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Spatiotemporal biocontrol and rhizosphere microbiome analysis of Fusarium wilt of banana

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Abstract

The soil-borne fungus *Fusarium oxysporum* f. sp. c*ubense* tropical race 4 (*Foc* TR4) causes Fusarium wilt of banana (FWB), which devastates banana production worldwide. Biocontrol is considered to be the most efficient approach to reducing FWB. Here we introduce an approach that spatiotemporally apply *Piriformospore indica* and *Streptomyces morookaensis* strain Sm4-1986 according to their respective strength to increase biocontrol efficacy of FWB. *P. indica* successfully colonizes banana roots and promotes lateral root formation, and it also inhibits *Foc* TR4 growth inside the banana plants and reduces FWB. *S. morookaensis* strain Sm4-1986 secrets different secondary compounds, of which xerucitrinin A (XcA) and 6-pentyl-α-pyrone (6-PP) show the strongest anti-*Foc* TR4 activity. XcA is able to chelate iron, an essential nutrient in pathogen-plant interaction that determines the output of FWB. 6-PP, a volatile organic compound, inhibits *Foc* TR4 germination and promotes banana growth. Biocontrol trials in the field demonstrated that application of *S. morookaensis* improves soil properties and increases soil rhizosphere-associated microbes that are beneficial to banana growth, and thus significantly reduces disease incidence of FWB. Our study suggests that optimal utilization of the different biocontrol strains increases efficacy of biocontrol and that operating the iron accessibility in the rhizosphere is a promising strategy to control FWB.

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Introduction

Bananas (*Musa* ssp.), originated in Southeast Asia and the Western Pacific[1,2], are now widely distributed throughout the humid tropics and sub-tropics where they provide a staple food for about 400 million people in the developing countries in Africa, Asia, and Latin America[3]. Bananas are the most exported fruit in the world, having production of 129 million tons and export trade value of 13.6 billion dollars in 2019[4].

Fusarium wilt of banana (FWB) caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*) is one of the most destructive disease in banana production worldwide, which has restricted banana production for more than a century[5]. The epidemic of FWB leads to the almost complete replacement of the *Foc* race 1 susceptible Gros Michel with the resistant Cavendish, which currently covers ca. 40% of the global production and may be well the only banana present on supermarket shelves of non-producer countries[3,6]. However, a newly emerged race of *Foc*, tropical race 4 (TR4), is virulent not only on Cavendish but also on all other banana cultivars. *Foc* TR4 caused serious losses in banana plantation worldwide, which resulted in abandonment of thousands of hectares of banana orchards. Currently, there are no effective methods to control FWB caused by *Foc* TR4.

The *Foc* pathogen can linger in soil for up to 30 years even in the absence of plant hosts, which makes it particularly difficult to be eliminated from the infected soil[7,8]. As being a vascular pathogen, *Foc* colonizes banana roots and reaches the vascular bundles[3], leading to the ineffectiveness of chemical control. Repeated use of chemical reagents has raised great concern for environmental pollution and human health. Breeding resistant cultivars is thought to be the most effective way to control FWB, but all commercial banana varieties tested are susceptible to *Foc* TR4, and they are propagated by cloning due to the nature of sterile triploid[9]. Thus, biological control of FWB has gained great interest[10].

Piriformospora indica is a well-known endophytic fungus that colonizes roots of a broad spectrum of plant species and confers diverse beneficial effects on host plants by promoting growth or enhancing disease resistance[11-13]. Besides, it also acts as a

biocontrol agent against plant pathogens including *Fusarium culmorum* and *Verticillium dahliae*[14,15]. Streptomyces are naturally abundant in soils, and it is likely that they will cause less damage to the surrounding ecosystem when applied. Many *Streptomyces* species have been used as biocontrol agents to protect plants against various diseases due to their ability to produce a range of secondary metabolites that can either inhibit the growth of phytopathogens or promote plant growth[16-18]. Additionally, application of Streptomyces improved the soil microbial communities and enhanced plant resistance to pathogens[19]. Rhizosphere is an important interface involved in exchange of resources including nutrients, compounds, etc. between the plants, the soil environment, and the microbes. It has been known that competition for essential nutrients such as iron in the rhizosphere is a crucial factor that determines the survival of microbes[20]. Rhizosphere microbial organisms that produce growth-inhibitory siderophores could suppress the pathogen growth and thus protect plants against pathogen infection[21]. The approach that takes advantage of the ability of microbes in restricting pathogens access to the essential nutrients is an effective strategy for biocontrol. In addition, the procedure of how to use biocontrol agents affects biocontrol efficacy when different biocontrol agents are used together.

Here, we present a spatiotemporal approach using *Piriformospore indica* in the endophytic compartments and *Streptomyces morookaensis* in the rhizosphere to controlling FWB. *S. morookaensis* strain 4-1986 (Sm4-1986) improves soil properties, shifts rhizosphere microbial structures, and suppresses *Foc* TR4 growth by secreting active compounds when applied to the field. *P. indica* colonizes and proliferates in the intracellular spaces within the banana roots to promote lateral root formation and restrict the growth and extension of *Foc* TR4 inside the banana roots. In addition, soil properties such as pH and iron content are also important factors affecting the control of FWB.

Results

Identification of metabolic compounds suppressing *Foc* TR4. *S. morookaensis* Sm4-1986 displayed antifungal activity against *Foc* TR4[22]. We reasoned that the anti-*Foc*

TR4 activity of Sm4-1986 might be due to the secreted compounds. In PDB medium, GFP-labeled *Foc* TR4 grew well showing strong fluorescence (Fig. 1a), on the contrary, addition of the Sm4-1986 supernatant significantly suppressed the growth of *Foc* TR4 as revealed by the great reduction of GFP intensity (Fig. 1b), suggesting the antifungal activity of Sm4-1986 supernatant against *Foc* TR4.

A set of metabolic compounds was isolated from Sm4-1986 supernatant and tested for the antifungal activity[23]. Xerucitrinin A (XcA) (Fig. 1c) and 6-pentyl-α-pyrone (6-PP) (Fig. 1d) are particularly interesting because they showed the strongest anti-*Foc* TR4 activity. Further analysis showed that 3 mM XcA completely suppressed the germination of GFP-labeled *Foc* TR4 spores and greatly reduced GFP fluorescence when compared with the untreated GFP-labeled *Foc* TR4 as examined by laser confocal microscopy (Extended Data Fig. 1a, b). Since Sm4-1986 was able to produce iron-chelating siderophores[22], we then examined the iron-chelating activity of the isolated compounds. Chrome Azurol Sulphonate (CAS) agar assay showed that there was a clear yellow zone around the Oxford cup, indicating a strong iron-chelating ability of XcA (Fig. 1e).

6-PP is another important compound isolated from Sm4-1986. *Foc* TR4 grew well in PDB medium as indicated by the consistently increasing optical density, however, the optical density of *Foc* TR4 culture decreased when 6-PP (0.96 mM final concentration) were added (Fig. 1f), suggesting the inhibition of 6-PP on the growth of *Foc* TR4. To further investigate the effect of 6-PP on *Foc* TR4 growth, we counted the number of *Foc* TR4 spores under microscope. 6-PP treatment significantly reduced the number of *Foc* TR4 spores and completely inhibited spore germination (Fig. 1g). Consistent with this, confocal observation showed that GFP fluorescence was greatly diminished after 6-PP treatment (Extended Data Fig. 1c). *Foc* TR4 is able to produce fusaric acid that acts as a virulent factor to increase environmental acidity[24]. We observed the pH value of *Foc* TR4 solution under normal condition was greatly decreased after 72 h cultivation, however, the pH value of *Foc* TR4 solution treated by 6-PP did not change even after cultivation for 96 h (Extended Data Fig. 1d).

To explore morphological and structural changes of *Foc* TR4 spores under the treatments of XcA and 6-PP, we used scanning electron microscope (SEM) and transmission electron microscope (TEM) to observe the shape of *Foc*TR4 spores. The surface of *Foc* TR4 spores became wrinkled when treated with XcA (Fig. 2a) and the spores were collapsed when treated by 6-PP treatment (Fig. 2b) when compared to the normal *Foc* TR4 spores (Fig. 2c). Furthermore, SEM observation showed that treatment with 6-PP for 24 h resulted in a thinner cell wall and a massive cytoplasm without integral organelles such as mitochondria (Fig. 2d, e) when compared with the normally grown *Foc* TR4 spores (Fig. 2f, g).

Since XcA and 6-PP were isolated from Sm4-1986 and each has antifungal activity against *Foc* TR4, we hypothesized that coexistence of these two compounds may have synergistic effect on suppressing *Foc* TR4. To test this hypothesis, we used response surface methodology (RSM) to analyze the interaction between XcA and 6-PP and to examine how these two compounds synergistically affect *Foc* TR4 germination at different concentrations. RSM analyses revealed that coexistence of XcA and 6-PP at lower concentrations was able to suppress *Foc* TR4 spore germination, indicating a synergistic effect of these two compounds (Fig. 2h and Extended Data Fig. 2). Given the inhibitory effect of XcA and 6-PP on *Foc* TR4, we therefore asked what are their effects on banana growth. Experiments with banana plantlets showed that lower concentrations of 6-PP (<150 μM) promoted banana plantlet growth although higher concentrations (>200 μM) showed side effects to banana plantlets (Extended Data Fig. 3). Regarding to XcA, banana plantlets grew well when treated for 65 days by 3 mM XcA, the concentration to inhibit *Foc* TR4 germination, when compared with the control. This indicates that XcA may be non-toxic to banana plants (Extended Data Fig. 4).

***P. indica* induces lateral root formation and suppresses *Foc* TR4 growth.** *P. indica* is symbiotically associated with a variety of host plants[25]. To explore the colonization pattern of *P. indica* in banana, we observed the *P. indica*-treated banana roots under microscope. *P. indica* entered banana roots primarily through root hairs (Extended

Data Fig. 5a) and, later, crossed cortex and endodermis, and then moved to stele and aggregated at the lateral root primordium initiation sites (Fig. 3a and Extended Data Fig. 5b). In agreement with these phenomena, *P. indica*-treated banana plantlets showed more lateral roots than untreated ones (Fig. 3b, c).

Foc TR4 is able to penetrate cortical parenchyma cells and reach the vascular bundle tissues of roots[26]. Therefore, inhibiting *Foc* TR4 growth and extension in the endophytic compartments of banana roots is an important part of FWB control. To investigate the interaction between *Foc* TR4 and *P. indica*, we co-cultured these two strains and observed their overlaid hyphae. SEM images showed that *P. indica* tightly clasped *Foc* TR4 and resulted in the collapse of *Foc* TR4 hyphae, suggesting an inhibitory effect of *P. indica* on *Foc* TR4 (Fig. 3d-f).

We then examined the effects of *P. indica* on growth of banana and control of Fusarium wilt disease. Inoculation with *P. indica* (1×10^6 chlamydospores/ml) promoted the growth of banana plantlets (Extended Data Fig. 6a, b and Supplementary Table 1). On the contrary, infection with *Foc* TR4 led to the occurrence of typical Fusarium wilt disease symptoms on banana plantlets (Extended Data Fig. 6c). However, if the banana plantlets were first inoculated with *P. indica* and then infected by *Foc* TR4, they showed less disease symptoms and grew better than *Foc* TR4-treated plantlets (Extended Data Fig. 6c, d and Supplementary Table 1). In agreement with the external symptoms, investigation of the internal symptoms of banana rhizomes showed that *P. indica* treatment greatly reduced the discoloration caused by *Foc* TR4 infection (Extended Data Fig. e-h).

***P. indica* and *S. morookaensis* synergistically control FWB in field.** Experiments in greenhouse demonstrated that both *P. indica* and *S. morookaensis* are able to promote banana growth and suppress *Foc* TR4[22]. We further investigated the biocontrol efficacy of these two strains in field.

The field trials were carried out in two consecutive years in a land that had been abandoned due to the severe infection of *Foc* TR4. The field and bananas plantlets were treated with Sm 4-1986 and *P. indica*, respectively, 7 days before transplantation. By

the end of the year, banana plants in the field were scored for Fusarium wilt disease incidence by investigating both the external and the internal disease symptoms, and the total Fusarium wilt disease incidence was reduced to 11.7% (164 of the 1,400 plants showed disease).

Banana plants with Fusarium wilt disease were cut down, and the sites were treated with *S. morookaensis* strain Sm4-1986 again in the next year. Banana plantlets were treated with *P. indica* 7 days before transplantation. By the end of the year, Fusarium wilt disease incidence of the treated banana plants was 9.1% (15 of the 164 diseased plants still remained disease), and most of the *P. indica*-treated banana plants grown in the Sm4-1986 treated sites did not show Fusarium wilt symptoms (Extended Data Fig. 7a). In contrast, all the untreated banana plants grown in the hotspots showed severe Fusarium wilt symptoms (Extended Data Fig. 7b),

Improved rhizosphere microbiome and soil property during the biocontrol of FWB. We next asked how the application of biocontrol strains changes the richness and diversity of rhizosphere microbiome during the biocontrol of FWB. ACE, Chao1, and Shannon indexes revealed that the rhizosphere soil of healthy plants generally harbored richer and more diverse microbial communities than that of the diseased plants, and continuous biocontrol application further increased the microbial richness and diversity (Supplementary Table 2). To further compare the structure of microbiota in the rhizosphere between the healthy and diseased plants, we applied principal coordinate analysis (PCoA) with bray-curtis distances to analyze the microbial data. The results revealed the clusters of the microbial communities in the rhizosphere between the healthy and diseased plants were clearly separated from each other (Extended Data Fig. 8a, b), and biocontrol of FWB further improved the separation of microbial communities (Fig. 4a, b).

The linear discriminant analysis effect size (LEfSe) method was used to identify differential biomarkers in the healthy and diseased rhizosphere soils. In bacterial community, the uncultured bacteria belonging to *Acidobacteriaceae, Acetobacteraceae*, and *Gammaproteobacteria* were biomarkers for diseased plants, whereas

Sphingomonadaceae and *Gemmatimonadeceae* could be used as biomarkers for healthy plants (Extended Data Fig. 9a). In fungal community, *Hydnodontaceae*, *Trechispora*, and *Morosphaeriaceae* were biomarkers for diseased plants, in contrast, *Leptodiscella*, *Acrospermales* and *Cladorrhinum* could be used as biomarkers for healthy plants (Extended Data Fig. 9b). To gain insight into the role of important microbial species behind pathogen suppression, we identified the potential driver taxa in the microbiome networks between the case (healthy) and control (diseased) based on the NetShift analysis. *Chthoniobacter*, *Mesorhizobium*, *Dyella*, *Streptomyces*, and some uncultured bacteria in bacterial community (Fig. 4c) together with *Enterocarpus*, *Leptobacillium*, *Musidium*, and *Humicola* in fungal community (Fig. 4d) were as the keystone taxa behind pathogen suppression in the initial microbiome of diseased plants.

To explore the relationship between soil properties and occurrence of FWB in banana plants, we measured total nitrogen (TN), total phosphorus (TP), total ferrum (Fe^{3+}, TF), and pH values of the soils from the healthy and diseased plants (Supplementary Table 3). Redundancy analysis (RDA) showed that higher TN, TP, and pH were positively correlated with the healthy plants, whereas higher TF was positively correlated with the diseased plants, and soil moisture had no effects on the disease (Fig. 4e, f).

Iron is a key factor in the control of FWB. Since high TF is always positively associated with FWB, we, therefore, examined the role of iron in the control of FWB. We used ethylenediaminedi-*O*-hydroxyphenylacetic acid (EDDHA) compound, one of the most efficient iron-chelating agents, to reduce available iron in the medium[27, 28]. Addition of EDDHA (final concentration of 4 mM) completely suppressed the growth of *Foc* TR4 in comparison with the control on PDA (Fig. 5a, b). On the other hand, banana plantlets treated with the same concentration of EDDHA grew well and did not show much difference to the control (Fig. 5c, d). As expected, banana plantlets infected by *Foc* TR4 showed severe disease symptoms and died (Fig. 5e), and the presence of EDDHA protected banana plantlets from being infected by *Foc* TR4 (Fig. 5f). 8-Hydroxyquinoline (8HQ) is another well-known iron chelator, and 200 μM 8HQ

sufficiently suppressed *Foc* TR4 growth (Extended Data Fig. 10a, b). On the other hand, banana plantlets grown in the pots filled with *Foc* TR4-treated soil showed Fusarium disease symptoms (Extended Data Fig. 10c), but those plants in the pots that were treated with 200 μM 8HQ grew well and did not show Fusarium disease symptoms, indicating that the Fusarium wilt disease was successfully controlled (Extended Data Fig. 10d).

Discussion

P. indica, a versatile root endophytic symbiont, grows intracellularly in the root cortex but not in the central part of the roots beyond endodermis when associated with barley[11,29], however it is able to penetrate endodermis and reach the stele of banana roots. *Foc* TR4 penetrates the cortex parenchyma of the roots and enters the xylem catheters when it infects the banana plants[26] . If they encounter in banana roots, *P. indica* is able to restrict the growth of *Foc* TR4 and reduce the disease symptoms. This hypothesis was supported by the observations that *P. indica* is able to clasp *Foc* TR4 and application of *P. indica* on banana plantlets leads to the reduced Fusarium wilt disease. Control of *Foc* TR4 growth and extension in the endophytic compartments of bananas is an important step of the procedure for controlling FWB, and *P. indica* functions in this step and plays important roles in the control of FWB. It was reported that *P. indica* promotes plant growth by producing auxin[30,31]. The auxin-induced changes of endodermis cells are required for the initiation of lateral root primordia in underlying pericycle cells, and later the hormone auxin triggers lateral root development[32,33]. When colonized in banana roots, *P. indica* preferred to aggregate at the lateral root primordia and promote more lateral roots than untreated ones. More lateral roots enable better growing of plants, which enhances disease resistance to pathogens.

S. morookaensis was used to control FWB in the field. Analysis of the soil properties showed that iron increase is associated with higher incidence of FWB whereas pH increase is associated with the lower incidence of FWB, indicating that iron and pH are two important factors in the control of FWB. Consistent with these results,

previous reports have showed that iron competition in fungus-plant interactions is the most important mechanism for biocontrol of plant diseases[34,35]. However, banana is cultivated in tropical and subtropical areas where the soils are acidified and enriched with iron, which makes it difficult to control FWB. In these respects, any strategy that increases soil pH and/or decreases iron content may help reduce the incidence of FWB. Experiments using EDDHA and 8HQ confirmed the importance of iron in the control of FWB, which establishes a causal mechanistic link between iron utilization and FWB control. Siderophores are small molecules that can easily bind to ferric iron, restricting the accessibility to other microbes, therefore, microbial strains that produce siderophores and suppress *Foc* TR4 growth are particular attractive in the control of FWB. *S. morookaensis* strain Sm4-1986 produces different compounds that not only chelate iron but also suppress *Foc* TR4 growth. Sm4-1986 produces various compounds, of which XcA and 6-PP play important roles in control of FWB; the former not only chelates iron but also deforms *Foc* TR4 spores, and the latter promotes plant growth and inhibits *Foc* TR4 germination. Additionally, combinatorial utilization of these two compounds synergistically increases inhibition effects on the growth of *Foc* TR4, implying the efficient inhibition of *Foc* TR4 and potential utilization of these two compounds in the control of FWB. In agreement with this, application of *S. morookaensis* strain Sm4-1986 in the field greatly reduced the incidence of FWB.

The rhizosphere microbiome greatly affects the outputs of the interaction between plants and microbes. Application of a biocontrol agent may have important impacts on the composition, structure, and functionality of the rhizosphere microbiome. The biomarker microbes were significantly different after the treatment of *S. morookaensis* strain Sm4-1986. *Acidobacteriaceae* is acidophilic and extremely abundant in acidic environments, and *Acetobacteraceae* can oxidize ethanol to acetic acid in neutral or acidic environments. They are two biomarkers for the diseased plants and correspond to the low pH. However, *Cladorrhinum foecundissimum* is a biomarker for the Sm4-1986-treated healthy plants, and this endophyte increases uptake of phosphorus by plants and promotes growth of the colonized plants[36]. This result indicated that *S.*

morookaensis increased rhizosphere microbiome that are beneficial to banana growth and it is an efficient biocontrol agent to FWB.

Biocontrol is a comprehensive strategy that consists of many complex and interconnected factors which can influence the efficacy of biocontrol in the field. Combinatorial application of different strains is a good strategy to increase the biocontrol efficacy, but much attention must be paid to the procedures of how to apply the different strains. Various methods that deliver biocontrol strains to plants and soils also influences the consistency of biocontrol. Thus, optimizing the mode of delivery of biocontrol strains determines the success of biocontrol. The first layer of biocontrol is to reduce the infection rate of pathogen in the soil. *S. morookaensis* produces a set of secondary compounds with different functions in suppressing *Foc* TR4 growth and reducing *Foc* TR4 spore number, which, consequently, reduces the infection chance of banana plants in the field. The secondary layer is to restrict pathogen growth and extension in the endophytic compartments of banana plants in case the pathogen escapes the first layer and colonizes the roots. *P. indica* functions in this front and inhibits *Foc* TR4 growth in banana plants. Spatiotemporal application of *P. indica* and *S. morookaensis* to banana plants and the field is to take advantages of the characteristics of these two strains to increase biocontrol efficacy of FWB.

References

1. Davey, M. W. et al. A draft *Musa balbisiana* genome sequence for molecular genetics in polyploid, inter-and intra-specific *Musa* hybrids. *BMC Genomics* **14**, 683 (2013).
2. Wang, Z. et al. *Musa balbisiana* genome reveals subgenome evolution and functional divergence. *Nat. Plants* **5**, 810-821 (2019).
3. Bubici, G., Kaushal, M., Prigigallo, M. I., Gómez-Lama Cabanás, C. & Mercado-Blanco, J. Biological control agents against Fusarium wilt of banana. *Front. Microbiol*. **10**, 616 (2019).
4. FAOSTAT. http://www.fao.org/faostat/en/#home (2019).
5. Dusunceli, F. Global programme on banana Fusarium wilt disease: protecting

banana production from the disease with focus on tropical race 4 (TR4). Available online at http://www.fao.org (2017).

6. Dale, J. et al. Transgenic Cavendish bananas with resistance to Fusarium wilt tropical race 4. *Nat*. *Commun*. **8**, 1496-1504 (2017).

7. Buddenhagen, I. Understanding strain diversity in *Fusarium oxysporum* f. sp. *cubense* and history of introduction of 'tropical race 4' to better manage banana production. *Acta Hortic*. **828**, 193-204 (2009).

8. Maxmen, A. CRISPR might be the banana's only hope against a deadly fungus. *Nature* **574**, 15 (2019).

9. Hwang, S. C. & Ko, W. H. Cavendish banana cultivars resistant to Fusarium wilt acquired through somaclonal variation in Taiwan. *Plant Dis.* **88**, 580-588 (2004).

10. Spadaro, D. & Gullino, M. L. Improving the efficacy of biocontrol agents against soilborne pathogens. *Crop Prot*. **24**, 601-613 (2005).

11. Waller, F. et al. The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proc. Natl Acad. Sci. USA* **102**, 13386-13391 (2005).

12. Lee, Y. C. et al. Growth promotion of Chinese cabbage and Arabidopsis by *Piriformospora indica* is not stimulated by mycelium-synthesized auxin. *Mol. Plant Microbe Interact*. **24**, 421-431 (2011).

13. Ye, W. et al. Colonisation of Oncidium orchid roots by the endophyte P*iriformospora indica* restricts *Erwinia chrysanthemi* infection, stimulates accumulation of NBS-LRR resistance gene transcripts and represses their targeting micro-RNAs in leaves. *BMC Plant Biol*. **19**, 601-617 (2019).

14. Harrach, B.D., Baltruschat, H., Barna, B., Fodor, J. & Kogel, K. H. The Mutualistic fungus *Piriformospora indica* protects barley roots from a loss of antioxidant capacity caused by the necrotrophic pathogen *Fusarium culmorum*. *Mol*. *Plant Microbe Interact*. **26**, 599-605 (2013).

15. Sun, C. et al. The beneficial fungus *Piriformospora indica* protects Arabidopsis from *Verticillium dahliae* infection by downregulation plant defense responses.

BMC Plant Biol. **14**, 268-282 (2014).

16. Rose, S., Parker, M. & Punja, Z. K. Efficacy of biological and chemical treatments for control of Fusarium root and stem rot on greenhouse cucumber. *Plant Dis*. **87**, 1462-1470 (2003).
17. Olanrewaju, O. S. & Babalola, O. O. *Streptomyces*: implications and interactions in plant growth promotion. *Appl*. *Microbiol*. *Biotechnol*. **103**, 1179-1188 (2019).
18. Jing, T. et al. Newly isolated *Streptomyces* sp. JBS5-6 as a potential biocontrol agent to control banana Fusarium wilt: genome sequencing and secondary metabolite cluster profiles. *Front*. *Microbiol*. **11**, e602591 (2020).
19. Zhang, H. Y., Xue, Q. H., Shen, G. H. & Wang, D. S. Effects of actinomycetes agent on ginseng growth and rhizosphere soil microflora. *Chin. J. Appl. Ecol*. **8**, 2287-2293 (2013).
20. Hyakumachi, M. Studies on biological control of soilborne plant pathogens. *J. Gen. Plant Pathol*. **66**, 272-274 (2000).
21. Gu, S. et al. Competition for iron drives phytopathogen control by natural rhizosphere microbiomes. *Nat. Microbiol*. **5**, 1002-1010 (2020).
22. Zhu, Z., Tian, Z. & Li, J. A *Streptomyces morookaensis* strain promotes plant growth and suppresses Fusarium wilt of banana. *Trop*. *Plant Pathol*. **46**, 175-185 (2020).
23. Wu, G. Y. et al. Chemical constituents from the *Streptomyces morookaensis* strain Sm4-1986. *Nat. Prod. Res.* https://doi:10.1080/14786419.2021.1881095 (2021).
24. Liu, S. et al. Fusaric acid instigates the invasion of banana by *Fusarium oxysporum* f. sp. *cubense* TR4. *New Phytol*. **225**, 913-929 (2020).
25. Unnikumar, K. R., Sree, K. S. & Varma, A. *Piriformospora indica*: a versatile root endophytic symbiont. *Symbiosis* **60**, 107-113 (2013).
26. Dong, H. et al. Histological and gene expression analyses in banana reveals the pathogenic differences between races 1 and 4 of banana Fusarium wilt pathogen. *Phytopathology* **109**, 1029-1042 (2019).
27. Hernandez-Apaolaza, L. & Lucena, L. L. Influence of irradiation time and solution

concentration on the photochemical degradation of EDDHA/Fe^{3+}: effect of its photodecomposition products on soybean growth. *J. Sci. Food Agric*. **91**, 2024-2030 (2011).

28. Kovacs, K. et al. Revisiting the iron pools in cucumber roots: identification and localization. *Planta* **244**, 167-169 (2016).
29. Deshmukh, S. et al. The root endophytic fungus *Piriformospora indica* requires host cell death for proliferation during mutualistic symbiosis with barley. *Proc. Natl Acad. Sci. USA* **103**, 18450–18457 (2006).
30. Sirrenberg, A. et al. *Piriformospora indica* affects plant growth by auxin production. *Physiol*. *Plantarum* **131**, 581-589 (2007).
31. Varma, A., Kost, G. & Oelmüller, R. *Piriformospora indica* (Springer-Verlag Berlin Heidelberg, 2013).
32. Vermeer, J. E. M. et al. A spatial accommodation by neighboring cells is required for organ initiation in Arabidopsis. *Science* **343**, 178-183 (2014).
33. Wachsman, G., Zhang, J. Y., Moreno-Risueno, M. A., Anderson, C. T. & Benfey, P. N. Cell wall remodeling and vesicle trafficking mediate the root clock in Arabidopsis. Preprint at *bioRxiv* https://doi.org/10.1101/2020.03.10.985747 (2020).
34. Scher, F. M. & Baker, R. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness of Fusarium wilt pathogens. *Phytopathology* **72**, 1567-1573 (1982).
35. Lopez-Berges, M. S. et al. Iron competition in fungus-plant interactions: The battle takes place in the rhizosphere. *Plant Signal. Behav*. **8**, e23012 (2013).
36. Gasoni, L. & de Gurfinkel, B.S. The endophyte Cladorrhinum foecundissimum in cotton roots: phosphorus uptake and host growth. *Mycol. Res*. **101**, 867-870 (1997).

Methods

Culturing microbe. *Piriformospore indica* was a gift from Prof. Kai-Wun Yeh of Institute of Plant Biology of National Taiwan University, Taiwan. *Streptomyces morookaensis* strain Sm4-1986 was purchased from China General Microbiological

Culture Collection Center (CGMCC# 4.1986), Beijing, China. GFP-tagged *Foc* TR4 was a gift from Dr. Gan-Jun Yi of Fruit Research Institute of Guangdong Academy of Agricultural Sciences, Guangdong, China.

P. indica was propagated on PDA (potato dextrose agar). A mycelial plug (5 mm in diameter) was placed in the center of a PDA plate and cultured in an incubator at 28 ℃ in the dark for a week. *P. indica* on PDA plate was refreshed once a month. For liquid culture, a small mycelial plug (5 mm in diameter) was punched from mycelium margin of the stock plate and cultured in a 250 ml Erlenmeyer flask containing 100 mL PDB (potato dextrose broth) at 28 ℃ with a rotation speed of 200 rpm for 7 days. The culture was harvested and homogenized in a blender, and then filtered with cheese cloth. Spore number was determined using a Malassez hemocytometer and the filtrate solution was adjusted to 1 x 10^6 chlamydospores/ml for use. To observe lateral root formation, we grew banana plantlets in glass bottles containing 1/2 Hoagland medium[37] for a month. *P. indica* inoculum of 1 x 10^6 chlamydospores/ml was used to inoculate the banana plants. To observe *P. indica* colonization patterns in banana roots, we used *P. indica* solution of 1 x 10^5 chlamydospores/ml. For field trials, five mycelium plugs were cultured in a 1000 ml Erlenmeyer flask containing 500 ml PDB for 10 days, and then the whole culture was collected and diluted by 5 times with water for use. Banana plantlets were placed into the solution by dipping the roots for 5 sec, and then transplanted 7 days later.

S. morookaensis strain Sm4-1986 was propagated on PDA medium at 28 ℃ in the dark for a week. A mycelium plug (5 mm in diameter) from the margin of a growing colony of *S. morookaensis* strain Sm4-1986 was inoculated into 200 ml PDB in a 500 ml Erlenmeyer flask and cultured at 28 ℃ in a rotary shaker at 200 rpm for 10 days. The fermentation liquid was collected for banana plantlet inoculation. For field trials, five mycelium plugs (1 cm in diameter) were inoculated into 500 ml PDB in a 1000 ml Erlenmeyer flask and cultured at 28 ℃ in a rotary shaker at 200 rpm for 10 days. The whole fermentation broth was collected and diluted by 3 times with water and then applied to the field.

Foc TR4 and a GFP-tagged *Foc* TR4 displayed similar growth characteristics and virulence to bananas were used[38]. *Foc* TR4 was used for inoculating banana plantlets and GFP-tagged *Foc* TR4 for observing the fluorescence. *Foc* TR4 or GFP-tagged *Foc* TR4 was cultured on PDA at 28 ℃ in the dark for 7 days. A mycelium plug (5 mm in diameter) from the margin of a growing colony of *Foc* TR4 or GFP-tagged *Foc* TR4 was put in a 250 ml Erlenmeyer flask containing 100 mL PDB and cultured at 28 ℃ with a rotation speed of 200 rpm for 5 days. The culture was filtered with cheese cloth, and the number of conidia in the filtrate was counted using a Malassez hemocytometer and adjusted to 1 x 10^6 conidia/ml for use.

Banana material. All banana plantlets used in this study are Cavendish cultivar 'Brazilian' banana plants (*Musa acuminate* L. AAA group)[39], which were purchased from the Center of Tissue Culture, South China Botanical Garden, Guangzhou, China. Banana plantlets at different growth stages were used in different experiments.

Compound isolation. *S. morookaensis* strain 4-1986 was fermented in PDB on a large-scale at 28 ℃ for a week with a rotation speed of 200 rpm. Xerucitrinin A, 6-pentyl-α-pyrone, and other compounds were isolated from the fermentation broth. Compound classification and structure elucidation were described in detail by Wu et al.[23].

Interaction between *P. indica* and *Foc* TR4. *P. indica* and *Foc* TR4 were co-cultured on a glass slide, which was first covered by a thin layer of PDA. A mycelium plug of *P. indica* was placed on one end of the glass slide that was covered by a petri dish which was then cultured in an incubator at 28 ℃ for 3 days. After that, a *Foc* TR4 plug was placed on the other end of the glass slide, and the petri dish was covered and incubated at 28 ℃ for another 4 days. During the period of cultivation, the leading hyphae of *P. indica* and *Foc* TR4 could meet somewhere between the two plugs. A small disc containing the overlapped hyphae of *P. indica* and *Foc* TR4 was sliced out from the place where they met and then subjected to scanning electron microscope observation[40]. Hyphae from the individually cultured *P. indica* and *Foc* TR4 were used as controls.

Effects of XcA and 6-PP on morphology of *Foc* TR4 spores. A plug of *Foc* TR4 mycelia was cultured in 50 ml PDB in a 100 ml Erlenmeyer flask shaken in a rotary

shaker with 200 rpm at 28 °C for 5 days. The culture was filtered using sterile gauze, and the filtrate was centrifuged at 1000 rpm for 5 min to collect conidia, which were then resuspended in PDB and adjusted to 1×10^7 conidia/ml. Aliquot 200 μl of the conidial suspension into the cells of a 96-well plate. XcA or 6-PP was added to the cells containing *Foc* TR4 conidia to make up the final concentrations of 3 mM for XcA and 0.96 mM for 6-PP. Each treatment was repeated three times. After 72 hr incubation at 28 °C in darkness, conidia were subjected to scanning electron microscope observation[41].

Effects of 6-PP on ultrastructure of *Foc* TR4 spores. *Foc* TR4 mycelia were cultured in 1000 ml PDB at 28 °C for 5 days. The culture was filtrated with sterile gauze, and the filtrate was centrifuged at 1000 rpm for 5 min to collect conidia, which were then resuspended in 1000 ml PDB and adjusted to 1×10^7 conidia/ml. 6-PP was added to the culture making up the final concentration of 6-PP at 0.96 mM. The conidial culture was incubated at 28 °C for 24 h in darkness and then centrifuging at 3000 rpm for 10 min at 4 °C to collect spores[42]. The collected conidia were subjected to transmission electron microscope observation.

Effects of XcA and 6-PP on growth of banana plantlets. Micro-propagated banana plantlets were grown on 1/2 MS in glass tubes containing either 3 mM XcA or different concentrations of 6-PP. The glass tubes were put in the tissue culture room at 26 ℃ with a photoperiod of 14/10 h light/dark. Banana plantlets were observed and photographed at the indicated time.

Scanning electron microscope. Conidial samples or mycelial discs were fixed in a solution containing 2.5% glutaraldehyde and 2% paraformaldehyde for 2 h, and then subjected to vacuum pumping to let samples sink to the bottom of tube. After that, the samples were stored in a refrigerator for 12 h. Pouring off the solution and washed samples three times with 0.1 M phosphate buffer (80 g NaCl, 32.3 g $Na_2HPO4\cdot12H_2O$, and 4.5 g $NaH_2PO4\cdot2H_2O$ in 1000 ml ddH_2O, pH 7.2) at 4 ℃; 40 min per washing. Washed samples were vapor-fixed with 1% (w/v) aqueous osmium tetroxide for 2.5 h at room temperature, and then followed by washing three times with 0.1 M phosphate

buffer at 4 ℃, and each time for 5 min. After washing, samples were subjected to gradient dehydration in different concentrations of ethanol as in the order of 30%, 50%, 70%, 80%, and 90% at 4 ℃; each for 10 min, and finally kept in 100% ethanol for 50 min. At the end of dehydration, samples were dried in a critical point dryer (Leica EM CPD300, Germany). The dried samples were sputter-coated with gold palladium in a sputter coaster (Leica EM ACE600, Germany) [42]. Conidial or hyphae morphology of *Foc* TR4 or *P. indica* was observed by SEM (JSM-6360LV, Japan).

Transmission electron microscope. 6-PP-treated *Foc* TR4 spores were fixed in the solution containing 2.5% glutaraldehyde and 2% paraformaldehyde for 3 h. The samples were then washed four times with 0.1 M phosphate buffer (80 g NaCl, 32.3 g, $Na_2HPO4{\cdot}12H_2O$, and 4.5 g $NaH_2PO4{\cdot}2H_2O$ in 1000 ml ddH_2O, pH 7.2); 15 min for each time, and then washed twice again with 0.1 M phosphate buffer; 30 min for each. Washed samples were post-fixed in 1.0% osmium tetraoxide (in 0.1 M phosphate buffer) for 4 h. The fixed samples were washed six times again with 0.1 M phosphate buffer for a period of 2 h. During this period, the washing buffer was changed every 15 min for the first four washing times and 30 min for the last two times. After washing, samples were pre-stained with 0.5% uranyl acetate (in 0.1 M phosphate buffer) for overnight. All the above steps were carried out at 4 ℃.

On the next day, spore samples were washed six times with 0.1 M phosphate buffer at 4 ℃; 15 min for the first 4 times and 30 min for the last two times. After washing, samples were dehydrated in gradient ethanol solutions (30%, 50%, 70%, 80%, 90%) at room temperature (RT) for 20 min each, and final in 100% ethanol for 30 min with one change. After dehydration, samples were passed in three changes of epoxypropane: epikote 812 (3:1 for 30 min, 1:1 for 60 min, and 1:3 for 150 min) at RT, and then in epikote 812 at RT for 3 h. Finally, samples were kept in fresh epikote 812 for overnight. In next day, samples were kept in fresh epikote 812 at RT for 7 h, and then were picked up to put in a small plastic box containing epikote 812. Samples were hardened in an oven at 60 ℃ for 12 h. Finally, the hardened blocks containing samples were sectioned into ultrathin sections of about 70 nm[43], which were observed under a transmission

electron microscope (Tecnai G2 SpiriBio TWIN).

Colonization pattern of *P. indica* in banana roots. Banana plantlets were cultured in 1/2 Hoagland medium[37] containing *P. indica* at the concentration of 1 x 10^5 chlamydospores/ml for two weeks. Banana roots were harvested and washed thoroughly with running tap water, and then cut into 1 cm segments, which were stained with 0.01% acid fuchsin-lactic acid for 5 min and destained in lactic acid for 1 min, and then sectioned with an automatic vibrating slicing machine (Leica VT1200S, Germany)[44]. The sections were observed under an optical microscope (Leica DMI3000, Germany) and images were taken.

Greenhouse experiments. Experiments with banana plantlets in pots were carried out in greenhouse at 25 ℃/18 ℃ (day/night) with a photoperiod of 14 h /10 h (light/dark). Prior to inoculation, all plantlets were maintained in greenhouse for a week to adapt environments. Plantlets with similar size were selected for inoculation. Each treatment contained 15 plantlets and was repeated three times. To inoculate plantlets, 50 ml inoculum were used for each pot. *P. indica* of 1 x 10^6 chlamydospores/ml or *Foc* TR4 of 1 x 10^6 conidia/ml was used for inoculation. 8QH was used at the concentration of 200 μM. After inoculation, banana plantlets were observed at the indicated time.

Field trials. The field trials were carried out in a land of 0.8 hectares in Longmen County (23°72'77"N, 114°25'49"E), Huizhou City, Guangdong Province, China. The small farmer had planted banana (Brazilian variety) in the land for more than 10 years and recently abandoned it because of the high incidence of Fusarium wilt caused by *Foc* TR4. Fifteen plants in a line were treated as a block, and the treatment and control blocks were arranged alternately in field. Banana transplantation holes of the treatment block were treated with *S. morookaensis* strain 4-1986 a week before transplantation. On the other hand, banana plants at the seven-leaf stage were treated with *P. indica* by submerging the roots into the inoculation solution. Banana transplantation was carried out in late February 2019. In the following days, standard irrigation and fertilization practices were applied to banana plants, and other managements were followed the normal farm operations in banana orchard. At the end of the year, external symptoms

of Fusarium wilt disease were investigated[45]. Plants showing external symptom of leaf chlorosis were cut down, and the pseudostems were chopped off to examine the internal symptoms[46]. The sites were treated with *S. morookaensis* strain 4-1986 again. New banana plants were treated with *P. indica* and transplanted into the treated hotspots in 2020. Fusarium wilt disease incidence of bananas plants was investigated at the end of the year. External and internal symptoms were examined to determine whether the plants were actually infected by *Foc* TR4.

Soil property analysis. Soil samples were collected from the evenly distributed four sites around a plant. pH values of each sample (soil material : distilled water = 1 : 10, w/v) were measured three times using a pH meter (PHS-25, Shanghai Inesa Instrument Co. Ltd., China). Total nitrogen (TN), total phosphorus (TP), and total ferrum (TF) were detected using an Elemental analyzer (PE2400, PerkinElmer, USA).

Rhizosphere microbial Illumina MiSeq sequencing. Rhizosphere soils were collected from the roots that were obtained from the evenly distributed four sites around a plant. The bacterial community composition of the rhizosphere soil was assessed by sequencing the V3-V4 region of the 16S rRNA gene using the universal primers 338F/806R (forward primers 5' -ACTCCTACGGGAGGCAGCA-3' and reverse primers 5' -GGACTACGCGGTATCTAAT-3'). The fungal ITS1 region was amplified using primer set ITS1 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3')[47]. The PCR reactions were carried out in a 50 μL reaction mixture containing 1.5 μL each primer, 1 μL dNTP, 10 μL Buffer, 0.2 μL Q5 High Fidelity DNA Polymerase, 10 μL High GC Enhancer and 40 ng soil DNA template. The PCR conditions for bacteria were initiated at 95 °C for 3 min, followed by 25 cycles of denaturation at 95 °C for 45 s, annealing at 50 °C for 45 s, and extension at 68 °C for 90 s, followed by a final elongation at 68 °C for 7 min, and then held at 4 °C. The PCR conditions for fungi were initiated at 98 °C for 2 min, followed by 30 cycles of denaturation at 98 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 1 min, followed by a final elongation at 72 °C for 5 min, and then held at 4 °C. The PCR products were pooled and visualized on 1.8% agarose gels, purified using a MinElute®

PCR Purification Kit according to the manufacturer's instructions, and quantified using QuantiFluorTM-ST (Promega, USA).

High-throughput sequencing was carried out on the Illumina MiSeq platform (BioMarker Technologies Co. Ltd, China). After pyrosequencing, raw sequences were processed with Prinseq (PRINSEQlite 0.19.5) to remove low-quality data and improve the syncretic rates of the subsequent sequence. Split sequences for each sample were merged using FLASH V1.2.7[48]. Using Usearch with a cut-off of 97% similarity, the OTUs were clustered and the taxonomic classification was performed using RDP Classifier (Version 2.2, based on Bergey's taxonomy) with the classification threshold set at 0.8. The sequences were taxonomically identified by a BLASTn search of a curated NCBI database.

Statistical analysis. All data analyses were analyzed using the SPSS 20.0 program (SPSS Inc., USA), and the significance between treatments was assigned at $P < 0.05$ using a one-way analysis of variance (ANOVA) with LSD test. Alpha diversity indices, including Chao 1, ACE, and Shannon, were calculated using the OTU table in QIIME[48].

The soil microbiome composition was ordinated by principal coordinates analysis (PCoA) using bray-curtis distance. Differences between microbiome composition of healthy and diseased plants were calculated by using PERMANOVA and ANOSIM. Bray-curtis distances are sensitive to rare OTUs and thus emphasize differences in the presence or absence of taxa[49]. R package (v3.2.0) was used to draw the graph of redundancy analysis (RDA).

Linear discriminant analysis was used to explore the most discriminating OTUs between health and diseased conditions using LEfSe[50]. Two screening criteria were used: (1) linear discriminant analysis with a score of ≥ 3.0 (healthy condition relative to diseased condition) and (2) significance test with $P < 0.05$.

We also used the NetShift method to identify potential keystone driver taxa based on differences in network interactions between healthy and diseased plant microbiome (https://web.rniapps.net/netshift)[51].

Accession numbers for sequence data. Accession numbers of the rhizosphere microbiome data determined at the end of the field experiment in 2019 (33 samples, paired end reads).

treatment	Bacterial			Fungi		
	Accession no	Paired end read 1	Paired end read 2	Accession no	Paired end read 1	Paired end read 2
Diseased	SAMN27606370	abDiseased1_1.fq	abDiseased1_2.fq	SAMN27606453	afDiseased1_1.fq	afDiseased1_2.fq
Diseased	SAMN27606371	abDiseased2_1.fq	abDiseased2_2.fq	SAMN27606454	afDiseased2_1.fq	afDiseased2_2.fq
Diseased	SAMN27606372	abDiseased3_1.fq	abDiseased3_2.fq	SAMN27606455	afDiseased3_1.fq	afDiseased3_2.fq
Healthy	SAMN27606373	abHealthy1_1.fq	abHealthy1_2.fq	SAMN27606456	afHealthy1_1.fq	afHealthy1_2.fq
Healthy	SAMN27606374	abHealthy2_1.fq	abHealthy2_2.fq	SAMN27606457	afHealthy2_1.fq	afHealthy2_2.fq
Healthy	SAMN27606375	abHealthy3_1.fq	abHealthy3_2.fq	SAMN27606458	afHealthy3_1.fq	afHealthy3_2.fq
Biotreated	SAMN27606376	abBiotreated1_1.fq	abBiotreated1_2.fq	SAMN27606459	afBiotreated1_1.fq	afBiotreated1_2.fq
Biotreated	SAMN27606377	abBiotreated2_1.fq	abBiotreated2_2.fq	SAMN27606460	afBiotreated2_1.fq	afBiotreated2_2.fq
Biotreated	SAMN27606378	abBiotreated3_1.fq	abBiotreated3_2.fq	SAMN27606461	afBiotreated3_1.fq	afBiotreated3_2.fq
Diseased	SAMN27605964	abA01_1.fq	abA01_2.fq	SAMN27606065	afA01_1.fq	afA01_2.fq
Diseased	SAMN27605965	abA02_1.fq	abA02_2.fq	SAMN27606066	afA02_1.fq	afA02_2.fq
Diseased	SAMN27605966	abA03_1.fq	abA03_2.fq	SAMN27606067	afA03_1.fq	afA03_2.fq
Diseased	SAMN27605967	abA04_1.fq	abA04_2.fq	SAMN27606068	afA04_1.fq	afA04_2.fq
Diseased	SAMN27605968	abA05_1.fq	abA05_2.fq	SAMN27606069	afA05_1.fq	afA05_2.fq
Diseased	SAMN27605969	abA06_1.fq	abA06_2.fq	SAMN27606070	afA06_1.fq	afA06_2.fq
Diseased	SAMN27605970	abA07_1.fq	abA07_2.fq	SAMN27606071	afA07_1.fq	afA07_2.fq
Diseased	SAMN27605971	abA08_1.fq	abA08_2.fq	SAMN27606072	afA08_1.fq	afA08_2.fq
Diseased	SAMN27605972	abA09_1.fq	abA09_2.fq	SAMN27606073	afA09_1.fq	afA09_2.fq
Diseased	SAMN27605973	abA10_1.fq	abA10_2.fq	SAMN27606074	afA10_1.fq	afA10_2.fq
Diseased	SAMN27605974	abA11_1.fq	abA11_2.fq	SAMN27606075	afA11_1.fq	afA11_2.fq
Diseased	SAMN27605975	abA12_1.fq	abA12_2.fq	SAMN27606076	afA12_1.fq	afA12_2.fq
Healthy	SAMN27605976	abB01_1.fq	abB01_2.fq	SAMN27606077	afB01_1.fq	afB01_2.fq
Healthy	SAMN27605977	abB02_1.fq	abB02_2.fq	SAMN27606078	afB02_1.fq	afB02_2.fq
Healthy	SAMN27605978	abB03_1.fq	abB03_2.fq	SAMN27606079	afB03_1.fq	afB03_2.fq
Healthy	SAMN27605979	abB04_1.fq	abB04_2.fq	SAMN27606080	afB04_1.fq	afB04_2.fq
Healthy	SAMN27605980	abB05_1.fq	abB05_2.fq	SAMN27606081	afB05_1.fq	afB05_2.fq
Healthy	SAMN27605981	abB06_1.fq	abB06_2.fq	SAMN27606082	afB06_1.fq	afB06_2.fq
Healthy	SAMN27605982	abB07_1.fq	abB07_2.fq	SAMN27606083	afB07_1.fq	afB07_2.fq
Healthy	SAMN27605983	abB08_1.fq	abB08_2.fq	SAMN27606084	afB08_1.fq	afB08_2.fq
Healthy	SAMN27605984	abB09_1.fq	abB09_2.fq	SAMN27606085	afB09_1.fq	afB09_2.fq
Healthy	SAMN27605985	abB10_1.fq	abB10_2.fq	SAMN27606086	afB10_1.fq	afB10_2.fq
Healthy	SAMN27605986	abB11_1.fq	abB11_2.fq	SAMN27606087	afB11_1.fq	afB11_2.fq
Healthy	SAMN27605987	abB12_1.fq	abB12_2.fq	SAMN27606088	afB12_1.fq	afB12_2.fq

Accession numbers of the rhizosphere microbiome data determined at the end of the field experiment in 2020 (33 samples, paired end reads).

treatment	Bacterial			Fungi		
	Accession no	Paired end read 1	Paired end read 2	Accession no	Paired end read 1	Paired end read 2
Diseased	SAMN27606490	bbDiseased1_1.fq	bbDiseased1_2.fq	SAMN27606507	bfDiseased1_1.fq	bfDiseased1_2.fq
Diseased	SAMN27606491	bbDiseased2_1.fq	bbDiseased2_2.fq	SAMN27606508	bfDiseased2_1.fq	bfDiseased2_2.fq
Diseased	SAMN27606492	bbDiseased3_1.fq	bbDiseased3_2.fq	SAMN27606509	bfDiseased3_1.fq	bfDiseased3_2.fq
Healthy	SAMN27606493	bbHealthy1_1.fq	bbHealthy1_2.fq	SAMN27606510	bfHealthy1_1.fq	bfHealthy1_2.fq
Healthy	SAMN27606494	bbHealthy2_1.fq	bbHealthy2_2.fq	SAMN27606511	bfHealthy2_1.fq	bfHealthy2_2.fq
Healthy	SAMN27606495	bbHealthy3_1.fq	bbHealthy3_2.fq	SAMN27606512	bfHealthy3_1.fq	bfHealthy3_2.fq
Biotreated	SAMN27606496	bbBiotreated1_1.fq	bbBiotreated1_2.fq	SAMN27606513	bfBiotreated1_1.fq	bfBiotreated1_2.fq
Biotreated	SAMN27606497	bbBiotreated2_1.fq	bbBiotreated2_2.fq	SAMN27606514	bfBiotreated2_1.fq	bfBiotreated2_2.fq
Biotreated	SAMN27606498	bbBiotreated3_1.fq	bbBiotreated3_2.fq	SAMN27606515	bfBiotreated3_1.fq	bfBiotreated3_2.fq
Diseased	SAMN27606096	bbA01_1.fq	bbA01_2.fq	SAMN27606338	bfA01_1.fq	bfA01_2.fq
Diseased	SAMN27606097	bbA02_1.fq	bbA02_2.fq	SAMN27606339	bfA02_1.fq	bfA02_2.fq
Diseased	SAMN27606098	bbA03_1.fq	bbA03_2.fq	SAMN27606340	bfA03_1.fq	bfA03_2.fq
Diseased	SAMN27606099	bbA04_1.fq	bbA04_2.fq	SAMN27606341	bfA04_1.fq	bfA04_2.fq
Diseased	SAMN27606100	bbA05_1.fq	bbA05_2.fq	SAMN27606342	bfA05_1.fq	bfA05_2.fq
Diseased	SAMN27606101	bbA06_1.fq	bbA06_2.fq	SAMN27606343	bfA06_1.fq	bfA06_2.fq
Diseased	SAMN27606102	bbA07_1.fq	bbA07_2.fq	SAMN27606344	bfA07_1.fq	bfA07_2.fq
Diseased	SAMN27606103	bbA08_1.fq	bbA08_2.fq	SAMN27606345	bfA08_1.fq	bfA08_2.fq
Diseased	SAMN27606104	bbA09_1.fq	bbA09_2.fq	SAMN27606346	bfA09_1.fq	bfA09_2.fq
Diseased	SAMN27606105	bbA10_1.fq	bbA10_2.fq	SAMN27606347	bfA10_1.fq	bfA10_2.fq
Diseased	SAMN27606106	bbA11_1.fq	bbA11_2.fq	SAMN27606348	bfA11_1.fq	bfA11_2.fq
Diseased	SAMN27606107	bbA12_1.fq	bbA12_2.fq	SAMN27606349	bfA12_1.fq	bfA12_2.fq
Healthy	SAMN27606108	bbB01_1.fq	bbB01_2.fq	SAMN27606350	bfB01_1.fq	bfB01_2.fq
Healthy	SAMN27606109	bbB02_1.fq	bbB02_2.fq	SAMN27606351	bfB02_1.fq	bfB02_2.fq
Healthy	SAMN27606110	bbB03_1.fq	bbB03_2.fq	SAMN27606352	bfB03_1.fq	bfB03_2.fq
Healthy	SAMN27606111	bbB04_1.fq	bbB04_2.fq	SAMN27606353	bfB04_1.fq	bfB04_2.fq
Healthy	SAMN27606112	bbB05_1.fq	bbB05_2.fq	SAMN27606354	bfB05_1.fq	bfB05_2.fq
Healthy	SAMN27606113	bbB06_1.fq	bbB06_2.fq	SAMN27606355	bfB06_1.fq	bfB06_2.fq
Healthy	SAMN27606114	bbB07_1.fq	bbB07_2.fq	SAMN27606356	bfB07_1.fq	bfB07_2.fq
Healthy	SAMN27606115	bbB08_1.fq	bbB08_2.fq	SAMN27606357	bfB08_1.fq	bfB08_2.fq
Healthy	SAMN27606116	bbB09_1.fq	bbB09_2.fq	SAMN27606358	bfB09_1.fq	bfB09_2.fq
Healthy	SAMN27606117	bbB10_1.fq	bbB10_2.fq	SAMN27606359	bfB10_1.fq	bfB10_2.fq
Healthy	SAMN27606118	bbB11_1.fq	bbB11_2.fq	SAMN27606360	bfB11_1.fq	bfB11_2.fq
Healthy	SAMN27606119	bbB12_1.fq	bbB12_2.fq	SAMN27606361	bfB12_1.fq	bfB12_2.fq

654 **References**

655 37. Hoagland, D.R. & Arnon, I. The water-culture method for growing plants without
656 soil. *Calif. Agric. Exp. Stat. Circ.* **347**, 1-32 (1950).
657 38. Li, C.Y. et al. Transcriptome profiling of resistant and susceptible Cavendish
658 banana roots following inoculation with *Fusarium oxysporum* f. sp. *cubense*
659 tropical race 4. *BMC Genomics* **13**, 374 (2012).
660 39. Dong, H. H., Li, Y. F., Fan, H. Y., Zhou, D. B. & Li, H. P. Quantitative proteomics
661 analysis reveals resistance differences of banana cultivar 'Brazilian' to *Fusarium*
662 *oxysporum* f. sp. *cubense* races 1 and 4. *J. Proteomics* **203**, 103376 (2019).
663 40. Rabiey, M., Ullah, I. & Shaw, M. W. The endophytic fungus *Piriformospora indica*
664 protects wheat from Fusarium crown rot disease in simulated UK autumn
665 conditions. *Plant Pathol*. **64**, 1-12 (2015).
666 41. Zuo, C. et al. The toxic mechanism and bioactive components of Chinese leek root
667 exudates acting against *Fusarium oxysporum* f. sp. *cubense* tropical race 4. *Eur. J.*
668 *Plant Pathol*. **143**, 447-460 (2015).
669 42. Benhamou, N., Rey, P., Picard, K. & Tirilly, Y. Ultrastructural and cytochemical
670 aspects of the interaction between the mycoparasite *Pythium oligandrum* and
671 soilborne plant pathogens. *Phytopathology* **89,** 506-517 (1999).
672 43. Xing, F. et al. Growth inhibition and morphological alterations of *Fusarium*
673 *verticillioides* by cinnamon oil and cinnamaldehyde. *Food Control* **46**, 343-350
674 (2014).
675 44. Brundrett, M. *Methods in Mycorrhiza Researc*h. (Mycologue Publications, 1994).
676 45. Ploetz, R. C., Haynes, J. L. & Vasquez, A. Responses of new banana accessions in
677 South Florida to Panama disease. *Crop Prot*. **18**, 445-449 (1999).
678 46. Almadhoun, H. R. & Abu-Naser, S. S. Banana knowledge based system diagnosis
679 and treatment. *Int. J. Acad. Pedag. Res*. **2,** 1-11 (2018).
680 47. Feng, J. et al. *Streptomyces griseorubens* JSD-1 promotes rice straw composting
681 efficiency in industrial-scale fermenter: Evaluation of change in physicochemical
682 properties and microbial community. *Bioresource Technol.* **321**, e124465 (2021).

48. Magoc, T. & Salzberg, S.L. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**, 2957-2963 (2011).
49. Peiffer, J. A. et al. Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proc. Natl. Acad. Sci. USA* **110**, 6548-6553 (2013).
50. Segata, N. et al. Metagenomic biomarker discovery and explanation. *Genome Biol.* 12, R60 (2011).
51. Kuntal, B. K., Chandrakar, K., Sadhu, S. & Mande, S. S. 'NetShift': A methodology for understanding 'driver microbes' from healthy and disease microbiome datasets. *ISME J.* **13**, 442-454 (2019)

Acknowledgements

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Data availability

All sequence data generated in this study have been deposited in NCBI SRA database and the accession numbers are reported in the Methods.

Code availability

All code or algorithms used in this study are published and referenced in the Methods.

Figure Legends

Fig. 1 Antifungal activity of *S. morookaensis* strain Sm4-1986 against *Foc* TR4. a, GFP-tagged *Foc* TR4 cultured in PDB for 36 h and photographed. b, The supernatant of *S. morookaensis* strain Sm4-1986 inhibits growth of *Foc* TR4. Strain Sm4-1986 was cultured in PDB for 10 days, and the supernatant of culture was collected and added to PDB (1:1, v/v), in which GFP-tagged *Foc* TR4 was cultured for 36 h and photographed. c, Structure of xerucitrinin A. d, Structure of 6-pentyl-α-pyrone. e, Xerucitrinin A chelates iron assayed by Chrome Azurol Sulphonate (CAS). There is yellow zone around the Oxford cup in the center of the plate, indicating the iron-chelating ability. f, 6-PP suppresses propagation of *Foc* TR4. *Foc* TR4 and 0.96 mM 6-PP-treated *Foc* TR4 were cultured in PDB, respectively, and the optical densities of the culture solution were monitored at the indicated time-points. Mean of triplicate and standard deviation were shown. g, 6-PP treatment significantly reduces the number of *Foc* TR4 spores and completely inhibits *Foc* TR4 spore germination. Spores were counted using a Malassez hemocytometer at 24 h of cultivation. Mean of triplicate and standard deviation was shown, **, $P < 0.01$.

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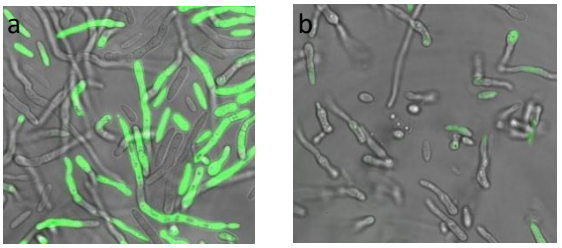

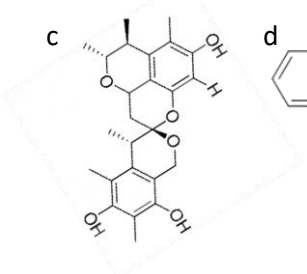

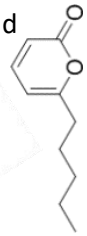

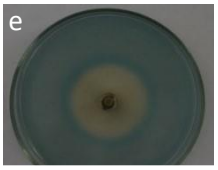

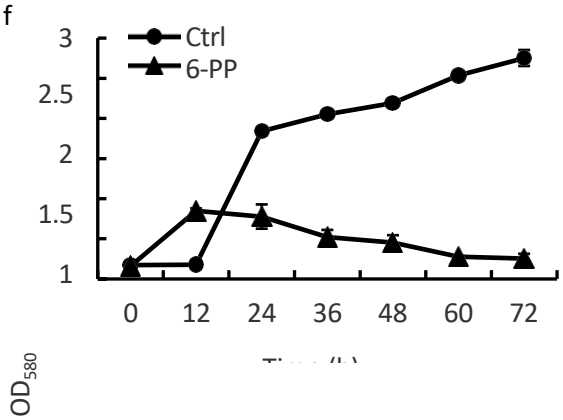

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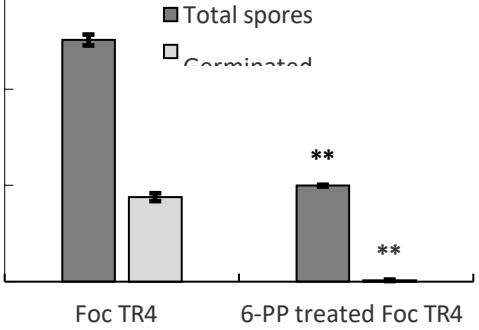

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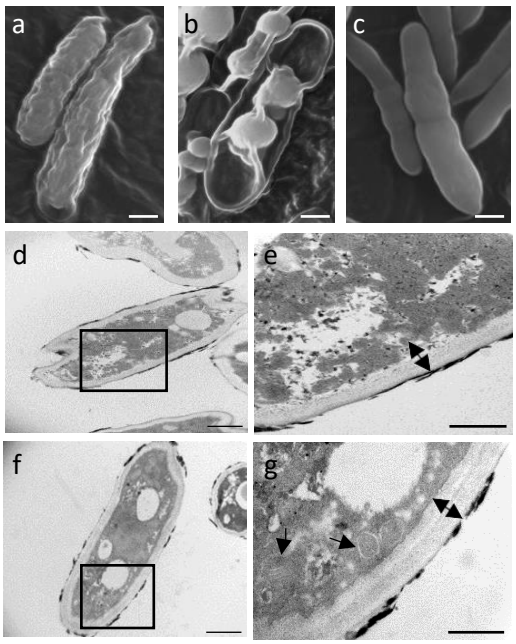

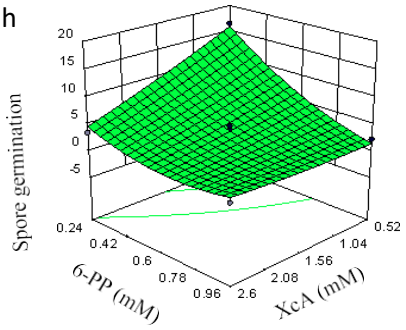

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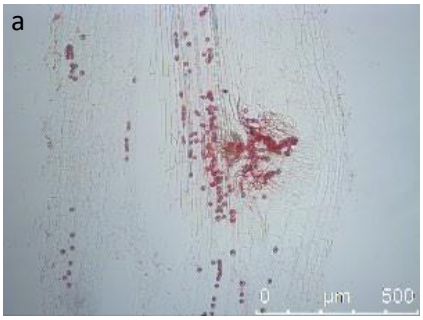
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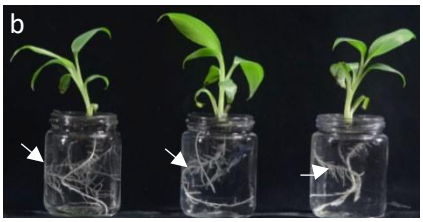
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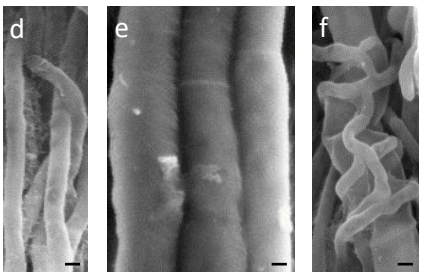
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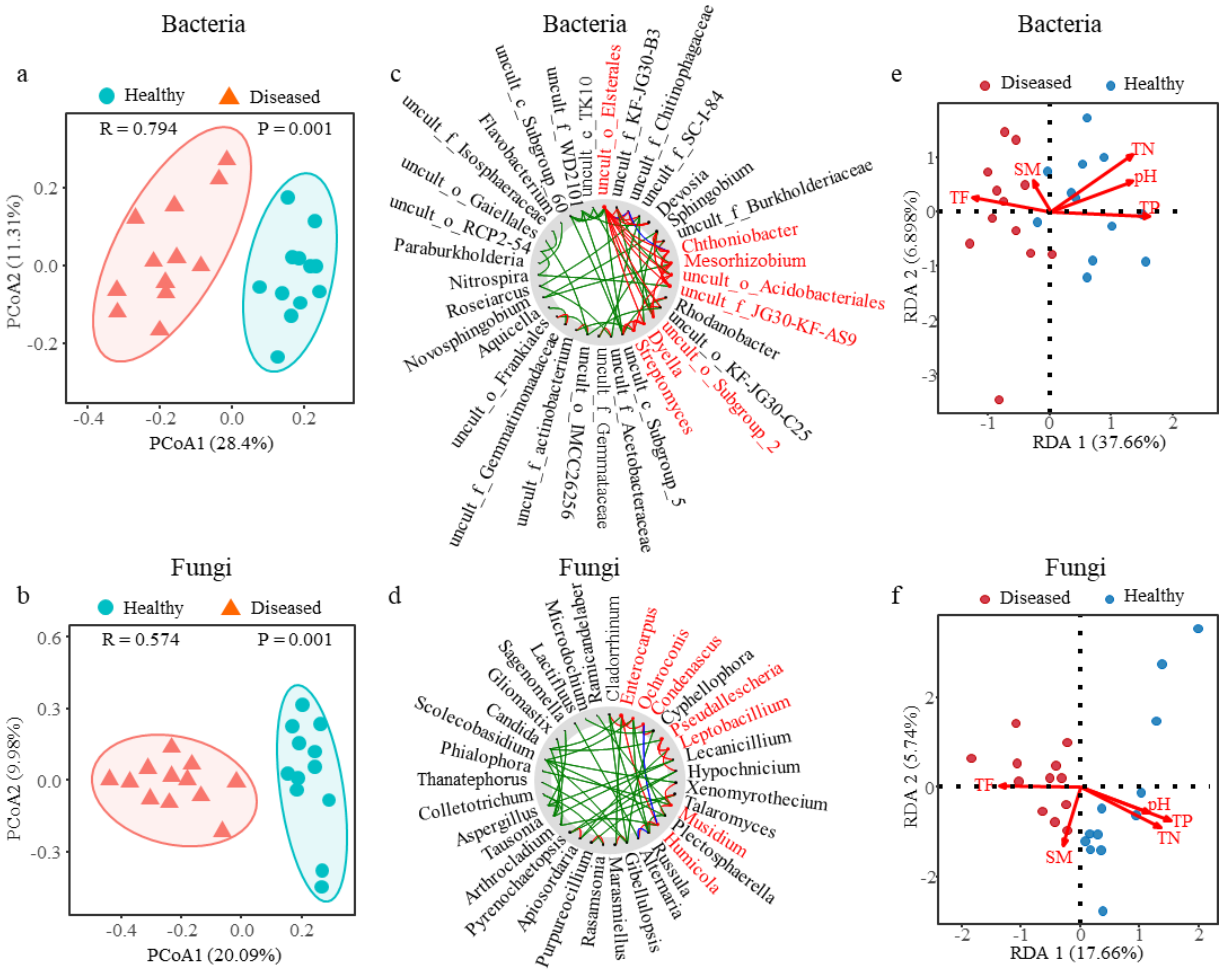

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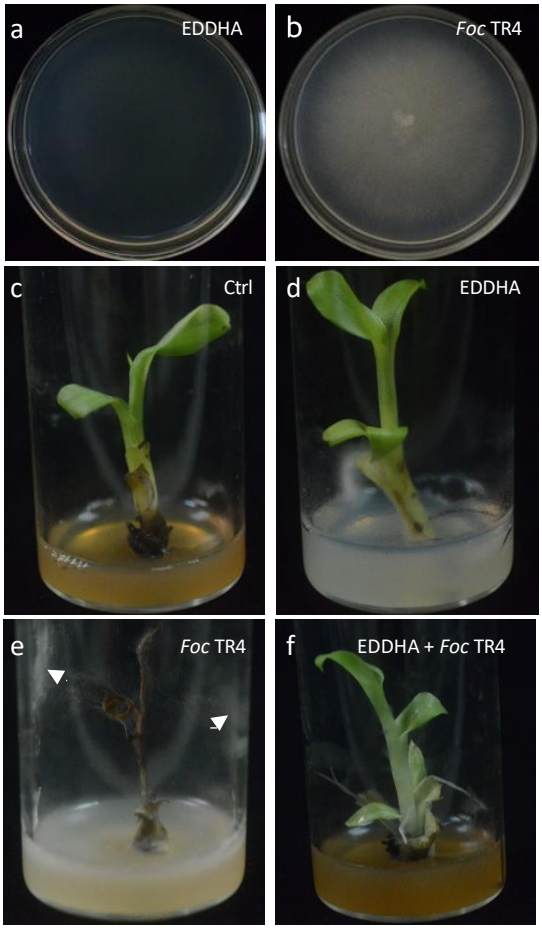

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Reviewer #2 (Remarks to the Author):

In this work authors studied the biocontrol mechanism of microbiome analysis against Fusarium wilt of banana (FWB) using advanced methods such GFP-tagged, TEM, SEM, and Illumina MiSeq sequencing. What I think is an interesting job, as a contribution to the solution of FW, a global issue in banana production systems. But I think in results section, it would make it easier to understand by extrapolate the results to the field settings and thus would make more sense. It would also be important to explain how the results impact small scale farmer's matrix in terms of production/ yield/ economy etc. where authors conducted field trails. Also, as authors mentioned that they got Piriformospore indica as a gift from Taiwan and Streptomyces morookaensis was purchased from Microbiological Culture Collection Center, Beijing, China which I feel require more work to examine the adaptability of these foreign strains in local soils. How long they will survive, what is the shelf life and impact on indigenous beneficial microbial communities. Similarly soil physico-chemical and biological properties should be studied more in detail and not only pH, total nitrogen (TN), total phosphorus (TP), and total ferrum (TF) and effects with microbial culture inoculations.

Manuscript review
Title: Spatiotemporal biocontrol and rhizosphere microbiome analysis of Fusarium wilt of banana
The manuscript is an interesting account of efforts towards exploring the use of *P. indica* and *S. morookaensis* as potential biocontrol agents in managing Fusarium wilt Tropical Race 4 through promotion of vigorous plant growth and secretion of secondary compounds respectively.
The manuscript title is reflective of the main ideas within the article. The introduction is systematically written and the significance of the manuscript is well explained. Although the results and conclusions are accurate and supported by the content, the authors lack a systemic presentation of this section. They need to write it out more elaborately and succinctly. The methods used in this study are appropriate. References need to be synchronized. Overall, this work is original and logiacal.
Specific comments were inserted directly in the manuscript.

REVIEWERS' COMMENTS:

Reviewer #2 (Remarks to the Author):

Many thanks for revisions, accepted in current form.