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Antagonistic effects of *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum* f.sp. *cubense* race 4: Indirect evidence for the role of antibiosis in the antagonistic process

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***Fusarium oxysporum* f.sp. *cubense* is the causal pathogen of wilt disease of banana. A cost-effective measure of control for this disease is still not available. *Streptomyces violaceusniger* strain G10 acts as an antifungal agent antagonistic towards many different phytopathogenic fungi, including different pathogenic races of the *Fusarium* wilt pathogen. In an attempt to understand the mode of action of this antagonist in nature, the interaction between *S. violaceusniger* strain G10 and *F. oxysporum* f.sp. *cubense* was first studied by paired incubation on agar plates. Evidence for the *in vitro* antibiosis of strain G10 was demonstrated by inhibition zones in the ''cross-plug'' assay plates. Microscopic observations showed lysis of hyphal ends in the inhibited fungal colonies. Culture of strain G10 in liquid media produces antifungal metabolites, which showed *in vitro* antagonistic effects against *F. oxysporum* f.sp. *cubense* such as swelling, distortion and excessive branching of hyphae, and inhibition of spore germination. An indirect method was used to show that antibiosis is one of the mechanisms of antagonism by which strain G10 acts against *F. oxysporun* f.sp. *cubense* in soil. This study suggests the potential of developing strain G10 for the biological control of *Fusarium* wilt disease of banana.**
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Introduction

Wilt disease of banana caused by *Fusarium oxysporum* f.sp. *cubense* (FOC) is one of the most destructive diseases of banana in the tropics [28]. The Cavendish banana cultivars grown commercially are highly resistant to FOC race 1 and race 2, but susceptible to the newly emerged FOC race 4 [17,27]. Although a rather tedious procedure of injecting the fungicide carbendazim into corm tissues of banana has been attempted, there are no effective chemical control measures for this disease [17,36]. Other control methods such as field sanitation, soil treatments with fumigants, flood fallowing, organic amendments and crop rotation with nonhosts of the fungus have rarely provided long-term control in any production area [27]. Currently, the selection of resistant cultivars is generally accepted as a method of controlling the disease, but research is often hampered by insufficient knowledge of genetic variability in strains of FOC race 4 [26].

Some microorganisms, including actinomycetes [23,24], *Pseudomonas fluorescens* [36] and other bacteria [32], have been reported to be potential antagonists of FOC. More recently, antagonistic strains of *P. fluorescens* and *Trichoderma viride* have been used successfully to reduce the *Fusarium* wilt incidence and to produce higher yields of banana in field trials [28]. These microorganisms can be developed as potential biocontrol agents against FOC by understanding the type of interactions between the fungal pathogen and the control agent [22]. Generally, mechanisms through which microorganisms inhibit fungal pathogens in the rhizosphere are: (i) competition for nutrients, oxygen or space; (ii) parasitism or the physical destruction of fungal cell walls by the action of hydrolytic enzymes produced by the antagonist; (iii) antibiosis or the inhibition of one microorganism by diffusible compound(s) produced by another, and/or, by a synergistic combination of these modes of action [1,2].

Actinomycetes of the genus *Streptomyces* are well known for their ability to suppress growth of a wide variety of fungal pathogens [4,5,40,42]. *Streptomyces* species have been used extensively in the biological control of several formae speciales of *F. oxysporum*, which caused wilt disease in many plant species such as cotton [30], carnation [16], asparagus [37], French bean [6] and tomato [7]. Many antibiotics produced by actinomycetes have been used directly or have been assumed to be responsible for the biocontrol potential of the producing strain [33,37,39]. *Streptomyces* are also known for their ability to produce fungal cell wall-degrading enzymes such as cellulases, hemicellulases, chitinases, amylases and glucanases [39,42]. The role of these hydrolytic enzymes in antifungal activity and biocontrol ability of *Streptomyces* has been investigated [21,41].

Previously, a marine-derived streptomycete was shown to exhibit strong *in vitro* antagonism toward several plant pathogenic fungi [10]. The isolate, referred to as strain G10, showed strong antagonism toward FOC race 4. When tested for its biocontrol ability, banana seedlings treated with strain G10 showed significantly less severe leaf wilting and rhizome discolouration due to FOC race 4 infection in a greenhouse assay [10]. Here we report the isolation, selection and identification of strain G10, as well as the antagonistic effects of this strain against FOC race 4 in agar. The present study also indirectly investigated whether diffusible

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antifungal metabolites produced by strain G10 could be considered responsible for its inhibitory action against the *Fusarium* wilt pathogen of banana.

Materials and methods

Fungal pathogens

Fungal strains used in the *in vitro* antagonism assay were: *Pyricularia oryzae* MPO 293 (provided by the Malaysian Agricultural Research and Development Institute, Seberang Prai); FOC races 1, 2 and 4 (provided by Prof. Dr. Mak Chai, University of Malaya); *Rhizoctonia solani* R1 and *Phytopthora palmivora* P 250 (provided by Syngenta R&D Station, Rembau, Malaysia). All cultures were grown on potato dextrose agar (PDA) plates at 27°C. Cultures were stored on PDA slants at 4°C.

Isolation of actinomycetes

Actinomycetes were isolated from sand, sediment and leaf litter samples collected from various locations on a coastal sandbar, located across a stretch of beach about 7 km from the coastal town of Port Dickson (2°31′N, 102°48′E), West Malaysia. Samples were suspended in sterile saline (0.9% NaCl, wt/vol) solution and treated for 30 min at 70°C. The serial dilution spread plate technique was used, where dilution 10^{-4} was plated onto starch–casein (SC) agar [25]. To minimize the growth of fungal contaminants, 50 μg/ml cycloheximide and 50 μg/ml nystatin were added to the isolation medium, which was prepared in half-strength artificial sea salt solution to enhance the isolation of marine-derived actinomycetes. Dilution plates were incubated at 28±2°C for 2–4 weeks. Actinomycete colonies were picked and streaked onto inorganic salts–starch (ISP4) agar plates [35]. Pure colonies were transferred to fresh ISP4 plates, incubated at 28±2°C until they sporulated and stored at 4°C until used. Stock cultures were stored as spore suspensions in 30% (vol/vol) glycerol at −20°C.

In vitro antagonism assay

A modified "cross-plug" assay method [25] was used to test the ability of these isolates to inhibit the growth of several plant pathogens. A 5-mm-diameter ISP4 agar plug, fully covered with a grown lawn of an actinomycete, was placed at the centre of assay plates and incubated at 28±2°C for 5 days. This was done to allow the culture to be established on the agar surface and to sporulate prior to inoculation of the plates with fungal strains. For each test fungus, two 5-mm-diameter PDA plugs covered with actively growing mycelium were placed about 2 cm from both sides of the actinomycete colony. Fungal plugs were also placed on uninoculated assay plates as controls. After 2 and 4 days of incubation at 27±2°C, the plates were examined for inhibition of growth.

Characterization of the selected antagonistic actinomycete

The selected isolate, strain G10, was characterized on the basis of cell wall 2,6-diaminopimelic acid type [13], spore chain morphology, cultural and physiological characteristics [35]. The strain was further identified by analysis of its 16*S* rDNA sequences as described previously [10,34].

Scanning electron microscopic studies of the antagonistic effects of strain G10 on mycelial growth of FOC race 4 on agar plates

Microscopic studies of the *in vitro* antagonistic effects of strain G10 on FOC race 4 were carried out on bioassay plates similar to those used in the "cross-plug" assay. Morphology of fungal mycelium along the edges of the inhibited colonies facing strain G10 was examined under a dissecting microscope at ×15 magnification. After 4 days of incubation, blocks of mycelial samples were collected from the inhibited fungal colony edge and fixed overnight in 2% aqueous osmium tetraoxide at 4°C. Following three washes with distilled water, the samples were dehydrated in a graded ethanol series and critical point dried in liquid carbon dioxide. Samples were then mounted on aluminium stubs, sputter-coated with gold (Biorad Cool Sputter Coater E5100) and examined with a Phillips scanning electron microscope (SEM model 515). Mycelial samples were collected from at least five assay plates and within each plate from different points of the inhibition zones to obtain a representative sampling of the antagonistic effects.

Extraction of metabolites produced by strain G10 in liquid medium

A 5-ml spore suspension of strain G10 was inoculated into 50 ml of seed medium in a 250-ml flask. The seed medium contained (wt/vol): 2% soluble starch, 0.4% glucose, 0.4% yeast extract, 0.5% malt extract, 0.5% peptone and 0.5% $CaCO_3$, prepared in half-strength artificial sea salt solution (pH=7.2). After incubation at 28±2°C for 48 h in a rotary shaker, 10 ml of seed inoculum was transferred into 100 ml of production medium in a 500-ml flask. The production medium contained (wt/vol): 2% cornstarch, 0.4% glucose, 1% cornsteep solids and 0.5% $CaCO_3$, prepared in half-

Table 1 *In vitro* antagonism of selected actinomycete isolates against fungal pathogens on "cross-plug" assay plates

Isolate code	Antagonism after 96 h toward					
	R. solani	FOC			*Phytophthora palmivora*	*Py. oryzae*
		Race 1	Race 2	Race 4		
G10	+	++	++	++	++	++
G16	+	nd	nd	+	++	++
G20	+	nd	nd	+	+	++
G21	+	nd	nd	+	++	++
G27	+	nd	nd	+	+	++

+Hyphal growth retarded with obvious zone of inhibition on the side facing the actinomycete colony.
++Total inhibition of hyphal growth in the area of plate facing the actinomycete colony.
nd, not done.

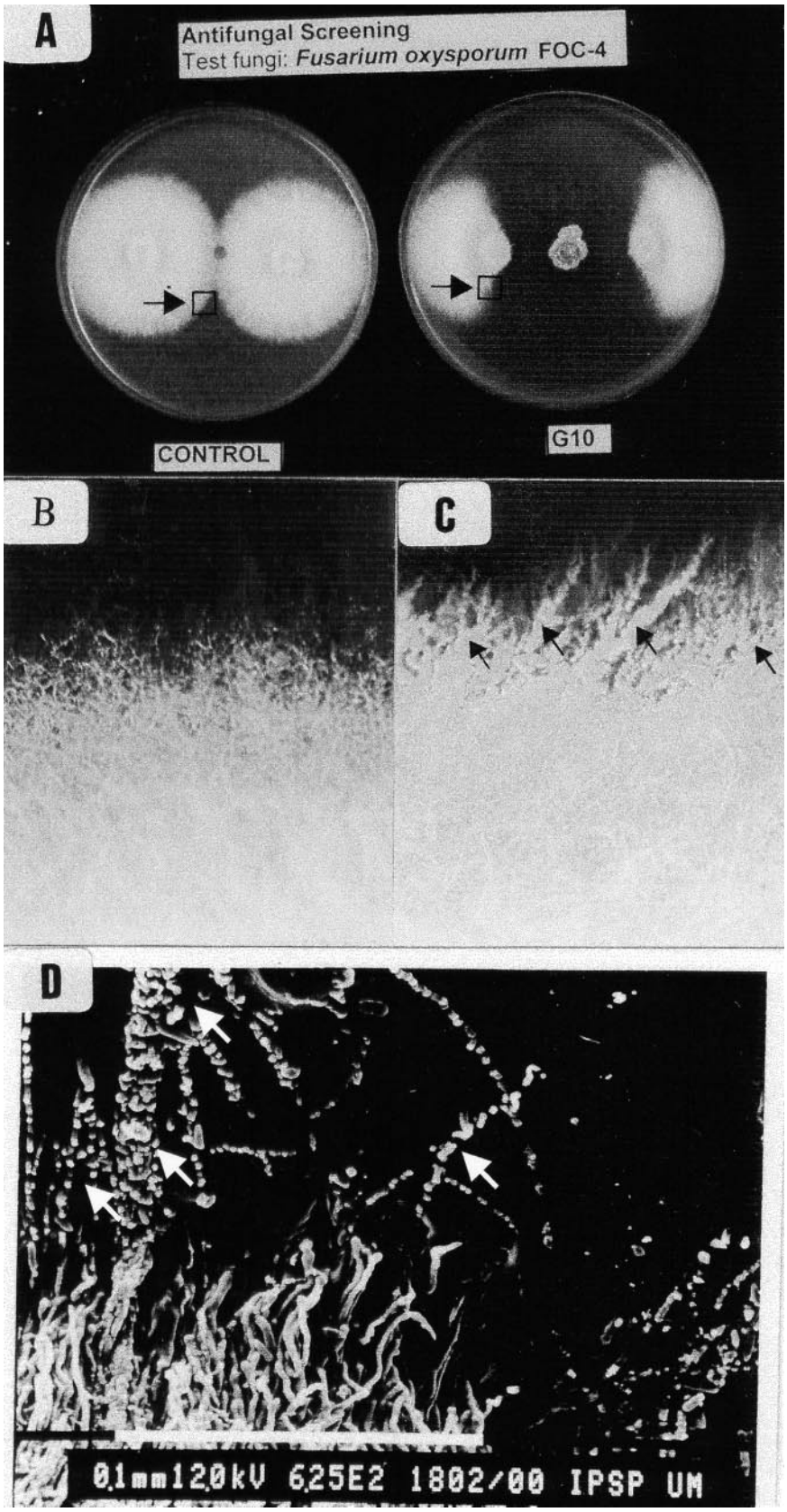

Figure 1 Antagonism of strain G10 to FOC race 4 on agar. (A) "Cross-plug" assay plates after 4 days of paired incubation (arrow showing the source of fungal mycelial samples for microscopic studies). (B) Hyphal ends of FOC race 4 colonies in control plate (×15). (C) Hyphae showing thickened and bulbous structures (arrowed) at the edges of the inhibited fungal colonies in the paired culture plate (×15). (D) SEM of disintegrated or lysed (arrow) hyphal ends of FOC race 4 in paired culture plates.

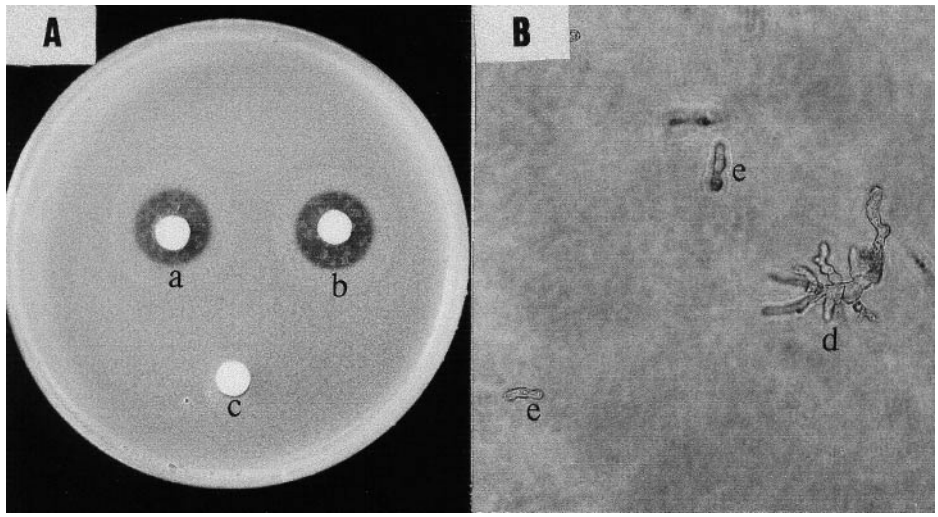

Figure 2 *In vitro* antagonistic effects of metabolites of strain G10 on FOC race 4. (A) Paper disc assay plate seeded with fungal spores; discs containing 0.5 mg of crude extracts of strain G10 (a,b) and solvent control (c). (B) Light micrograph of fungal germ tube and spores at the margin of inhibition zones produced by the crude extracts (×400); swollen and distorted germ tube which branched more freely (d) and spores showing PEI (e).

strength artificial sea salt solution (pH=7.2). After 5 days of incubation, the whole culture broth was lyophilized and extracted with a mixture of dichloromethane:methanol (1:1, vol/vol) to obtain crude extracts.

In vitro antagonistic effects of strain G10 metabolites on mycelial development and spore germination of FOC race 4

Crude extracts from strain G10 were tested for antibiosis using the paper disc method of Gunji *et al* [11]. About 0.5 mg of crude extract in 20 μl of 10% (vol/vol) methanol was applied to sterile paper discs and placed on test plates inoculated with spores of FOC race 4 at a concentration of 1×10^6 spores/ml. Control discs contained 20 μl of 10% (vol/vol) methanol. Plates were incubated at 27±2°C and observed for the presence of an inhibition zone. After 24 h, morphological changes on fungal mycelia occurring in the margin of the inhibition zones were examined under a compound microscope at ×400 magnification. Experiments were repeated twice.

A modified method of El-Abyad *et al* [5] was used to study spore germination of FOC race 4 in the presence of metabolites of strain G10. Fungal spore suspension (1×10^6 spores/ml) was prepared from fresh culture plates. Two pieces of 18-mm-diameter plain agar (1.8%, wt/vol) discs were placed on a sterile glass slide in a Petri dish layered with sterile moistened filter paper. About 0.5 mg of strain G10 crude extracts in 20 μl of 10% (vol/vol) methanol was placed on the agar disc. Then, 20 μl of fungal spore suspension was added and mixed with the crude extracts on the agar disc. Control agar discs containing 20 μl of sterile distilled water mixed with fungal spore suspension and 20 μl of 10% (vol/vol) methanol mixed with spore suspension were also prepared. After 8 h of incubation at 27±2°C, agar discs were covered with coverslips and examined under a compound microscope at ×200 magnification. Percentage spore germination was calculated by counting 100 spores on each agar disc. Two plates were prepared for each treatment. The experiment was repeated twice. The results obtained were analysed statistically using analysis of variance (ANOVA), and significance was determined using multiple range analysis at the 95% LSD level.

Direct observation of the antagonistic effects of strain G10 on spores of FOC race 4 in a soil environment

A buried slide technique [38] was used. About 50 g of soil (pH=7.6) was sieved, air-dried and sterilized in 250-ml conical flasks by autoclaving at 121°C for 30 min. A 5-ml spore suspension of strain G10 containing 3×10^8 colony-forming units (CFU)/ml was prepared from ISP4 plates. Spores, washed thoroughly with sterile distilled water, were inoculated into the sterile soil and incubated at 28±2°C for 7 days.

A 10-ml spore suspension of FOC race 4 containing 2×10^6 spores/ml was mixed in 100 ml of sterile 1.8% (wt/vol) molten agar, and coated on sterile microscope glass slides by dipping them. When the agar layer had set, slides were carefully inserted vertically into beakers containing sterile soil alone (control) or strain G10-inoculated soil, and incubated at 27±2°C. At the end of each specified incubation period (2, 3, 4, 5 and 7 days), the slides were removed carefully and examined immediately under a compound

Table 2 Effect of crude extracts of strain G10 on spore germination of FOC race 4

Treatment (sample)[x]	Mean percentage germination (%) after 8 h[y]	
	Experiment 1	Experiment 2
Sterile distilled water	95.0±4.16a[z]	99.7±0.50a[z]
10% (vol/vol) methanol	100.0±0.00a	98.2±1.71a
0.5-mg crude extract in 10% (vol/vol) methanol	43.0±13.66b	43.7±9.98b

[x]20 μl of spore suspension (1×10^6 spores/ml^{-1}) of FOC race 4 mixed with 20 μl of each sample (treatment) on water agar discs.
[y]Mean percent spore germination was calculated by counting 100 spores on each agar discs; four replicate discs per treatment. Experiments were repeated twice (1 and 2).
[z]Means (mean±SD) with the same letter within a column are not significantly different ($P<0.05$).

microscope at ×400 magnification. Each observation period involved analysis of two beakers for each treatment. Each beaker contained three spore-coated glass slides. After the second day of incubation, the number of spores that germinated and formed germ tubes on the control and test slides was compared visually under a light microscope.

Results

Isolation, selection and characterization of antagonistic actinomycete

Thirty-eight strains were isolated and classified into streptomycete-like strains (abundant aerial mycelium with powdery spores) or nonstreptomycete strains (small and compact, or slimy colonies coloured orange, red-brown or brown to black). The streptomycete-like isolates were then grouped into different colour groups based on such gross morphological differences as aerial mycelium colour and pigmentation on inorganic salts–starch agar medium [35]. Twenty-five isolates, representatives from each colour group of actinomycetes, were selected for the *in vitro* antagonism assay against phytopathogenic fungi. Five isolates (G10, G16, G20, G21 and G27) inhibited the growth of all fungi tested (Table 1). The remaining isolates were either weak or not antagonistic. One isolate, G10, showed strong antagonism towards three of four fungal pathogens. Moreover, it was the only isolate that strongly inhibited FOC race 4 and also FOC races 1 and 2 (Table 1).

Strain G10 produced whitish to gray aerial mycelium and rugose ornamented spores in spiral chains. LL diaminopimelic acid (DAP) was found in strain G10 as a component of the cell wall. The morphological characteristics and the presence of DAP permitted a clear assignment of strain G10 to the genus *Streptomyces* [13,35]. Based on these phenotypic properties and results from the phylogenetic or 16*S* rDNA analysis (unpublished data), strain G10 was assigned to the *Streptomyces violaceusniger* clade [34].

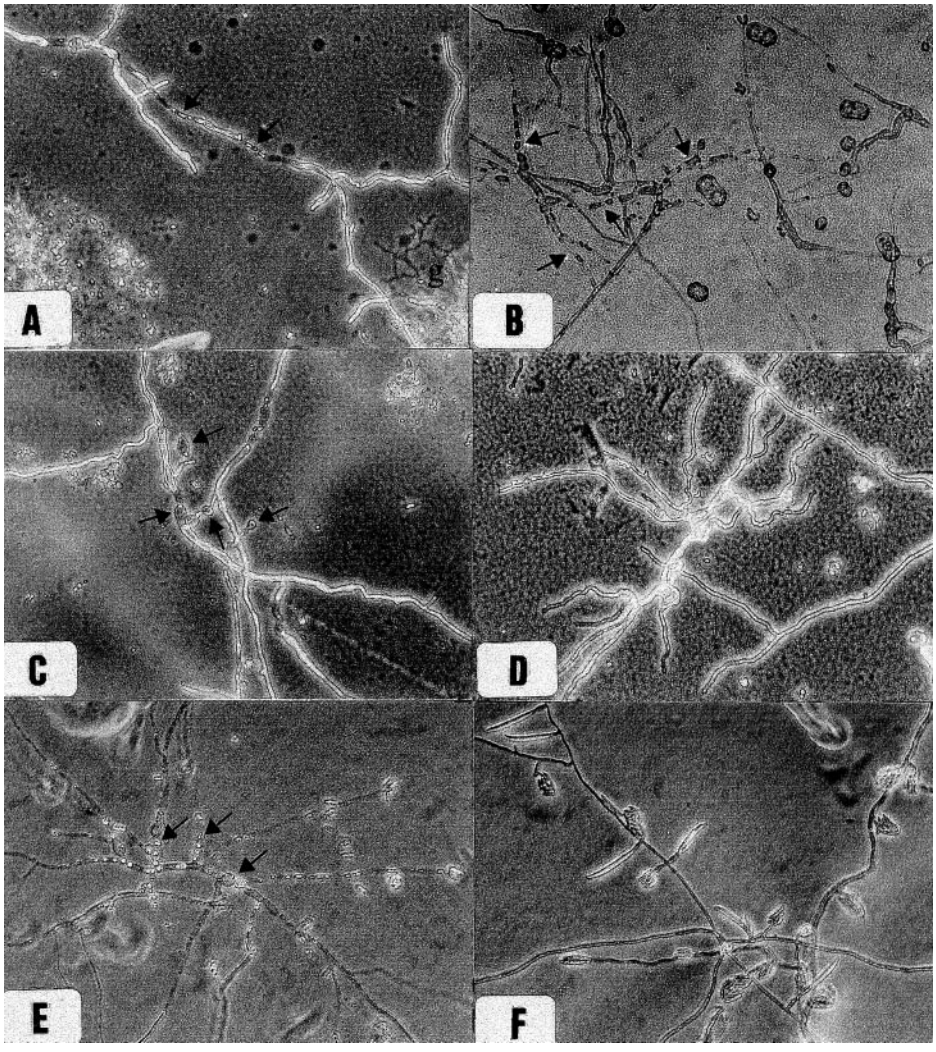

Figure 3 Hyphal development of FOC race 4 in strain G10-inoculated soil culture and in uninoculated control soil (×400). (A) Early stages of hyphal distortion and lysis (arrowed) after 2 days in soil culture; soil particles colonized with strain G10 (g). (B) Distortion and lysis of hyphae (arrowed) noted more frequently after 4 days in soil culture. (C) Formation of hyphal protuberances and swellings (arrowed) after 5 days in soil culture. (D) Branched hyphal appearance also noted after 5 days in soil culture. (E) Swollen (arrow), distorted and massive lysis of hyphae after 7 days in soil culture. (F) Healthy hyphal growth with abundant sporulation after 5 days in uninoculated control soil.

Antagonistic effects of strain G10 on mycelial growth of FOC race 4 on agar plates

Microscopic studies done on the "cross-plug" assay plates of FOC race 4 indicated that there was no direct contact between strain G10 and the inhibited fungal colonies. This suggested that the inhibition of FOC race 4 by strain G10 was most likely due to excreted, diffusible antifungal metabolites in the agar medium. Inhibition of fungal mycelial growth was evident after 2 days of incubation, but the antagonistic effects were more pronounced after 4 days of incubation (Figure 1A). When observed under a dissecting microscope, fungal mycelium along the edges of the colonies facing strain G10 appeared thickened with bulbous-like formation along the ends (Figure 1C). On the control plate, however, fungal mycelium showed regular radial growth (Figure 1B). When examined by SEM, peripheral areas of the inhibited colonies showed lysed or greatly disintegrated hyphal ends (Figure 1D).

The in vitro effects of metabolites of strain G10 on mycelial development and spore germination of FOC race 4

Crude extracts of strain G10 at a concentration of 0.5 mg/disc produced inhibition zones with a diameter of about 13 mm on paper disc assay plates seeded with FOC race 4 spores. The control disc with 10% (vol/vol) methanol gave no inhibition zones (Figure 2A). Observation of fungal spores in the margin of the inhibited region showed that a majority of the FOC race 4 spores had germinated to the post-emergence inhibition (PEI) stage (Figure 2B). This is the inhibition of development just after spore germination [38]. Although some germination occurred, the germ tubes were unable to develop fully. In most cases, further hyphal development ended up in the formation of highly distorted, swollen and frequently more branched hyphal appearances (Figure 2B). The distortion and excessive branching, a typical example of which is seen in Figure 2B, are associated with stunting [3]. It is typical that the diameter of the stunted hyphae is much greater than normal.

Strain G10 metabolites, at a concentration of 0.5-mg crude extracts in 10% (vol/vol) methanol, significantly ($P<0.05$) reduced spore germination of FOC race 4 when assayed on plain agar discs (Table 2). After 8 h of incubation, spore germination of the fungus was reduced by about 57% in each of the two experiments. In contrast, a high degree of germination occurred in both experiments when spores were mixed with either distilled water (95–99.7%) or with 10% (vol/vol) methanol (98.2–100%). The presence of solvent in the crude extract sample did not have any negative effect on spore germination.

Antagonistic effects of strain G10 on spores of FOC race 4 in a soil environment

Results from the buried slide technique showed that the spores of FOC race 4 on glass slides buried in uninoculated sterile soil germinated normally and the germ tubes developed into long hyphal strands. By the fifth day, healthy and extensive hyphal growth with abundant sporulation was evident on the control slides (Figure 3F). However, in the sterile soil in which strain G10 was grown, fungal spore germination was reduced compared to the control slides. The exact percentage of spore germination was not calculated in this study because the presence of soil particles on the slides made it difficult to count ungerminated spores. Therefore, the extent of fungal spore germination was compared visually using a compound microscope.

A lytic effect caused by strain G10 was first detected in the fungal hyphae after 2 days of incubation. Hyphal distortion, like swelling or bulbous growth, was also observed. By the fourth day, distortion and lysis of the hyphae were more frequently noted. Other morphological effects, such as abnormal branching of hyphae and the formation of hyphal protuberances, were also seen. Overall, very little sporulation was observed in the FOC race 4 culture. After incubation periods longer than 7 days, only fragments of the fungal hyphae remained in the soil. Some of the typical effects of strain G10 on FOC race 4 in soil are illustrated in Figure 3A–E.

Discussion

The majority of actinomycetes isolated in this study belong to the streptomycete-like group (data not given). The predominance of streptomycetes in any actinomycete population is well known [4,25]. This could also be due to the fact that no special pretreatment techniques were adopted to enhance the isolation of rare actinomycetes or nonstreptomycetes that have been reported to be present in sediments [25]. In the *in vitro* antagonism assay, all of the antagonistic actinomycetes were streptomycetes (unpublished data). The assay, therefore, showed promising results for the use of streptomycetes as antifungal agents against phytopathogenic fungi. Similar results have been reported previously in actinomycete screening studies [4,41]. In previous studies, it was noted that some microorganisms which inhibited pathogens on agar also did so in soil, whereas those ineffective on agar were also ineffective in soil [9]. Thus, *in vitro* assays appear to be useful in identifying which actinomycete antagonists might function in soil.

Strain G10 was selected for one trait often associated with biocontrol agents — the ability to produce extracellular metabolites active against several phytopathogenic fungi, including FOC race 4. In the *in vitro* assay, preinoculation of strain G10 was done to allow growth and sporulation of the culture prior to inoculation with FOC race 4. Thus, the antagonism between strain G10 and the pathogen may have involved production of secondary metabolites in agar [4]. The *in vitro* studies further indicated that culture of strain G10 in liquid media produced extracellular metabolites that showed clear zones of inhibition against FOC race 4. In their crude form, these metabolites inhibited spore germination and hyphal development of FOC race 4, and induced morphological changes such as swollen and distorted germ tubes that branched more freely than normal conidia.

In this study, an indirect approach was used to show that strain G10 produced diffusible metabolites, which have inhibitory effects on the growth of FOC race 4 in a soil environment. A direct observation *via* the buried slide technique was used to determine the antagonistic effects of strain G10 on FOC race 4, when fungal spores were incubated in a strain G10-inoculated soil culture. Strain G10 was incubated for 7 days in the soil culture prior to the buried slide experiment. This period of incubation may be required to allow the antagonist to either become established or multiply in soil, or to bring about the mechanism of antagonism. Rothrock and Gottlieb [33] had shown that the population of *S. hygroscopicus* subsp. *geldanus*, a strong antagonist of the root rot-causing *R. solani*, and antibiotic concentration in soil increased with the time of incubation. Generally, the inhibitory effects of strain G10 on spore germination and development of FOC race 4 in soil closely resembled those observed in the presence of the actinomycete metabolites *in vitro*. These effects include inhibition of fungal spore

germination, swelling, distortion and excessive branching of the fungal hyphae.

In all cases, there was a definite decrease in the development of FOC race 4 in the presence of strain G10. Some decrease in the degree of inhibition of FOC race 4 was displayed by strain G10 in soil, when compared to the degree of inhibition by the antifungal metabolites of strain G10 in the paper disc and spore germination assays. This was indicated by a slightly more extensive hyphal development of FOC in the strain G10-inoculated soil culture, compared to that observed in the *in vitro* assays. Variations in the antifungal potential of strain G10 in agar and in soil might be due to variations in the concentration of the antifungal metabolites produced by strain G10 under different growth conditions [29]. Medium composition may also cause a difference in the type of bioactive compounds that are produced [39]. For example, different types of carbon sources in the growth medium have been shown to have an important impact on both the production of secondary metabolites by streptomycetes, and the interactions of bacterial cells and the fungal hyphal cell surface [42].

Evidence for the role of competition and parasitism in the biocontrol of plant diseases has been convincing. However, antibiosis is much less clearly established due to the lack of methods for a meaningful evaluation of the production and function of compounds mediating antibiosis in soil [9,33]. Previously, Fravel [9] considered antibiosis as a type of antagonism mediated by specific or nonspecific metabolites of microbial origin, by lytic agents, enzymes, volatile compounds or other toxic substances. Fungal cell wall-degrading enzymes produced by an antagonist were, therefore, thought to be involved simultaneously in parasitism and antibiosis. Antibiosis is particularly considered to provide an advantage in biological disease control because compounds mediating antibiosis can diffuse rapidly in nature, and direct contact between the antagonist and pathogen is not necessary [12].

Antibiotics have been implicated repeatedly in the antagonism of fungi by actinomycetes [4,5,29,30,33,38,40,42]. There are many reports related to antibiotic substances that induced such malformations such as stunting, distortion, swelling, hyphal protuberances or the highly branched appearance of fungal germ tubes [3,11,29,31]. Using this criterion, Stevenson [38] found that antibiotics of some soil actinomycetes caused similar effects on hyphae of *Helminthosporium sativum*, in culture and in soil. Swellings and lysis of hyphal tips have also been seen in the presence of antibiotics of other actinomycetes [31]. Several species from the *S. violaceusniger* clade produced antifungal antibiotics such as niphithricins [8], spirofungin [14], azalomycin F complex, guanidylfungins and malonylniphimycin [15]. Previously, a species from this clade that produced geldanamycin in culture and in soil was able to biocontrol diseases caused by *R. solani* [33]. In other studies, a strain of *S. violaceusniger* that produced at least three antifungal antibiotics has been reported to suppress damping-off of lettuce caused by *Pythium ultimum* [39]. On the other hand, *Streptomyces* are also known for their ability to cause lysis of fungal hyphae by producing chitinases and glucanases [18-21,41]. Chitin in fungal cell walls is normally in a highly rigid, crystalline state. In the hyphal apex, however, the chitin is sensitive to chitinases [21].

Although no direct evidence of parasitism was observed during the SEM and buried slide studies, strain G10 was capable of causing lysis of FOC race 4 hyphae in the "cross-plug" assay plates and in soil culture. Lysis was not observed in the margin of the inhibitory zones produced by strain G10 metabolites on paper disc assay plates. However, the metabolites markedly reduced spore germination of FOC race 4 in the glass slide assay (Table 2). In their studies, Lockwood and Lingappa [20] had indicated that the ability of actinomycetes to lyse living mycelium in agar or in soil was apparently related to their ability to inhibit spore germination as determined by production of inhibition zones on agar. According to these authors, the correlation between these two processes suggests that inhibition of spore germination and destruction of mycelium was brought about by a similar mechanism. This could either be a lytic enzyme that destroys germ tubes or otherwise prevents germination, or an antibiotic that induces autolysis of the fungal hyphae. Autolysis results in self-digestion of the protoplasm and cell walls by enzymes of the fungus itself [18]. However, autolysis induced by antibiotics and toxins from antagonistic microorganisms may not result in complete lysis of fungal mycelium in soil. Final dissolution of the remains of the empty cell wall would then depend on hydrolytic enzymes, which in soil would be of microbial origin [20]. No studies have been done to determine if strain G10 produced hydrolytic enzymes under the influence of fungal cell walls. The antifungal biocontrol agent, *S. lydicus* WYEC108, produces high levels of chitinase in the presence of chitin from fungal cell walls as carbon source [21].

This study indirectly demonstrated that antibiosis mediated by diffusible metabolites was likely involved in the antagonism of strain G10 against FOC race 4 in soil. In these studies, the antagonistic effects were studied in sterile soils. However, further studies are needed to determine whether inhibition of fungal spore germination, and hyphal swelling, distortion and lysis will be detected when FOC race 4 is placed in strain G10-inoculated nonsterile soils. In view of the much higher concentration of antagonistic organisms in natural soils, a more rapid rate of destruction is to be expected [38]. Under certain circumstances, however, the antagonistic organisms may antagonize each other and thus become ineffective [7]. If antibiotics are involved, some of these compounds can be inactivated due to biological degradation, adsorption on clays and reactions with organic matter in soil. Despite all the *in vitro* assays, conclusive evidence as to the exact role of antibiotics and/or hydrolytic enzymes could not be reached in the absence of purified compounds. Secretion of enzymes and antibiotics may interact synergistically in the process [2]. Further studies are needed to characterize the antibiotic substances of strain G10, study the antagonistic effects of purified compound(s), determine the enzyme production and understand the regulation of their production. These studies will aid in the manipulation and development of strain G10 as a potential biological control agent for the *Fusarium* wilt pathogen of banana.

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