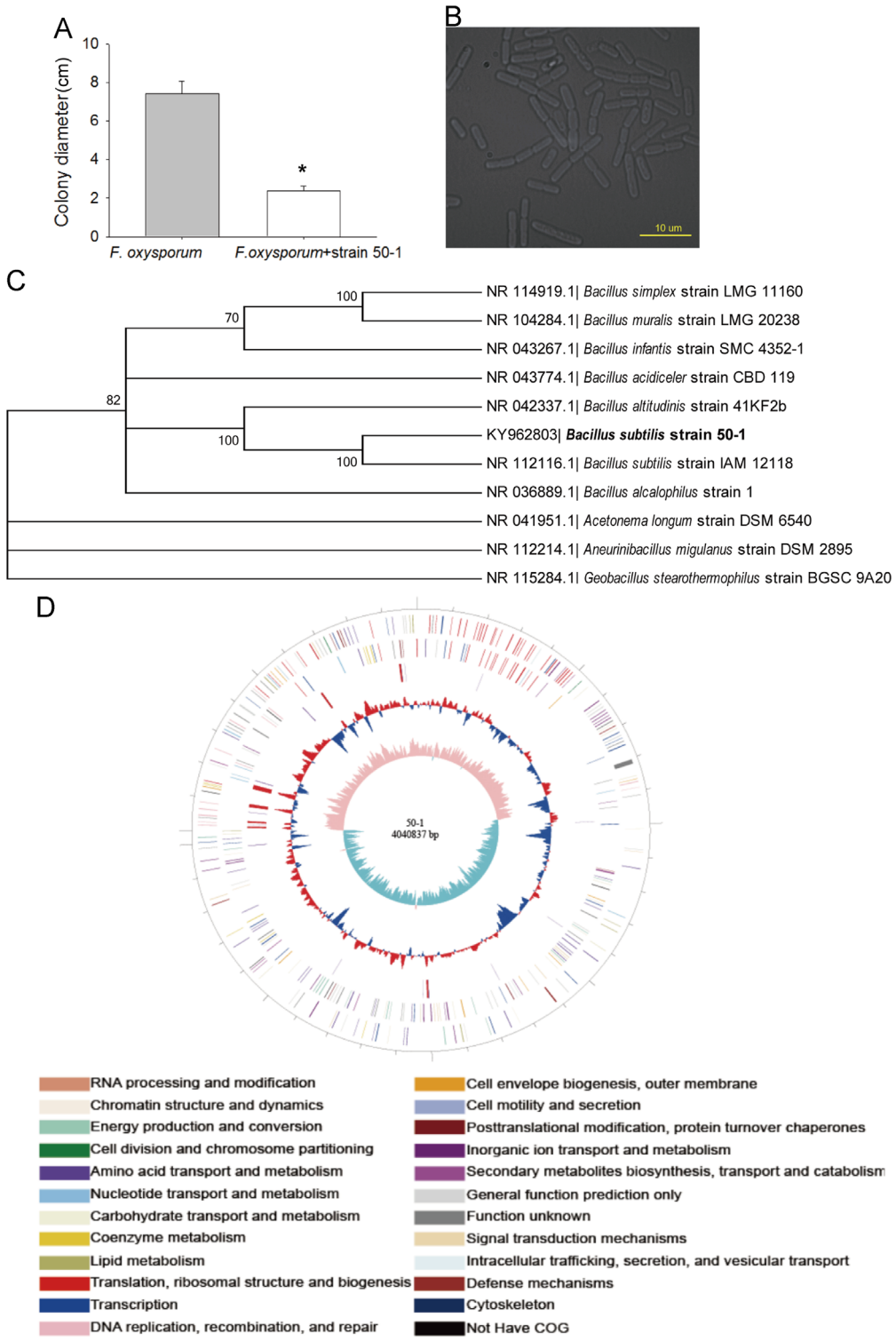

Figure 5 *Bacillus subtilis* 50-1 antagonized *Fusarium oxysporum*. (A) Colony diameter measured in a dual culture. (B) Morphological features of bacterium 50-1. (C) Relationships of 16S rRNA sequences between *B. subtilis* strain 50-1 (black body) and published 16S rDNA sequences. (D) Genome map of strain 50-1. The six circles (outer to inner) represent the scale line, forward strand CDSs (color by COG categories), reverse strand CDSs (color by COG categories), RNA genes, GC content, and GC skew. From outside to center: genome size, genes on the forward strand (color by COG categories), genes on the reverse strand (color by COG categories), RNA genes (tRNAs, orange; rRNAs, red), GC content (red and blue), and GC skew. Only bootstrap values higher than 70% are shown. Bars represent the mean $\pm$ SE ($n=3$). Asterisks denote significant differences between the colony diameters of *F. oxysporum* and *F. oxysporum* + strain 50-1 at $P < 0.05$.

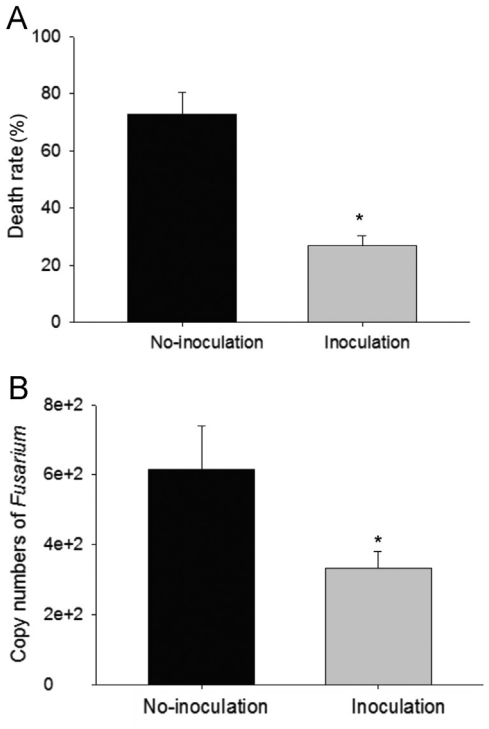

Figure 6 Inoculation of strain 50-1 in ginseng replanting soils. (A) Death rate of ginseng after inoculation with strain 50-1. (B) Copy numbers of *Fusarium* in soils. No-inoculation and inoculation represent the samples inoculated with cultures containing inactivated and activated strain 50-1, respectively. Data are presented as mean ± SD ($n = 3$). Asterisks denote significant differences between the no-inoculation and inoculation at $P < 0.05$.

(Fig. 4A). The relative abundance of Microascales, *Helicoma*, and Tubeufiaceae was higher in the soils of 4-y seedling than those in the soils of 2- and 3-y seedlings at the flowering stage (Fig. 4B). The relative abundance of Tremellales, Acrospermales, *Occultifur*, *Acrospermum*, Cystobasidiales, Cystobasidiaceae, and Cystobasidiomycetes was markedly higher in the soils of 4-y seedlings than those in the soils of 2- and 3-y seedlings in the fruiting stage (Fig. 4C). The relative abundance of Zygomycota was lower in the soils of 3- and 4-y seedlings than those in the soils of 2-y seedlings; in addition, the abundance of Tremellomycetes, Chytridiomycota, and Sordariales significantly increased in the soils of 4-y seedlings than those in the soils of 2- and 3-y seedlings at the root growth stage (Fig. 4D).

High-throughput sequencing analysis revealed that the relative abundance of *Fusarium* increased in the soils of adult ginseng plants (Fig. 4E). The abundance of *Fusarium* increased by 22.5%–25.0%, 35.7%–50.0%, and 18.2%–36.4% in the soils of 3- and 4-y seedlings in the flowering, fruiting, and root growth stages, respectively, relative to that in the soils of 2-y seedlings at equivalent growth stages. The results of qPCR analysis showed that the abundance of *Fusarium* also showed similar trends as those revealed by high-throughput sequencing analysis (Supplementary Information Fig. S4).

3.5. *Bacillus subtilis 50-1 was responsible for the biocontrol of F. oxysporum*

Dual culture techniques were used to isolate microbial antagonists against *F. oxysporum* for the control of ginseng root rot (Fig. 5). Antagonistic bacterium, namely, *B. subtilis* 50-1, was isolated. This strain exhibited a broad spectrum of growth inhibition activity against *F. oxysporum*, thereby resulting in 67.8% inhibition percentage (Fig. 5A). Strain 50-1 is a gram-positive, oxidase- and catalase-positive, rod-shaped bacterial species (Fig. 5B and Supplementary Information Table S4). The analysis of the 16S rRNA sequences revealed that strain 50-1 belonged to *B. subtilis* with the bootstrap value of 100% (Fig. 5C). Strain 50-1 was further evaluated in accordance with genome sequencing. The complete genome of strain 50-1 comprised a circular chromosome of 4,040,837 bp in length with 43.86% GC content (Fig. 5D and Supplementary Information Table S5). The total numbers of genes were 4,193, which covered 88.6% of the genome and encoded 3,176 proteins. COG function classification revealed that strain 50-1 has roles in amino acid transport and metabolism; carbohydrate transport and mechanism; and secondary metabolite biosynthesis, transport, and catabolism (Supplementary Information Fig. S5).

3.6. *Inoculation of biocontrol bacteria decreased ginseng death rate in replanting soil*

Analyzing the results of the pot experiment revealed that the death rate of ginseng and the relative abundance of *Fusarium* significantly decreased by 63.3% and 46.1% in replanting soils inoculated with strain 50-1, respectively (Fig. 6). Furthermore, the height and leaf area of the plants increased by 62.7% and 22.5%, respectively (Supplementary Information Fig. S6). These results revealed that inoculation with biocontrol bacteria alleviated the ginseng replanting problem.

4. Discussion

The replanting problem is a common and severe issue faced in the cultivation of medicinal plants. The biomass and tumor quality of *Rehmannia glutinosa* decrease as a result of replanting problems[42]. The survival rate of ginseng seedlings is lower than 25% after 3 years of replantation[43]. The replanting problem is caused by multiple factors, and changes in the soil microbial community influence soil health and crop yield[44,45]. The composition of the soil microbial community is governed by plant species and growth[46]. We previously used high-throughput sequencing technology to show that microbial diversity and composition change in soils cultivated with American ginseng relative to those in soils cultivated with traditional crops[16]. In the present study, we revealed that ginseng seedlings of different ages and developmental stages drive changes in the rhizospheric microbial community. We also screened for antagonistic bacteria against root rot and confirmed that biological control is a remarkably potent approach to decreasing the occurrence of root rot.

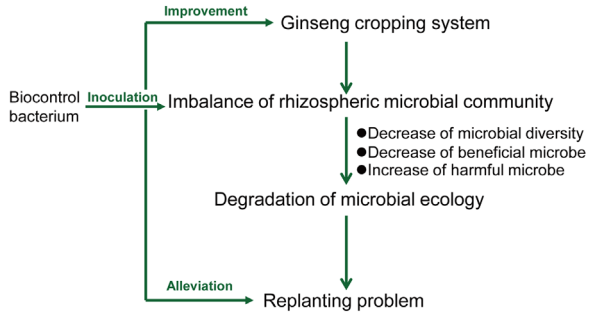

Figure 7 Schematic model demonstrated ginseng plants droved the imbalance of microbial community and biological bacterium alleviated replanting problem.

The *H′* and Chao1 values obtained in this study revealed that bacterial diversity was low, whereas fungal diversity was high, in the rhizosphere of adult ginseng seedlings in the root growth stage. A similar study reported that increasing ginseng cultivation ages decreases bacterial diversity and increases fungal diversity[12]. The diversity of microbial communities in the rhizosphere of *Pseudostellaria heterophylla* decreases with the increasing number of cropping years[47]. The developmental stage of crops is an important drive of microbial community structure[17]. Additionally, microbial diversity is critical to the maintenance of soil health and quality, as well as serves as a sensitive bioindicator of soil health[48]. For example, the death rate of notoginseng and fungal diversity are significantly and negatively correlated, suggesting that fungal diversity is a potential bioindicator of soil health[14]. Microbial diversity and root disease suppression are related[49,50]. The decrease in bacterial diversity in response to adult plants in the root growth stage is a possible indicator of ecological variations and functional impairment.

The microbial compositions of the rhizospheres of ginseng plants at different ages and developmental stages were different. Changes in microbial dynamics occur in the rhizosphere during ginseng growth[4]. Soybeans in the vegetative stage of growth affect the structure of bacterial communities, with bacterial communities are likely further altered during later growth stages[51]. Microbial community structures are highly divergent during the young plant stage of tomato but become homogeneous during the flowering and senescence stages of the plant[46]. In our study, the relative abundance of microbial community decrease, such as *Luteolibacter*, Cytophagaceae, *Luteibacter*, *Sphingomonas*, and Sphingomonadaceae, while that of *Brevundimonas*, Enterobacteriaceae, *Pandoraea*, Cantharellales, *Dendryphion*, *Fusarium*, and Chytridiomycota increased in the soils of adult ginseng plants compared with those in the soils of 2-year-old seedlings. Another paper reported similar results, *i.e.*, the population of microbe decreases, whereas that of microorganisms increases with the increasing number of cropping years[47]. The soil microbial community is an important bioindicator of soil function[52]. Changes in functional groups revealed that the microecological environment of the rhizosphere gradually degrades with the increasing age of ginseng.

Soil characteristics and plant species can influence the rhizospheric microbial community[53,54]. In our study, field-grown ginseng plants appeared healthy without any signs of disease during the growing season (Supplementary Information Fig. S7). The pH, available *K*, and organic matter contents of rhizosphere soils from ginseng plants of different ages and developmental stages were not significantly different (Supplementary Information Table S5). Plant species could influence rhizobacterial communities[55]. Root exudates are a main drive of the changes in rhizospheric microbial communities during plant growth[53]. The richness of the rhizospheric microbial community in an *Arabidopsis* system is enhanced by the high cumulative levels of sugars secreted during the early developmental stages of the plant[56]. Root exudates also contain allelochemicals that disturb the balance of a microbial community[15]. Our results showed that the diversity of rhizospheric microbial communities markedly changed as the ginseng plants entered the root growth stage. This phenomenon likely resulted from the influence of different root types and root exudates. The composition of *Arabidopsis* root exudates changes throughout the plant development; for example, root exudates produced by tomato plants during the reproductive stage are more phytotoxic than those produced during the vegetative stage[57,58]. The ginseng root rapidly grows after 3 years of cropping prior to harvest, especially during the root growth stage. The root growth stage of ginseng is possibly characterized by a specific but distinctive root exudation pattern that drives different bacterial communities.

In the present study, *B. subtilis* 50-1 was isolated and acted as an effective antagonist against *F. oxysporum*. Inoculation results revealed that the biocontrol bacterium decreased ginseng morbidity and alleviated the replanting problem. Numerous studies have reported that *Bacillus* strains are potent biological control agents of plant diseases[22,59]. The biocontrol efficacy of *B. amyloliquefaciens* 54 against bacterial fruit blotch has been proven in greenhouse experiments[60]. *B. megaterium* (B5), *B. cereus* sensu lato (B25), and *Bacillus* sp. (B35) exhibit antagonistic activity against *F. verticillioides*[61]. Bioactive constituents produced by microbial strains can attenuate the negative effects of pathogens and abiotic stresses on plants[22]. Thus, microbial antagonists could be used for the efficient and environmentally friendly control of plant pathogens. Moreover, the genome sequencing analysis of *Ganoderma lucidum* revealed key genes that encode for cytochrome P450s, which are involved in secondary metabolism[62]. The genome sequence of strain 50-1 would help provide insight into the pathways of functional bacteria and facilitate their exploration. However, information related to the biocontrol functions of strain 50-1 requires further analysis.

5. Conclusions

Ginseng cropping induced changes in rhizospheric microbial communities and decreased bacterial diversity. These effects could collectively cause microecological degradation, which consequently results in replanting problems. However, inoculation with a biocontrol bacterial strain alleviated the replanting problem and improved the growth of ginseng (Fig. 7). Given that the replanting issues that underlie this work are common to many perennial medicinal plants, our work provides crucial insight into the replanting problem within the framework of rhizospheric microbial dynamics. Moreover, we confirmed that inoculation with microbial antagonists is an effective soil bioremediation method that alleviates the replanting problem associated with Chinese medicinal plants.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.apsb.2017.12.011.

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