Newly Isolated \textit{Streptomyces} sp. JBS5-6 as a Potential Biocontrol Agent to Control Banana Fusarium Wilt: Genome Sequencing and Secondary Metabolite Cluster Profiles

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Banana is a key staple food and fruit in countries all over the world. However, the development of the global banana industry is seriously threatened by Fusarium wilt disease, which is caused by \textit{Fusarium oxysporum} f. sp. \textit{cubense} (Foc). In particular, Foc tropical race 4 (Foc TR4) could infect more than 80\% of global banana and plantain crops. Until now, there were no commercial chemicals or resistant cultivars available to control the disease. Biological control using actinomycetes is considered a promising strategy. In this study, 88 actinomycetes were isolated from a banana orchard without symptoms of Fusarium wilt disease for more than 10 years. An actinobacterial strain labeled as JBS5-6 has exhibited strong antifungal activities against Foc TR4 and other selected 10 phytopathogenic fungi. Based on phenotypic and biochemical traits as well as complete genome analysis, strain JBS5-6 was assigned to \textit{Streptomyces violaceusniger}. Extracts of the strain inhibited the mycelial growth and spore germination of Foc TR4 by destroying membrane integrity and the ultrastructure of cells. The complete genome of strain JBS5-6 was sequenced and revealed a number of key function gene clusters that contribute to the biosynthesis of active secondary metabolites. Sixteen chemical compounds were further identified by gas chromatography-mass spectrometry (GC-MS). 5-hydroxymethyl-2-furancarboxaldehyde was one of the dominant components in strain JBS5-6 extracts. Moreover, fermentation broth of strain JBS5-6 significantly reduced the disease index of banana seedlings by inhibiting the infection of Foc TR4 in a pot experiment. Hence, strain JBS5-6 is a potential biocontrol agent for the management of disease and the exploitation of biofertilizer.

\textbf{Keywords:} biocontrol, \textit{Streptomyces}, banana fusarium disease, whole genome sequencing, GC-MS
INTRODUCTION

Banana Fusarium wilt is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc). It is a highly destructive disease that significantly affects the banana industry (Zhou et al., 2019). According to the pathogenicity against banana different cultivars, Foc pathogens can be divided into four physiological tropical races, namely Foc TR1, Foc TR2, Foc TR3, and Foc TR4 (Dita et al., 2010). It is anticipated that Foc TR4 in particular, could infect more than 80% of global banana and plantain crops, which causes the whole plant to wilt and die (Dita et al., 2018). Until now, there has been a lack of effective physical and chemical strategies to manage the disease (Ploetz, 2015). Biological control agents (BCAs) are considered a promising strategy to limit the spread of the disease. Biological control agents (BCAs) lack of effective physical and chemical strategies to manage wilt and die (Dita et al., 2018). Until now, there has been a lack of effective physical and chemical strategies to manage the disease (Ploetz, 2015). Biological control agents (BCAs) are considered a promising strategy to limit the spread of the disease. Biological control agents (BCAs) lack of effective physical and chemical strategies to manage the disease (Ploetz, 2015).

**collection of soil samples**

A banana orchard which had been without symptoms of banana wilt for more than 10 years was selected in Nanbao (109°51′17″E, 19°47′1″N) of Hainan Province, China. An approximate 10–20 cm soil layer of the banana plant rhizosphere was collected in May 2017. Ten individual banana plants with a distance of at least 5 m were randomly selected. Soil samples at four positions of each plant rhizosphere were collected. A total of 40 samples from each plant were mixed as a soil sample in a sterile plastic bag and stored at −20°C.

**Actinomycetes Isolation**

Actinomycetes were isolated by a serial dilution method (Williams et al., 1983). Rifampicin (50 mg/L) and nystatin (50 mg/L) were added to inhibit bacterial and fungal growth. To prepare soil suspension, a 10 g dried soil sample was transferred into a 250-ml bottle. 90 ml of sterile distilled water was added and cultured at 200 rpm for 30 min at 28°C. The soil suspension was then diluted from 10−3 to 10−5 fold, which were spread on the Gause’s no. 1 medium at 28°C for 7–15 days, respectively. Each colony was purified on the yeast extract-malt extract (ISP2) agar and kept in 20% (v/v) of glycerol at −80°C.

**The Selected Phytopathogenic Fungi**

A total of 11 phytopathogenic fungi were selected, including *Fusarium oxysporum* Race 4 (ATCC 76255, Foc TR4) from a banana, *Colletotrichum acutatum* (ATCC56815) from loquat, *Curvularia fallax* (ATCC 38579) from banana, *Fusarium oxysporum* sp. *cucumerinum* (ACCC306) from cucumber, *Pyricularia oryzae* (ATCC 52352) from rice, *Colletotrichum gloeosporioides* (ATCC 58222) from mango, *Fusarium graminearum* (ATCC 46779) from wheat, *Botryosphaeria dothidea* (ATCC 208829) from apple, *Curvularia lunata* (ATCC 42011) from maize, *Colletotrichum fragariae* (ATCC 58718) from strawberry and *Botrytis cinerea* (ATCC 11542) from grape. These fungi were kindly provided by the Institute of Environment and Plant Protection at the China Academy of Tropical Agricultural Sciences, Haikou, China.
Antifungal Activity Assay
Antagonistic actinomycetes were screened according to the inhibition ability against Foc TR4 using a conventional spot inoculation method (Qi et al., 2019). A phytopathogenic fungal disk (8.5 mm diameter) was placed in the center of the potato dextrose agar (PDA) plate. Four mycelial blocks (5 mm diameter) of each actinomycete were inoculated at four symmetrical points about 2.5 cm from the plate center (Yun et al., 2018). A fungal disk alone was used as a control. After incubation at 28°C for 5–7 days, the inhibition zone was recorded by measuring the distance between the fungal mycelium edge and the actinomycete disks. The inhibition percentage of the radial growth (PIRG) was calculated using the following formula: PIRG = [(C – T)/C] × 100, where C and T represented the radius of fungal mycelial growth in the control and the treatment groups, respectively. All strains were tested in triplicate experiments.

Construction of Phylogenetic Tree
The amplified 16S rRNA sequence was compared with those that have been deposited in public databases and the EzBiocloud server1 (Yoon et al., 2017). The similarity of pairwise sequences was also calculated. Other 16S rRNA sequences from representative taxa were obtained from GenBank databases using the CLUSTAL X software (Thompson et al., 1997). A phylogenetic tree was constructed using the neighbor-joining method of MEGA version 7.0 (Kumar et al., 2016). The evolutionary distance was estimated by bootstrap analysis.

Physiological and Biochemical Characteristics of Actinomycetes
The effects of different pH, temperature, and NaCl on microbial growth were measured on the ISP4 medium after incubation at 28°C for 14 days. The pH range was set from 3.0 and 11.0 at intervals of one unit. The temperature ranged from 4, 15, 20, 25, 28, 37, 40, and 45°C. NaCl tolerance for microbial growth was tested in the presence of 0–15% at intervals of one unit. Some biochemical characteristics, including H2S production, milk peptization, starch hydrolysis, nitrate reduction, tyrosinase production, and cellulose hydrolysis, were also measured according to the description of Qi et al. (2019). The carbon and nitrogen sources were tested according to the previous method of Chen et al. (2018).

Antifungal Activity of Streptomyces Extracts
The selected Streptomyces were inoculated in one liter of sterilized soybean liquid culture medium (SLM, 15 g of corn flour, 10 g of glucose, 0.5 g of K2HPO4, 0.5 g of NaCl, 0.5 g of MgSO4, 3 g of beef extract, 10 g of yeast extract, 10 g of soluble starch, 2 g of CaCO3, pH 7.2–7.4) at 180 rpm for 7 days at 28°C (Hong et al., 2009). The culture filtrate was extracted with EtOAc at a ratio of 1:1 (v/v). The mixture was filtered through the qualitative filter paper (Whatman no. 1). The organic phase (EtOAc) was separated from the filtered liquid media using a decantation funnel. The extracts were evaporated using a rotary vacuum evaporator (EYELA, N-1300, Japan), and then dissolved in 10% of dimethyl sulfoxide (DMSO) with a final concentration of 20.0 mg/mL. After being sterilized by filtration through a 0.22-µm sterile filter (Millipore, Bedford, MA, United States), the extracts were stored at −4°C.

Effect of Streptomyces Extracts on Growth Inhibition of Fungal Mycelia
A total of 11 phytopathogenic fungi were selected for analyzing the antifungal activities of Streptomyces extracts using a plate method (Sharma et al., 2016). The extracts were dissolved in 10% of DMSO (20.0 mg/mL) and added to the PDA plate with a final concentration of 200 μg/mL. An equivalent 10% of DMSO was used as a control. A fungal disk (5 mm diameter) was inoculated aseptically into the center of each Petri dish at 28°C. When the control mycelium reached the edge of the plate, the diameter of the mycelium was measured. Each assay was performed in three replicates. The inhibition percentage of mycelial growth was calculated using the following formula: mycelial inhibition percentage = [(C – T)/C] × 100, where C and T represented the colonial diameters of control and different treatments. 50% of the growth inhibition of

1https://www.ezbiocloud.net/identify
Determination of the Minimum Inhibitory Concentration (MIC) of Streptomyces on Phytopathogenic Fungi

The minimum inhibitory concentrations (MICs) of Streptomyces extracts on different phytopathogenic fungi were determined by a method of 96-well microtiter assay (Chen et al., 2018). Cycloheximide and Nystatin were used as positive controls. 10% of DMSO was used as a negative control. The different concentrations (50.0, 25.0, 12.5, 6.25, 3.125, and 1.563, and 0.781 µg/mL) were prepared for MIC tests. Each well contained 80 µL of the Roswell Park Memorial Institute (RPMI) mycological media, 100 µL of fungal suspension with 1.0 × 10⁵ CFU (colony-forming units)/mL, and 20 µL of Streptomyces extracts. The 96-well plates (Nunc MicroWell, Roskilde, Denmark) were covered with a plastic lid and incubated at 28°C for 12 h. Absorbance was measured at 620 nm using a microplate photometer (Packard Spectra Count, Packard Instrument Co., Downers Grove, IL, United States). The lowest concentration of growth inhibition was recorded as MIC.

Effects of Streptomyces Extracts on the Spore Germination of Phytopathogenic Fungi

The inhibitory activities of Streptomyces extracts on spore germination of phytopathogenic fungi were observed using light microscopy (Nikon, E2000MV, Japan). Each fungal strain was challenged in dose response to different extract concentrations (1/2 × EC₅₀, 1 × EC₅₀, or 2 × EC₅₀). The mixture (0.1 mL) of extracts and fungal spore suspension (10⁵ CFU/mL) at a ratio of 1:1 (v/v) was placed on a sterile glass slide and incubated in a moist chamber at 28°C for 20–24 h. For the control, we replaced extracts with sterile water. All experiments were performed in three replicates. We detected approximately 100 conidia in each field. The percentage of spore germination (PSG) was calculated as follows: PSG = (A – B)/A, where A and B represented the spore germination rate of control and treatment groups, respectively (Chen et al., 2018).

Effects of Streptomyces Extracts on the Hyphae Growth of Foc TR4

The hyphal growth of Foc TR4 treated with Streptomyces extracts was observed according to the method of Feng et al. (2019). A mycelial disk (4 mm diameter) was taken from the periphery of a colony growing on the PDA medium with 2 × EC₅₀ of extracts and incubated at 28°C for 24 h. 10% of DMSO treatment was used as a negative control. Foc TR4 hyphae were chosen from the plate margin, fixed in 2.5% of glutaraldehyde (0.1 mol/L of phosphate buffer, pH 7.2) at 4°C overnight, and post-fixed in 1% of OsO₄ for 2 h at 4°C. The samples were then dehydrated in a graded ethanol series, dried, and coated with gold powder. Hyphal growth was observed using SEM at an accelerating voltage of 3 kV.

Effects of Streptomyces Extracts on the Ultrastructure of Foc TR4

A 5-mm diameter mycelial disk was removed from the periphery of a colony that has been growing on the PDA medium for 24–72 h at 28°C. An equal volume of DMSO (10%, v/v) was used as a control. The sample was pre-fixed in 3% of fresh glutaraldehyde, post-fixed in 1% of osmium tetroxide solution, and dehydrated by transferring fixed specimens to a graded water-acetone series (50, 70, 80, 90, 95, and 100% for 10 min, respectively). Afterward, these samples were embedded in Epon 812 resin at 37°C for 12 h, 45°C for 12 h, and 60°C for 24 h, respectively. The embedded materials were sectioned with an ultramicrotome (EM UC6, Leica, Germany). These sections were double stained with saturated uranyl acetate and lead citrate and observed by a transmission electron microscopy (TEM, HT7700, Hitachi, Japan) under an operating voltage of 80 kV.

Effects of Streptomyces Extracts on the Physiological and Biochemical Characteristics of Foc TR4

Spore suspension of Foc TR4 with 10⁷ CFU/mL was cultured in 100 mL of the PDB medium at 180 rpm for 48 h at 28°C. Different concentrations (0, 5, 10, 25, 50, 100, and 200 µg/mL) of extracts were added and co-cultured at 180 rpm for 72 h at 28°C, respectively. Foc TR4 mycelia were collected by centrifugation at 5,000 rpm for 20 min and then washed three times with sterile water. One gram of Foc TR4 mycelia was ground in 5 mL of Tris–HCl on the ice. After centrifugation at 10,000 rpm for 20 min at 4°C, the supernatant was used for measuring N-acetylglucosamine content in the light of the standard curve (Hofmann and Roitsch, 2000). Two grams of mycelia were ground in 25 mL of sodium acetate (0.05 mol/L, pH 5.0). After centrifugation at 12,000 rpm for 15 min at 4°C, crude enzyme supernatant was used for measuring the activity of β-1,3-glucanase according to the method of Miller (1959). One unit of β-1,3-glucanase activity was defined as an enzyme amount that produced 1 μmol of reducing sugar per min. Soluble protein content was measured in the mixture (0.1 mL of supernatant, 0.9 mL of distilled water, and 5 mL of Coomassie brilliant blue G-250 dye). The absorbance was determined at 595 nm and protein content was calculated by comparing the standard curve (Bradford, 1976). The dried mycelia of Foc TR4 were weighed after treatment with different extract concentrations, respectively.

Genome Sequencing and Metabolite Prediction of the Selected Streptomyces

Streptomyces were cultured aerobically in the ISP4 medium at 28°C for 48–72 h. Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, United States) according to the standard manufacturer’s protocol. Purified genomic DNA was quantified by a TBS-380 fluorometer (Turner BioSystems Inc., Sunnyvale, CA, United States). Sequencing and assembly of the complete genome were performed by the Majorbio Bio-Pharm Technology Co.,
Streptomyces of the selected orchard, and passed through a twenty-mesh sieve, and sterilized into a greenhouse at 30°C, 60% humidity for 12 h dark/12 h light. The fermentation broth was filtered through two layers of sterile prewetted Mira cloth and was diluted to 50 folds. Banana seedlings (Foc TR4-susceptible Cavendish cultivar "Brazilian") were washed with sterile water and planted in plastic pot. Banana seedlings (inoculated with Foc TR4), CK2 (inoculated with sterile water), and treatment (inoculated with Foc TR4 and a fermentation broth of Streptomyces).

Assay of Biocontrol Efficiency After co-incubation for 35 days, the corms of the banana seedlings were cut to detect the infection degree of Foc TR4. Root sections were made using the hand-sliced method. The infection and colonization of Foc TR4 were observed using a laser scanning confocal microscope (ZEISS, LSM800, Germany) equipped with filter blocks with spectral properties matching those of GFP (488 nm excitation, 520–540 nm long-pass emission). The disease indexes of banana seedlings were measured after 35 d according to the following formula (Fang, 2007):

\[
\text{Disease Index} = \frac{\sum (\text{Number of diseased plants of each grade} \times \text{value of relative disease index})}{(\text{Total number inspected} \times 4)} \times 100
\]

The number of chlorotic leaves was used to evaluate the disease grade such as a healthy plant (grade 0), below 25% of chlorotic leaves (grade 1), 25–50% of chlorotic leaves (grade 2), 51–75% of chlorotic leaves (grade 3), and 76–100% of chlorotic leaves (grade 4).

Biocontrol efficiency was measured using the following formula:

\[
\text{Controlling efficiency (\%)} = \frac{\text{(disease index in CK1} - \text{disease index in the treated group)}/\text{disease index in CK1} \times 100}
\]

RESULTS

Actinomycetes Isolation and Antifungal Activity Assay

A total of 88 actinomycetes were isolated from the banana orchard without symptoms of banana wilt disease for more than 10 years. All isolates were screened against Foc TR4. Out of them, 17 actinomycetes showed antifungal activities during the preliminary experiment. In particular, a strain marked with JBS5-6 exhibited strong antifungal activity against Foc TR4 with the inhibition efficiency of 60.46% (Figure 1A and Supplementary Table 1).

Cultural and Morphological Characteristics of Strain JBS5-6

Strain JBS5-6 can grow well on ISP3, ISP4, ISP5, and ISP6 media with pH 5.0–9.0 and less than 3% of NaCl at 28–45°C (Supplementary Tables 2, 3). The strain could resolve nitrate and hydrolyze hippurate, but could not produce soluble pigment, H₂S, and tyrosinase. Moreover, it also cannot hydrolyze starch, solidify milk, decompose cellulose, and liquefy gelatin (Supplementary Table 3). SEM analysis showed that the straight
and long aerial mycelia produced about 4–6 spores at the end of the short branch. Smooth and cylindrical spores first formed spiral chains, and then gathered together like a bunch of grapes (Figure 1B).

**Phylogenetic Construction of Strain JBS5-6**

The complete 16S rRNA gene (1509 bp) of strain JBS5-6 was amplified and was submitted to the GenBank database with the accession number MN384963. By analysis of EzBioCloud, the strain showed a 99.93% similarity with the standard strain *S. violaceusniger* NBRC13459. A phylogenetic tree was constructed using the neighbor-joining method of MEGA version 7.0. These selected strains could be divided into four major clusters (Figure 1C). Strain JBS5-6 formed a well-delineated subclade with *S. violaceusniger* and the bootstrap value was over 45. Combining the morphological, physiological, and biochemical characteristics, strain JBS5-6 was assigned to the genus *Streptomyces*.

**Carbon and Nitrogen Utilization of Strain JBS5-6**

Strain JBS5-6 could fully utilize 17 carbon sources including α-lactose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, D-sorbitol, D-trehalose, D-xyllose, melibiose, D-mannitol, inositol, melezitose, rhamnose, ribose, maltose, and sucrose (Supplementary Table 4). However, it could not use L-arabinose and melitose as carbon sources. Furthermore, the strain could fully utilize L-serine, glycine, and histidine as nitrogen resources, but could not utilize L-arginine, L-phenylalanine, methionine, tryptophan, (+)-cysteine, phenylalanine, valine, and glutamate (Supplementary Table 4).

**Antifungal Activity Assay of Strain JBS5-6**

To assess whether strain JBS5-6 had a broad-spectrum antifungal activity, the 11 phytopathogenic fungi were selected (Supplementary Table 5). The percentage of inhibition of mycelial growth ranged from 70.37 to 88.89%. Compared with untreated hypha, more than 80% of inhibitory abilities were demonstrated against Foc TR4 (ATCC 76255), *C. acutatum* (ATCC 56815), *C. gloeosporioides* (ATCC 58222), and *C. lunata* (ATCC 42011). The maximum inhibition percentage of mycelial growth was detected against *C. lunata* (ATCC 42011) (88.98 ± 1.47%), followed by Foc TR4 (ATCC 76255) (87.22 ± 1.47%). The minimum inhibition percentage was observed against *F. oxysporum* sp. cucumerinum (ACCC 30220) (72.22 ± 0.56%), followed by *P. oryzae* (ATCC 52352) (69.44 ± 2.42) and *B. dothidea* (ATCC 208829) (70.37 ± 2.31). Based on the acquired toxicity curves, EC\(_{50}\) values of strain JBS5-6 extracts were measured against 11 pathogenic fungi (Supplementary Table 5). Its extracts exhibited the best antifungal activity against *C. gloeosporioides*, *C. lunata*, and *B. cinerea*, and Foc TR4 with an EC\(_{50}\) value of 106.64 ± 108.96, 129.27 ± 136.92 µg/mL, respectively. These results indicate that strain JBS5-6 could have a potentially excellent inhibitory ability against the selected 11 pathogenic fungi.

**Strain JBS5-6 Extracts Significantly Inhibit Spore Germination and Hyphal Growth of Foc TR4**

The morphological characteristics of Foc TR4 hypha after treatment with strain JBS5-6 extracts were detected by SEM (Figure 2A). In the control PDA plate with 10% of DMSO, Foc...
TR4 mycelia displayed smooth external surfaces, rounded apexes, and regular morphology. Most of the formed fungal mycelia appeared to be parallel and adherent. In the treatment group, Foc TR4 mycelia exhibited a deformed and wrinkled external surface and a varied hyphal diameter. The mycelia showed an undulation and deformation structure along with the hyphal borders. Some ruptured bubbles at the top of mycelia were also detected. Similarly, strain JBS5-6 extracts also inhibited significantly the hyphal growth of the selected phytopathogenic fungi (Supplementary Table 5).

Additionally, strain JBS5-6 extracts were used to analyze their effects on spore germination of the tested pathogenic fungi (Figure 2B). The results showed that spore germination was significantly reduced ($P < 0.05$) after treatment with different concentration extracts. At 2 × EC$_{50}$, strain JBS5-6 extracts effectively inhibited the conidial germination of Foc TR4, C. acutatum, C. gloeosporioides, and C. lunata with the inhibition percentages of 80.81, 83.25, 82.23, and 82.38%, respectively (Supplementary Table 5). The percentage of spore germination gradually decreased with the increase of concentration of extracts. The minimum inhibition percentage of spore germination was 64.31 ± 0.98% against P. oryzae (ATCC 52352), followed by 65.13 ± 0.38% against B. dothidea (ATCC 208829). By contrast, 10% of DMSO (v/v) treatment did not inhibit the spore germination of the tested pathogenic fungi.

### Effects of Strain JBS5-6 Extracts on the Ultrastructure of Foc TR4 Cells

Ultrastructure of hyphal cells treated with 2 × EC$_{50}$ of strain JBS5-6 extracts was observed by TEM. In the 10% of the DMSO treatment group, cell wall, membrane, and organelles were intact and well defined. Organelles such as vesicles, vacuoles, lipid bodies, and mitochondria were observed clearly (Figure 2C). After treated with strain JBS5-6 extracts, the cell wall of Foc TR4 mycelia became incrassated with a rough surface. Hyphal cells showed more low density granules and less high density granules. In addition, the cytoplasmic heterogeneity and disintegrated organelles were also observed in the treated cells (Figure 2C).

### Effects of Strain JBS5-6 Extracts on the Physiological and Biochemical Characteristics of Foc TR4

The dry weight of Foc TR4 mycelia gradually decreased with the concentration increase of strain JBS5-6 extracts. No significant difference was observed between the 10 and 25 µg/mL treatment groups. Although 50 and 100 µg/mL extracts significantly inhibited the increase of dry weight of Foc TR4 mycelia, there was no obvious difference between them (Figure 3A). Compared with the control group, the soluble protein contents of Foc TR4 mycelia exhibited a decreasing tendency with the increase of extract concentrations (Figure 3C). No significant difference in soluble protein content was detected between 10 and 25 µg/mL extract treatments. The lowest soluble protein content was observed in 200 µg/mL of the treatment group. In addition, N-acetylglucosamine and β-glucanase activities of Foc TR4 significantly increased along with the increase of extract concentrations, suggesting that the cell wall of Foc TR4 was seriously damaged by strain JBS5-6 extracts (Figures 3B,D).

### Minimum Inhibitory Concentration of Strain JBS5-6

The MICs of strain JBS5-6 extracts were determined against 11 phytopathogenic fungi by the 96-well microtiter assay. Compared with the antifungal activity of cycloheximide and azoxystrobin, MICs ranged from 50 µg/mL to 1.563 µg/mL. The low MICs were detected against Foc TR4 with 6.25 µg/mL and F. graminearum with 3.125 µg/mL. The lowest MIC was 1.563 µg/mL against C. gloeosporioides, suggesting that extracts had a strong fungicidal activity that inhibits the growth of this pathogen (Supplementary Table 6). However, 10% of DMSO had
clusters, and other gene clusters (one oligosaccharide cluster, three siderophore gene clusters, two arylpolyene-ladder gene clusters, three bacteriocin gene clusters, ten NRPS gene clusters, six terpene gene clusters, one PKS gene clusters, one type II PKS gene cluster, two type III PKS gene clusters, one hserlactone gene cluster, one indole cluster, six lantipeptide gene clusters, one olosaccharide cluster, three siderophore gene clusters, and other gene clusters (Supplementary Table 8).

By alignment of antiSMASH software and GenBank, above 70% of similarity gene clusters producing twenty chemicals were annotated in Supplementary Table 9, including 12 gene clusters with 100% similarity. The structures and functions of predicted chemicals were demonstrated (Supplementary Figure 2). Genome analysis further revealed that nine gene clusters were involved in the biosynthesis of antimicrobial metabolites, including azalomycin F3a, amipurimycin, curamycin, halostocansanolide A, myxothiazol, echoside A-E, bicornutin A1-A2, icosalide A-B, and nigericin. In addition, two gene clusters may participate in the biosynthesis of anticancer agents (nigericin and azalomycin F3a). One gene cluster was responsible for the production of cytotoxic agents (rhizomide A-C and luminmide). Furthermore, two gene clusters were involved in the biosynthesis of desferrioxamine B and E, which were used as the iron chelation removing excess iron in the environment. Notably, other unmatched gene clusters were also found in the strain JBS5-6 genome, which might participate in the biosynthesis of some key secondary metabolites (Supplementary Table 9).

Identification of the Active Compound Candidates of Strain JBS5-6 by GC-MS

The active compounds of strain JBS5-6 extracts were analyzed by GC-MS. A total of 16 chemical compounds were identified by alignment of the NIST library based on retention time, molecular mass, and the molecular formula (Table 1). These compounds mainly contained hydrocarbons, flavonoids, polymeric aldehyde, acids, pyrrolizidine, and phenol esters. Their chemical structures and functions were predicted in Supplementary Figure 1, including: (1): 1,3-dimethyl-benzene; (2): 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-; (3): 5-hydroxymethyl-2-furancarboxaldehyde; (4): Benzeneacetic acid; (5): Methyl 1-methylpyrrole-2-carboxylate; (6): 5-Acetyl-2-furanmethanol; (7): N-Methylpyrrole-2-carboxylic acid; (8): phenylethyl ester of caffeic acid; (9): 1H-Indene,2,3-dihydro-1,1,3-trimethyl-3-phenyl-; (10): 4-methyl-N-(tetrahydro-2-oxo-3-furanyl)-Benzenesulfonamide; (11): 2,5-Piperazinedione,3,6-bis(2-methylpropyl)-; (12): Phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-; (13): Pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(phenylmethyl)-; (14): 2-Cyclohexen-1-one-1,3, 3-methyl-4,4-diphenyl-; (15): n-Hexadecanoic acid and (16): Pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-. The peak area of compounds represented the proportion to their quantities in extracts (Table 1). Among these compounds, the peak area of 5-hydroxymethyl-2-furancarboxaldehyde was 52.75%, indicating that it was the dominant compound in strain JBS5-6 extracts.

Fermentation Broth of Strain JBS5-6 and Reducing the Disease Index of Banana Seedlings

In our pot experiments, the visual external wilt symptoms (chlorotic leaves) of banana seedlings in the CK1 group (plantlets inoculated with Foc only) was first observed on the seventh day. After that, leaf chlorosis began to transfer from old leaves to new inhibitory effect on the selected phytopathogenic fungi in the control group.

Genome Sequencing and Metabolite Prediction of Strain JBS5-6

By sequencing and assembly, the genome of strain JBS5-6 consisted of 11,161,721 bp with 71.40% of GC content, including 9840 genes and 73 rRNA genes (Figure 4A and Supplementary Table 7). Furthermore, an ANI calculated for genomes of strain JBS5-6 and S. violaceusniger NBRC 13459T was 97.52, which was above the threshold value of 95–96% for species delineation (Richter and Rosselló-Móra, 2009). Strain JBS5-6 was identified as S. violaceusniger. Among a total of identified 9767 protein-coding genes, 73.85% and 45.38% of them were annotated into COG and KEGG functional categories, respectively (Figures 4B,C). For COG categories, the highest ratio was the metabolism process (39.12%), followed by information storage and processing (17.48%) as well as cellular processes and signaling (12.20%). Notably, 31.20% of the high proportion showed in the poorly characterized category. Sixty-five biosynthetic gene clusters coding for secondary metabolites were detected in strain JBS5-6 genome, mainly including 23 type I PKS gene clusters, one type II PKS gene cluster, two type III PKS gene clusters, ten NRPS gene clusters, six terpene gene clusters, two arylpolyene-ladder gene clusters, three bacteriocin gene clusters, two butyroloaceta gene clusters, one ectsone gene cluster, one hserlactone gene cluster, one indole cluster, six lantipeptide gene clusters, one olosaccharide cluster, three siderophore gene clusters, and other gene clusters (Supplementary Table 8).
FIGURE 4 | Analysis of genome structure and metabolic pathway of strain JBS5-6. (A) Circular map of strain JBS5-6 chromosome. From outside to center, rings 1 and 4 show protein-coding genes colored by COG categories on forward/reverse strand. Rings 2 and 3 showed CDS, tRNA, and rRNA on forward/reverse strand. Ring 5 represented the G + C content, followed by G + C skew in ring 6. (B) COG annotation of strain JBS5-6 genome. (C) Pathway annotation of strain JBS5-6 genome according to the KEGG database. The vertical axis represented the level two classification of KEGG pathway. The horizontal axis represented the gene number annotated in this classification. Different colors of the columns represented different classifications of KEGG pathway.

Young leaves. On day 35, fusarium wilt disease spread rapidly to the whole plant. The leaves with chlorotic symptoms collapsed and a large area of banana corm gradually decayed (Figure 5A). The disease index in the CK1 group reached 71.30 ± 7.24. Although few plantlets in the treatment group of fermentation broth of strain JBS5-6 showed chlorotic symptom on the margin of lower leaves, vascular discoloration was not observed on cutting suckers horizontally (Figure 5A). Only a slight brown in the middle of the banana corm was detected. The disease index was 25.78 and the control efficiency was 64.94% (Figure 5C). The laser scanning confocal microscope was used to future monitor the characteristics of Foc TR4 infection. In the control group, the vascular tissue was infected by Foc TR4 seriously and a number of vascular cells were full of GFP-tagged Foc TR4 spores. However, only a few vascular cells with the GFP-tagged Foc TR4 spores were observed in the treatment group (Figure 5B). These results indicated that the fermentation broth of strain JBS5-6 could inhibit the infection of Foc TR4 and reduce the disease index of banana seedlings. Based on these findings, strain JBS5-6 could improve the resistance of banana seedlings to Foc TR4.

DISCUSSION

Plant diseases caused by phytopathogenic fungi can cause heavy losses for agriculture production. Banana is an important food crop in the tropics and subtropics, and fusarium wilt is one of the most destructive diseases affecting agricultural production of this fruit. Biological control is a promising strategy for combating fusarium wilt, due to the advantages of high efficiency, broad spectrum, and the fact that it is relatively environmentally friendly (Zhou et al., 2013; Wei et al., 2020). Previous studies have indicated that specific microbial groups, such as Pseudomonas, Streptomyces, Flavobacterium, etc., are closely related to plant disease suppression (Mendes et al., 2011; Cha et al., 2016; Kwak et al., 2018). Similarly, we also found that some functional microbes existed in the selected banana orchard without symptoms of banana wilt for more than 10 years (Zhou et al., 2019). Streptomyces and its natural antibiotics were beneficial for constructing a stable disease-suppressive soil microbial community and reducing the incidence index of banana wilt disease (Cha et al., 2016). In our present study, strain JBS5-6 was isolated from the disease-suppressive soil in the above banana orchard. Strain JBS5-6 exhibited a strong antifungal ability against Foc TR4 and the selected 10 different phytopathogenic fungi. Similar studies have also shown that Streptomyces exhibit great potential in suppressing plant diseases caused specifically by fungal pathogens (Qin et al., 2017; Chen et al., 2018).

Accumulated evidence indicates that Streptomyces species can provide a rich source of natural products that may have potential BCAs (Newman and Cragg, 2016). Most secondary metabolites are used as antibiotics, antitumor agents, antioxidation substances, pesticides, and plant-growth-promoting substances, etc, (Qi et al., 2019). In
our study, strain JBS5-6 extracts exhibited a strong inhibition ability on mycelial growth and the cellular integrity of phytopathogenic fungi (Figure 2 and Supplementary Table 5). Similarly, morphological abnormalities in *F. oxysporum* fungal hyphae were observed after treatment with supernatant of *Streptomyces bikiniiensis* HD-087 (Zhao et al., 2014). It was supported by the significant increase of N-Acetylglucosamine content and β-glucanase activity in the treated Foc TR4 mycelial cells (Figure 3). *Streptomyces* sp. M4 treatment caused swelling of fungal mycelia (Sharma et al., 2016). We did not find swollen mycelia in Foc TR4 treated with strain JBS5-6 extracts. This might be because the swollen morphology was an earlier symptom of abnormalities (Wei et al., 2020). The antifungal ability of *Streptomyces* might be related to antibiotics, nutrient competition, degradative enzyme biosynthesis, nitrous oxide production, and quorum quenching (Cohen and Mazzola, 2006). However, the antifungal mechanism of strain JBS5-6 on Foc TR4 still needs to be further investigated.

Additionally, actinomycetes can produce a wide range of bioactive secondary metabolites. Approximately two-thirds of available antibiotics have been isolated from actinomycetes (Wang et al., 2015). The genome sequencing of actinomycetes has also revealed numerous cryptic secondary metabolite gene clusters of unknown and known functions (Figure 4). These gene clusters are responsible for the production of microbial natural products. Biosynthetic gene cluster encoding for polyketides and non-ribosomal peptides were identified in strain JBS5-6 genome, mainly including 23 type I PKS gene clusters, one type III PKS gene cluster, two type III PKS gene clusters, and 10 NRPS gene clusters. The encoding of the genes PKS-I and NRPS might play a role in the production of antifungal activity from strain JBS5-6. Passari et al. (2015) and Sharma et al. (2016) also showed that actinomycetes possessing antifungal activity are positively related to two biosynthetic pathways.

In order to further uncover some important chemicals produced by strain JBS5-6, some gene clusters that encode antibiotic metabolites were identified (Supplementary Figure 2). These include the amipurimycin gene cluster of strain JBS5-6, which showed 90% similarity with the corresponding genes in *S. novoguineensis*. Amipurimycin is a kind of peptidyl nucleoside antibiotic and a potential new source of antimicrobials (Serpi et al., 2013). Yuan et al. (2013) obtained seven azalomycin F analogs (1–7) from a broth of mangrove *Streptomyces* sp. 211726, which were used successfully against *Candida albicans*, *Staphylococcus aureus*, *Fusarium moniliforme*, and *F. oxysporum* (Arai, 1968; Chandra and Nair, 1995; Xu et al., 2018). Additionally, one gene cluster of strain JBS5-6 had 83% similarity with the gene involved in the production of nigericin. The compound belonged to a fungistatic polyether with a high inhibitory effect for mycelial growth of *Pythium* and *Phytophthora* (Myskiw et al., 2010). Interestingly, three gene clusters in strain JBS5-6 genome shared 100, 80, and 90% of similarity with desferrioxamine E, desferrioxamine B, and coelichelin, respectively. They were involved in the production of siderophores. Recent research has provided evidence that the secreted siderophores could suppress the pathogen in *vitro* and protect plants from pathogen infection by changing rhizosphere

### Table 1: Compounds identified from the crude extract of strain JBS5-6 through QCM-MS.

| No. | Predicted compounds                                         | RT (min) | Similarity (%) | Area (%) | MF | MM | Activity |
|-----|-------------------------------------------------------------|----------|----------------|----------|----|----|----------|
| 1   | 1,3-dimethyl-benzene                                       | 4.55     | 29.67          | 1.49     | C  | 18 | Organic-intermediate |
| 2   | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl          | 12.00    | 96.23          | 2.78     | C  | 144| Antioxidant and antifungal activity |
| 3   | Methyl 1-methylpyrrole-2-carboxylate                        | 16.54    | 66.70          | 1.08     | C  | 138| Antimicrobial activity |
| 4   | 5-hydroxymethyl-2-furancarboxaldehyde                       | 17.35    | 66.70          | 1.08     | C  | 138| Antimicrobial activity |
| 5   | Phenethyl ester of caffeic acid                             | 23.06    | 39.14          | 0.60     | C  | 144| Antifungal activity |
| 6   | 1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl             | 29.01    | 17.58          | 0.97     | C  | 100| Antimicrobial activity |
| 7   | 4-methyl-N-(tetrahydro-2-oxo-3-furanyl)-Benzenesulfonamide  | 29.16    | 8.69           | 0.91     | C  | 110| Antimicrobial activity |
| 8   | 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)                | 29.26    | 65.08          | 1.04     | C  | 110| Antimicrobial activity |
| 9   | Pyrrolo[1,2-a] pyrazine-1,4-dione,hexahydro-3(phenylmethyl) | 31.73    | 96.49          | 5.05     | C  | 110| Antimicrobial activity |
| 10  | 2-Cyclohexen-1-one, 3-methyl-4,4-diphenyl                  | 35.7     | 34.57          | 0.21     | C  | 110| Antimicrobial activity |
| 11  | n-Hexadecanoic acid                                        | 26.53    | 71.52          | 8.95     | C  | 110| Cytotoxic, Anti-Inflammatory Property |
| 12  | 1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl             | 29.01    | 17.58          | 0.97     | C  | 110| Antimicrobial activity |
| 13  | 4-methyl-N-(tetrahydro-2-oxo-3-furanyl)-Benzenesulfonamide  | 29.16    | 8.69           | 0.91     | C  | 110| Antimicrobial activity |
| 14  | 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)                | 29.26    | 65.08          | 1.04     | C  | 110| Antimicrobial activity |
| 15  | Pyrrolo[1,2-a] pyrazine-1,4-dione,hexahydro-3(phenylmethyl) | 31.73    | 96.49          | 5.05     | C  | 110| Antimicrobial activity |
| 16  | n-Hexadecanoic acid                                        | 26.53    | 71.52          | 8.95     | C  | 110| Cytotoxic, Anti-Inflammatory Property |
microbiome members (Gu et al., 2020). Hence, strain JBS5-6 might produce some important secondary metabolites to improve its antifungal activity.

To further identify antifungal compounds, GC-MS was used to analyze the secondary metabolites of strain JBS5-6 extracts in our study. A total of 16 chemical compounds were detected, including hydrocarbons, flavonoid, polymeric aldehyde, acids, pyrrolizidine, and phenol esters (Table 1 and Supplementary Figure 1). Of these, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-(DDMP) isolated from Schizophyllum commune received much attention for its antifungal activity against rubberwood fungi (Teoh and Don, 2014). Recent reports showed that benzenecacetic acid exhibited a high inhibition activity against Pseudomonas aeruginosa and Penicillium notatum (Valsalam et al., 2019). Caffeic acid phenethyl ester was identified as having multiple biological activities, including antioxidant, antimicrobial, anti-inflammatory, and antitumor effects (Pittalà et al., 2018). Hexadecanoic acid exhibited an antibiotic activity by damaging the cell walls of phytopathogenic fungi (El Mokni et al., 2019). Interestingly, these compounds identified by GC-MS were different from those synthesized by the above gene clusters (Table 1 and Supplementary Figure 2). This might be due to the difference of identification methods and alignment databases (Wei et al., 2020). Thus, we propose that these compounds could altogether contribute to the broad-spectrum antifungal activity of strain JBS5-6. This result was supported by the pot experiment, as part of which a fermentation broth of strain JBS5-6 significantly improved the resistance of banana seedlings to Foc TR4.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/, SAMN15515113 https://www.ncbi.nlm.nih.gov/genbank/, MN384963.

AUTHOR CONTRIBUTIONS

TJ, DZ, XZ, WW, and JX developed the ideas and designed the experiment. DZ, JX, and WW supervised the research and provided funding support. TJ, DZ, MZ, TY, DQ, YW, and XZ performed the experiments. TJ, DZ, TY, DQ, YW, XZ, WW, and JX analyzed the data. TJ, DZ, WW, and JX prepared the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Key Research and Development Program of China (2017YFD0202105), the Natural Science Foundation of Hainan (2019RC293), the China Agriculture Research System (CARS-31), the National Natural Science Foundation of China (31770476), and Central
Public-interest Scientific Institution Basal Research Fund for Chinese Academy of Tropical Agricultural Sciences (No. 1630052016005).

ACKNOWLEDGMENTS

We thank Zhufen Gao, Kai Li, Yankun Zhao, and Wen Tang for their help in this work.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.602591/full#supplementary-material

Supplementary Figure 1 | Chemical structures of the identified compounds of strain JBS5-6 extracts using GC-MS.

Supplementary Figure 2 | Genome-wide analysis of gene clusters related to the biosynthesis of secondary metabolites using the online antiSMASH v4.2.0 software.

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