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ORIGINAL PAPER

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Evaluation of *Streptomyces* sp. strain g10 for suppression of *Fusarium* wilt and rhizosphere colonization in pot-grown banana plantlets

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Abstract *Streptomyces* sp. strain g10 exhibited strong antagonism towards *Fusarium oxysporum* f.sp. *cubense* (Foc) races 1, 2 and 4 in plate assays by producing extracellular antifungal metabolites. Treating the planting hole and roots of 4-week-old tissue-culture-derived 'Novaria' banana plantlets with strain g10 suspension (10^8 cfu/ml), significantly ($P < 0.05$) reduced wilt severity when the plantlets were inoculated with 10^4 spores/ml Foc race 4. The final disease severity index for leaf symptom (LSI) and rhizome discoloration (RDI) was reduced about 47 and 53%, respectively, in strain g10-treated plantlets compared to untreated plantlets. Reduction in disease incidence was not significant ($P < 0.05$) when plantlets were inoculated with a higher concentration (10^6 spores/ml) of Foc race 4. Rhizosphere population of strain g10 showed significant ($P = 0.05$) increase of more than 2-fold at the end of the 3rd week compared to the 2nd week after soil amendment with the antagonist. Although the level dropped, the rhizosphere population at the end of the 6th week was still nearly 2-fold higher than the level detected after 2 weeks. In contrast, the root-free population declined significantly ($P = 0.05$), nearly 4-fold after 6 weeks when compared to the level detected after 2 weeks. Neither growth-inhibiting nor growth-stimulating effects were observed in plantlets grown in strain g10-amended soil.

Keywords Actinomycete biocontrol agent · *Fusarium oxysporum* f.sp. *cubense* · *Fusarium* wilt control · *Streptomyces violaceusniger* · Rhizosphere

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Introduction

Banana and plantain varieties are often attacked by pathogens, of which the most serious is *Fusarium oxysporum* f. sp. *cubense* or Foc [39]. The pathogen survives in soil and penetrates roots from where it spreads slowly to the corm [28]. Major symptoms of wilt develop when the fungus invades the vascular tissues of the petiole and leaves, resulting in leaf chlorosis and necrosis. Longitudinal sections of the pseudostem show signs of vascular discoloration, which are more distinct in the corm tissue [27]. Earlier, some workers have successfully employed antagonistic bacteria isolated from flax wilt-suppressive soil [42], fluorescent pseudomonads [34] and *Trichoderma viride* [28] to control *Fusarium* infection of banana. So far, Meredith [24] was the only one to attempt to evaluate the use of antagonistic actinomycetes in controlling banana wilt in field experiments.

Streptomyces spp., well known as sources of secondary metabolites, antibiotics and lytic enzymes, have been studied extensively as potential biological control agents against fungal phytopathogens such as *Pythium ultimum* [9], *Fusarium oxysporum* [10], *Sclerotinia homeocarpa* [41] and *Phytophthora fragariae* [44]. In screening for biocontrol agents against the root-rot pathogen of wheat, Stevenson [38] reported that the most promising *Streptomyces* isolates were those producing antibiotics against *Helminthosporium sativum*. Yuan and Crawford [47] also showed that *Streptomyces lydicus* WYEC108, a potent biocontrol agent for controlling seed and root

rot, was selected for its strong in vitro activity against *Pythium ultimum*. According to Weller [45], microorganisms that colonize the rhizosphere are ideal for use as biological control agents against soil-borne diseases. Pathogens encounter antagonism from rhizosphere microorganisms before and during primary infection, and also during secondary spread on the root. The ability of the control organism to maintain a sufficient population density in the rhizosphere for a sufficient length of time is critical to the success of the biocontrol method [32].

Previously, Getha et al. [13] tested the in vitro antifungal antagonism of actinomycetes isolated from Malaysian coastal resources. *Streptomyces* sp. strain g10, isolated from a coastal mangrove [*Rhizophora apiculata* (Blume)] stand, was among several strains that demonstrated strong activity against a range of phytopathogenic fungi. The strain, later assigned to the *Streptomyces violaceusniger* clade, produced secondary metabolites that strongly inhibited spore germination and hyphal development of Foc race 4 in plate assays [14]. Positive results obtained from the in vitro tests led to the present in vivo studies to evaluate the ability of strain g10 to protect banana seedlings against Fusarium wilt. It is also important to study the ability of this strain to survive in soil and adapt to the specific conditions of the host rhizosphere where extensive penetration of roots by Foc takes place.

In this paper, we characterized the in vitro antifungal activities of *Streptomyces* sp. strain g10 against different pathogenic races of Foc. The biocontrol ability of strain g10 against Foc in tissue-culture-derived banana plantlets was evaluated by the simple application technique of combining root-immersion and soil treatment (adding a suspension of strain g10 to soil) just before planting in the greenhouse. Further, we studied the rhizosphere-colonizing ability of the strain when incorporated into a soil-based delivery medium and inoculated into pot soil of banana plantlets.

Materials and methods

Fungal and bacterial inoculum

Fusarium oxysporum f.sp. *cubense* (Foc) races 1 and 2 were obtained from the Department of Genetics, University of Malaya. Foc race 4 isolated from susceptible banana rhizome tissues was obtained from United Plantation (UP) Berhad, Jendarata Estate, Teluk Intan, Perak. Working cultures were grown on potato dextrose agar (PDA) plates at 27 ± 2°C for 10 days. The aerial growth of Foc on PDA cultures was suspended in sterile distilled water and filtered through double-layered cheesecloth. Two inoculum concentrations (2.5×10^6 and 2.5×10^4 spores/ml) were prepared with the aid of a hemocytometer. The aerial growth of a 10-day-old culture of *Streptomyces* sp. strain g10 grown at 28 ± 2°C on inorganic salts-starch (ISP 4) agar was suspended in 5 ml sterile distilled water. The number of colony forming units (cfu) in the suspension was determined by dilution plate count method and an inoculum size of 10^8 cfu/ml strain g10 was used.

In vitro antagonism bioassay

Strain g10 was tested for its ability to inhibit the growth of different pathogenic races of Foc using the agar-streak method [9]. Strain g10 was streak-inoculated on one side of PDA plates in a 2-cm-wide band from the edge of the plate and incubated at 28 ± 2°C for 5 days. A 5-mm-diameter PDA plug from the growing mycelium edges of Foc culture (Foc races 1, 2 or 4) was then placed in the center of the test plate and further incubated at 27 ± 2°C. Control plates containing fungal plugs only were also prepared. All control and test plates were run in four replicates and repeated at least three times. Plates were scored after 2, 4, 6 and 8 days by measuring fungal colony radius on the control plate (γ_o) and the distance of fungal colony growth perpendicular to strain g10 colonies on test plates (γ). Percent inhibition of fungal linear growth was calculated from each test plate and recorded as an average as follows:

$$\text{Percent inhibition of fungal linear growth} = [1 - (\gamma/\gamma_o)] \times 100$$

Host plant, soil and experimental pot

The cultivar used was an early maturing Dwarf Cavendish banana called 'Novaria' [21], which is susceptible to Foc race 4. Tissue cultured plantlets, acclimatized for 4 weeks in a commercial nursery, were provided by G. Singh, United Plantation Berhad. Test plantlets were carefully uprooted and those with healthy white roots were selected for the in vivo biocontrol assay.

The experimental pot, modified from the "double-tray technique" [1], consisted of a perforated inner plastic pot (diameter 15 cm; height 12 cm) containing soil and a larger outer container for collecting drained water, and strain g10 and Foc race 4 washouts. The inner pot fits snugly into the outer container. Soil comprising a mixture of sand and clay (4:1, w/w) was autoclaved for 30 min at 121°C. Each pot was filled with 900 g sterile soil and 100 g autoclaved (121°C/30 min) compost (USM Super Compost, Agronas, Sdn. Berhad). The pH of this soil mix was 7.1. All pots were watered to saturation and placed in a greenhouse that received 12 h sunlight each day with fluctuating day temperatures of 30–35°C and night temperatures of 20–24°C.

In vivo biocontrol assay

An assay modified from the biocontrol experiment carried out by Sivamani and Gnanamanickam [34], was performed to test the ability of strain g10 to control the

severity of wilt in 'Novaria' plantlets caused by Foc race 4. *Fusarium* inoculum was tested at low (2.5×10^4 spores/ml) and high (2.5×10^6 spores/ml) concentrations. Thirty plantlets were numbered and grouped into six treatment groups. For treatment G, roots of the test plantlets were immersed in a suspension of strain g10 (10^8 cfu/ml) for about 1 h before replanting. In treatments GF4 and GF6, roots were immersed into strain g10 suspension and drained for about 10 min before immersing into low or high conidial suspension of Foc, respectively, for another hour. An additional 10 ml strain g10 cell suspension was added to the planting hole before planting strain-g10-treated plantlets [8]. In treatments F4 and F6, roots were immersed for 1 h in low or high conidial suspension of Foc, respectively, before replanting. Roots of control plantlets (group C) were immersed in sterile distilled water. The plantlets were watered once a day from the 2nd day onwards and fertilizer was not added to the soil.

A second biocontrol assay was carried out where plantlets were given only treatments GF4 (plantlets treated with strain g10 and inoculated with Foc) and F4 (plantlets inoculated with Foc only). Only one inoculum concentration of Foc race 4 was tested, i.e., 10^4 spores/ml. All other experimental conditions, such as treatment method, soil type, greenhouse conditions, duration of experiment and number of plantlets per treatment, were the same as in the first experiment.

Plantlets were uprooted and their fresh weight taken at the end of the 4th week. Disease evaluation was based on external examination for chlorosis of leaves graded according to the leaf symptom index (LSI) scale and internal examination for the extent of rhizome discoloration graded according to the rhizome discoloration index (RDI) scale, as reported by Brake et al. [4]. The LSI scale had five levels, while an eighth scale was added to RDI to differentiate dead plants (dried completely) from those showing 100% rhizome discoloration but still surviving (standing). The overall disease severity index (DSI) for each treatment was calculated as follows:

$$\mathrm{DSI} = \frac{\sum(\text{number of scale} \times \text{number of seedlings in that scale})}{\sum(\text{number of treated seedlings})}.$$

Re-isolation of pathogen from infected rhizome tissues (Koch's postulates)

Infected (discolored) rhizome tissues were used to re-isolate the pathogen [11], according to the methods of Windels [46]. Rhizome samples from healthy pathogen-free untreated plants and strain-g10-treated plants were also used for isolation to reconfirm the causal pathogen. Fungal colonies were examined microscopically and *F. oxysporum* was identified as described in Toussoun and Nelson [40].

Inoculation of *Streptomyces* sp strain g10 into a soil-based delivery medium

A suspension of strain g10 (5 ml; 10^8 cfu/ml) was inoculated into 250-ml flasks containing 50 ml sterile nutrient medium [20]. After incubation for 48 h at 28 ± 2°C and 160 rpm, the cell mass of strain g10 was centrifuged at 5,000 rpm for 15 min under sterile conditions and re-suspended twice in sterile distilled water. Soil comprising a mixture of sand and clay (4:1, v/v) collected from a commercial plant nursery, was air-dried and sieved before use. The delivery medium consisted of 5% (w/w) sago hampas in soil [37]; 500 g delivery medium (pH 6.85) was autoclaved at 121°C for 30 min in 1-l Erlenmeyer flasks. Sterile distilled water was added to the formulation to adjust the moisture content to 40% (w/w). After mixing thoroughly, 50 ml inoculum of strain g10 was added and incubated at 28 ± 2°C for 10 days. Two 1 g samples were taken from three randomly selected flasks and serial dilutions were made to determine the average concentration of viable cells of strain g10 in the delivery medium.

Incorporation of delivery medium containing propagules of *Streptomyces* sp strain g10 into pot soil

Tissue-culture-derived 'Novaria' plantlets, acclimatized for 6 weeks in a commercial nursery, were used as the test plantlets, and experimental pots were prepared according to the "double-tray technique". Twelve pots were each half filled with 500 g sterilized soil (sand:clay, 4:1) and watered to saturation. Then, 500 g delivery medium containing propagules of strain g10 was added to each pot and mixed well with the soil below to ensure even distribution of strain g10 around the plantlet roots [37]. Six control pots containing 500 g sterile soil and 500 g delivery medium without strain g10 were also prepared. One plantlet was planted in each pot and the pots were watered once a day from the 2nd day onwards. The experiment was carried out for 6 weeks under the same greenhouse conditions as described above.

Isolation of *Streptomyces* sp strain g10 from rhizosphere and root-free soil

Three test pots were examined for the presence of strain g10 at each sampling period (2, 3 and 6 weeks after planting). Two 10 g samples of root-free soil were taken at a depth of about 5 cm from the top of each pot and placed

in Erlenmeyer flasks containing 95 ml sterile saline solution. Plantlets were then removed from each pot and shaken gently to remove all but the most tightly adhering rhizosphere soil. Root segments of 10 g (weight of roots and rhizosphere soil) were taken from each of the harvested plantlets and placed in Erlenmeyer flasks containing 95 ml sterile saline solution. Flasks were shaken for 10 min at 200 rpm and 10-fold serial dilution of the suspensions was prepared to determine the populations of strain g10 found in the rhizosphere and root-free soil of banana plantlets [25, 29]. Soil suspensions plated on actinomycete isolation agar plates amended with cycloheximide, nystatin and novobiocin, were incubated at 28 ± 2°C. Heights were measured for the six control and six test plantlets left at the end of the 6th week.

Statistical analysis

Means of percent inhibition of fungal linear growth were analyzed using analysis of variance (ANOVA), and means at each incubation period were compared by Duncan's multiple range analysis ($P = 0.05$). Five replicate pots per treatment, with one plantlet per pot, were prepared in the in vivo biocontrol assay. Pots were arranged in a complete randomized design in the greenhouse. The mean values of plant fresh weight and wilt disease index were subjected to ANOVA and were compared by Duncan's multiple range analysis ($P = 0.05$). The mean values of plant fresh weight and disease index in the second assay were statistically analyzed using two-sample analysis at $P = 0.05$. Means of strain g10 population density in the rhizosphere and root-free soil from triplicate test pots were subjected to ANOVA and were compared by Duncan's multiple range analysis ($P < 0.05$). Average height of the control and test plantlets measured 6 weeks after the soil amendment studies were statistically compared using two-sample analysis at $P = 0.05$.

Results

In vitro antagonism bioassay

Strain g10 exhibited a strong degree of inhibition of linear growth of all three races of Foc tested. The strain showed 80–89% inhibition against Foc race 1, 87–94% against race 2 and 82–93% against race 4 within a period of 2–8 days of paired incubation (Fig. 1). Growth inhibition of race 4 increased significantly ($P = 0.05$) after the 4th day and there was no significant difference between percent inhibition of races 2 and 4 after 8 days of incubation.

In vivo biocontrol assay

The *Fusarium*-inoculated plantlets were monitored continually for wilt symptom development. The *Fusarium*-free control plantlets treated with strain g10 (G) and *Fusarium*-free control plantlets not treated with strain g10 (C), remained healthy, without indicating leaf chlorosis or rhizome discoloration. Although one plantlet in control group G showed some browning on the margin of lower leaves, there was no visible internal discoloration when the rhizome of this plantlet was sectioned longitudinally.

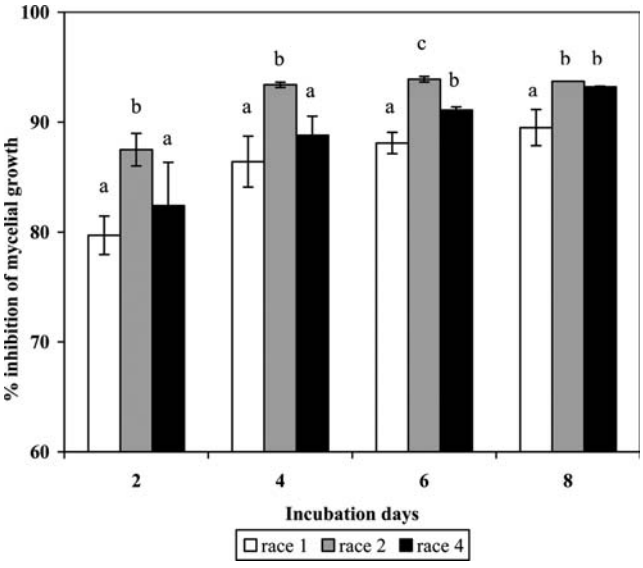

Fig. 1 Inhibition caused by *Streptomyces* sp. strain g10 on mycelial linear growth of *Fusarium oxysporum* f.sp. *cubense* (Foc) races 1, 2 and 4 on agar plates. For each incubation period, columns with different letters represent percent inhibitions that differ significantly from each other ($P < 0.05$). *Error bars* Standard deviation of the mean

It was observed that, at both Foc race 4 concentrations tested, the DSI for LSI and RDI were reduced in *Fusarium*-inoculated plantlets treated with strain g10 compared to *Fusarium*-inoculated plantlets grown in the absence of the control agent (Table 1). When plantlets were inoculated with the low concentration of Foc, severe leaf symptoms were observed already 2 weeks after inoculation in plantlets that were not treated with strain g10 (F4). Leaf chlorosis began with older leaves and progressed upward to younger leaves. At the end of the 4th week, two plantlets died, while all the others showed mild to severe leaf and rhizome symptoms. On the other hand, leaf symptoms in the *Fusarium*-inoculated plantlets treated with strain g10 (GF4) were seen clearly only in the 4th week. One plantlet showed severe leaf and rhizome symptoms while another showed very mild leaf chlorosis and restricted discoloration of the rhizome. None of the plantlets died after 4 weeks. The LSI and RDI of GF4 plantlets were significantly ($P = 0.05$) reduced (about 47 and 53%, respectively) when compared to the F4 plantlets (Table 1). This showed that plantlets treated with strain g10 prior to inoculation with Foc race 4 took longer to express wilt symptoms, thus providing initial control of wilt disease.

Table 1 Disease severity indices (DSI) and mean fresh weight of 'Novaria' plantlets grown in the presence and absence of *Streptomyces* sp. strain g10 after 4 weeks in the greenhouse (in vivo biocontrol assay 1). Means with the same letter within a column are not significantly different ($P < 0.05$). *Foc Fusarium oxysporum* f.sp. *cubense*, *LSI* leaf symptom index, *RDI* rhizome discoloration index (average of five plantlets for each treatment)

Foc race 4 (spores/ml)	Strain g10 treatment[e]	Fresh weight (g)[f]	DSI		Number of plantlets inoculated with Foc race 4	Number of infected plantlets	Number of dead plantlets
			LSI	RDI			
0	Untreated (C)	11.0 ± 2.84a	1a	1a	0	0	0
0	Treated (G)	9.0 ± 2.96ab	1.2a	1a	0	0	0
2.5×10^4	Untreated (F4)	5.5 ± 2.82cd	3.4b	6.4c	5	5	2
2.5×10^4	Treated (GF4)	7.7 ± 1.60bc	1.8a	3b	5	2	0
2.5×10^6	Untreated (F6)	3.7 ± 0.65d	4.6b	7.4c	5	5	3
2.5×10^6	Treated (GF6)	5.6 ± 1.83cd	3.8b	6.2c	5	5	1

[e] *C* Roots immersed in distilled water, *G* roots immersed in 10^8 cfu/ml *Streptomyces* sp. strain g10 suspension, *F* roots immersed in Foc race 4 spore suspension (F4, $\sim2.5\times10^4$ spores/ml; *F6* $\sim2.5\times10^6$ spores/ml), *GF* roots immersed in 10^8 cfu/ml strain g10 suspension and in Foc race 4 spore suspension (GF4, $\sim2.5\times10^4$ spores/ml; *GF6* $\sim2.5\times10^6$ spores/ml)

[f] Average fresh weight of five plantlets for each treatment (mean ± SD)

The disease, however, spread rapidly when plantlets were inoculated with the high concentration of Foc. After 2 weeks, all *Fusarium*-inoculated plantlets that were not treated with strain g10 (F6) succumbed to the disease, and by the end of the 4th week three were dead. All of the *Fusarium*-inoculated plantlets treated with strain g10 (GF6) were also affected after 2 weeks. Although strain g10 did not succeed in delaying disease symptom expression in these plantlets, only one died after 4 weeks. Reductions in the LSI and RDI of the GF6 plantlets were not statistically significant when compared to the F6 plantlets (Table 1; $P = 0.05$). It was apparent that treatment of plantlet roots and the planting hole with strain g10 provided better control at the lower Foc inoculum concentration.

At both Foc inoculum concentrations tested, the mean fresh weight was higher in plantlets grown in the presence of strain g10 compared to those grown in the absence of the control agent (Table 2). Despite the favorable influence of strain g10-treatment, the *Fusarium*-free control plantlets treated with strain g10 (G) showed lower plant fresh weight compared to *Fusarium*-free control plantlets that were not treated with strain g10 (C). However, the mean fresh weights of the two control groups were not statistically different ($P = 0.05$). The results from the second biocontrol assay also showed the expected significant reduction in DSI for LSI and RDI (Table 2; $P = 0.05$) in the *Fusarium*-inoculated plantlets treated with strain g10 compared to *Fusarium*-inoculated plantlets grown in the absence of the control agent. The DSIs of leaf symptom and rhizome discoloration were reduced about 55 and 51%, respectively. Improved plant growth in terms of fresh weight was also reflected in strain g10-treated plantlets. However, the differences in mean fresh weights of both groups were not statistically significant ($P = 0.05$).

Fungal mycelia that grew out on water agar plates containing discolored rhizome samples were identified as *F. oxysporum* [40]. *F. oxysporum* was not isolated from randomly selected rhizome samples from *Fusarium*-free untreated ©) and *Fusarium*-free treated (G) control plantlets, including the single plantlet from group G that showed some leaf browning. In comparison, 11 of the 12 rhizome samples examined from plantlets exposed to Foc and grown in the presence (GF4 and GF6) and absence (F4 and F6) of strain g10 were positive for the pathogen.

Table 2 DSI and mean fresh weight of 'Novaria' plantlets grown in the presence and absence of *Streptomyces* sp. strain g10 after 4 weeks in the greenhouse (in vivo biocontrol assay 2). Means with the same letter within a column are not significantly different ($P < 0.05$)

Foc race 4 (spores/ml)	Strain g10 treatment[c]	Fresh weight (g)[d]	DSI	
			LSI	RDI
2.5×10^4	Untreated (F)	3.9 ± 1.82 a	4 a	6.6 a
2.5×10^4	Treated (GF)	6.1 ± 1.55 a	1.8 b	3.2 b

[c] *F* Roots immersed in $\sim2.5\times10^4$ spores/ml Foc race 4 spore suspension, *GF* roots immersed in 10^8 cfu/ml *Streptomyces* sp. strain g10 suspension and in Foc race 4 spore suspension

[d] Average fresh weight of five plantlets for each treatment (mean ± SD)

Population of *Streptomyces* sp strain g10 in the rhizosphere and root-free soil

After 10 days of incubation, strain g10 sporulated and was well established in the soil-sago hampas delivery medium, with an average population density of about 6×10^7 cfu/g dry weight (data not shown). The estimated populations of strain g10 in the root-free soil and rhizosphere of 'Novaria' plantlets over a period of 6 weeks are shown in Table 3. Although the total number of strain g10 propagules declined in both soils after the 3rd week, the levels of strain g10 population were still much higher in the rhizosphere than in the root-free soil 3 and 6 weeks after planting. After 2 weeks, the rhizosphere and root-free soil population of strain g10 was about

Table 3 Population density of *Streptomyces* sp. strain g10 in rhizosphere and root-free soil of 'Novaria' plantlets sampled at different periods after soil amendment. Data on each sampling period are the average of three fresh root samples and six 1-g root-free soil samples taken from three replicate pots. Means followed by the same letter within a column are not significantly different ($P < 0.05$)

Number of days after soil amendment	Strain g10 population density[d]	
	Rhizosphere	Root-free soil
14	2.35 ± 0.63a	2.45 ± 0.14a
21	5.67 ± 0.65c	2.84 ± 0.56a
42	3.74 ± 0.74b	0.70 ± 0.01b

[d]Defined as (average cfu ± SD) $\times 10^6$ per gram dry weight of soil

Table 4 Effect of *Streptomyces* sp. strain g10 on plant height of 'Novaria' plantlets after soil amendments. Means followed by the same letter within a column are not significantly different ($P < 0.05$)

Treatment[c]	Average plant height (cm ± SD)[d]	
	0 day	42 days
Test	10.02 ± 0.35a	18.43 ± 0.97b
Control	9.98 ± 0.50a	17.78 ± 0.75b

[c]Test pots contained 500 g soil + 500 g delivery medium containing propagules of strain g10, control pots contained 500 g soil + 500 g sterile delivery medium without strain g10
[d]Average taken from six pots with one plantlet per pot

2.4×10^6 cfu/g dry soil. The rhizosphere population showed a significant ($P = 0.05$) increase of more than 2-fold when analyzed a week later. There was an almost 2-fold increase in the rhizosphere population of strain g10 at the end of the 6th week, when compared to the level detected after 2 weeks. In contrast, there was no significant ($P = 0.05$) difference in the root-free soil population of strain g10 at 2 and 3 weeks after soil amendment (Table 3). After 6 weeks, the population size declined significantly ($P = 0.05$), nearly 4-fold when compared to the level detected after 2 weeks. Plantlets grown in strain g10-amended and unamended soil showed normal growth (external), and there was no significant ($P = 0.05$) difference between the mean heights of plantlets from both groups at the end of the 6th week (Table 4).

Discussion

Results from the in vitro bioassay showed that the control plates supported excellent growth of Foc, and the radial growth of fungal colonies was easily measured over time. Therefore, differences in growth in the test plates could be attributed to diffusible antifungal metabolites produced by strain g10 in agar cultures. In the absence of the host plant, different species of *F. oxysporum* were able to survive in soil by the formation of chlamydospores [27]. Fungal plant pathogens that have an active saprophytic life in soil, or are suspected to survive in soil, were more tolerant to antibiotics produced by antagonistic actinomycetes [30]. Reduction in fungal linear growth rates indicated antibiotic sensitivity [30]. Therefore, strain g10 was grouped as strongly antagonistic to *Fusarium* wilt of banana as it showed 80–87% reduction in the linear growth of all three races of Foc within 2 days of paired incubation. Race 4 was used in the in vivo studies to investigate the disease suppressive ability of strain g10 since it was more destructive and has a wider host range than races 1 and 2 [16].

In vitro antagonism is not a clear indicator of the potential antagonism in actual field situations [5]. *Streptomyces* strains, however, have shown promising results in in vitro tests as well as reducing disease severity in vivo [7, 43]. In this study, 'Novaria' plantlets grown in sterile pot soil and inoculated with 10^4 spores/ml Foc showed significant wilt suppression when the roots and planting holes were treated with strain g10. Treating the roots with strain g10 prolonged the period before wilt symptom was expressed in some of the plantlets to about 4 weeks. This provided preliminary evidence that strain g10 has the ability to interfere with the banana wilt disease cycle and may be a potential biocontrol agent. Although we should be critical of results that were not obtained in natural soil (not sterilized), we should recognize that such results could nevertheless contribute to understanding of the nature of disease suppression by strain g10. Sterilization of the experimental soil ensured that the results were not complicated by the activities of other antagonists or plant pathogens originating from natural soil.

The mechanism(s) associated with the antagonistic effects of strain g10 against Foc race 4 in a soil environment was studied previously [14]. Inhibitory effects such as lysis, inhibition of fungal spore germination, and growth malformations like swelling, hyphal protuberances and abnormal branching of hyphae generally produced by antifungal metabolites [3], were observed in strain-g10-amended soil [14]. The latter study also showed that very few conidia were formed from the remains of undamaged Foc mycelium. Completion of a disease cycle is generally characterized by sporulation, which subsequently contributes to dissemination of the pathogen by water [2]. Reduction in fungal sporulation in the presence of strain g10 can result in a corresponding reduction in the inoculum-potential of the pathogen in soil. In all cases, there was a definite decrease in the development of Foc race 4 in the presence of strain g10 [14]. Many antibiotics produced by the genus *Streptomyces* have been used directly [17], or have been assumed to be responsible for the biocontrol potential of the producing strain [41]. Antifungal metabolites produced by the antagonists could reduce invasion and subsequent development of the wilt fungi [3]. However, the metabolites would have to be produced at the right time and at high enough levels to protect the infection sites from the pathogen [22].

In the present study, the reduction in disease severity attributed to treatment with strain g10 was not significant when plantlets were challenged with a high

concentration of Foc. The success of establishment of microorganisms in the rhizosphere was dependent on the delivery system [15]. In our study, strain g10 was applied to 'Novaria' roots by immersion and by application to the planting holes prior to inoculation with pathogen. However, there was no pre-inoculation to allow good root colonization and high antifungal activity of strain g10 in the possible infection sites before challenge with high levels of the pathogen were applied. Generally, *Streptomyces* spp. are slow growers, with growth and antibiotic production depending heavily on the types of carbon sources available in the rhizosphere. Moreover, effective concentrations of antibiotics are produced mostly during later stages of their growth phase [31]. Therefore, sufficient time is needed between the introduction of strain g10 and inoculation of Foc for better establishment and persistence of the streptomycete in the rhizosphere and disease suppression [31]. Mehrotra and Caludius [23] observed that root rot and wilt disease of *Lens culinaris* caused by *Sclerotium rolfsii* and *F. oxysporum* f.sp. *lentis*, respectively, was better suppressed when antagonistic microorganisms were incorporated into the soil 15 days earlier than sowing of seeds and pathogen.

Other factors may also contribute to the low level of protection exhibited by strain g10 when plantlets were challenged with high pathogen concentration. When the concentration of *Fusarium* exceeds certain critical levels, the chances of root-fungus contact are increased [39]. This allows more extensive penetration of roots and colonization of the xylem system such that normal field reactions of host resistance might not be expressed in strain g10-treated plantlets [39]. Resistant banana plants are known to become progressively more susceptible when some of the host resistance mechanisms are not effective against the fungus [3]. Therefore, a more dilute inoculum suspension of Foc would provide a good differential in disease severity between treated and untreated groups.

In the present study, strain g10 formulation was prepared using a mixture of soil:sago hampas (5%, w/w). Sago hampas is the fibrous residue obtained after the extraction of starch from rasped sago pith. The formulation had a porous and loose texture suitable to provide space for mycelial and plant root growth and spread when added to the potting mix. When delivered in this formulation, strain g10 has the ability to colonize and increase its numbers in the banana rhizosphere as the plantlets grow. The strain can be easily re-isolated from the rhizosphere even up to 6 weeks after soil amendments. On the other hand, population in the root-free soil declined significantly by the end of the 6th week.

Generally, it is known that abiotic and biotic factors in soil influence the propagation and survival of bacteria introduced into the rhizosphere. Soils rich in organic matter, with a neutral pH and moisture conditions favoring plant and microbial growth, efficiently support natural populations of introduced bacteria [6]. Although the final test is in field soil, the relative importance of these abiotic factors in rhizosphere colonization by strain g10 may be assessed by studies in sterile soil. This will provide a greater understanding of the environmental conditions that may influence colonization and the antagonist-pathogen interaction. *Streptomyces* sp. strain g10 was isolated from mangrove soil that is known to be rich in organic matter and leaf litter, and high in biological decomposition activities. Actinomycetes capable of growing on a large variety of complex organic compounds have been shown to produce enzymes that can degrade cellulose and lignin [19]. It has been reported that streptomycetes with efficient rhizosphere colonization ability also show high cellulolytic and amylolytic activities [26].

Many researchers have shown that actinomycetes found in the rhizosphere population [9, 12, 18], and endophytic streptomycetes found inside root tissues [33], have antagonistic potential and play an important role in plant health. Some endophytic and rhizosphere-colonizing microorganisms have shown the ability to reduce the susceptibility of plants to the pathogen by inducing systemic resistance in the host [22]. Therefore, the possible impact of induced host resistance in the mechanism of disease suppression by strain g10, in addition to antibiosis, should be investigated by using ''killed'' cells of strain g10 as a control treatment. According to Crawford et al. [9], actinomycetes may also play important roles in the mycorrhizal colonization of plant roots. All these reports point to the fact that actinomycetes are quantitatively and qualitatively important in the rhizosphere, where they may influence plant growth and protect plant roots against invasion by root pathogenic fungi. Thus, a greater understanding of the environmental conditions that may influence rhizosphere colonization by strain g10 or the interaction between the antagonist and the pathogen is needed. By altering the biotic factors in the rhizosphere soil to favor the activities of colonization and antibiosis, a long-term effect on biological control of banana wilt pathogen could be sustained.

In the in vivo biocontrol assay, the differences in mean fresh weights of *Fusarium*-free control plantlets that were grown in the presence of strain g10 and control plantlets grown in the absence of strain g10 may have been due to inadequate rinsing of cells to remove metabolites prior to root-immersion treatment. Smith et al. [36] also found that inadequate rinsing of *Streptomyces griseus* cells before using as a root-dip treatment for control of *Fusarium* diseases reduced the total plant weight of asparagus seedlings compared to untreated plants. Their results were supported by studies that showed the absence of phytotoxic effects from the antibiotic faeriefungin produced by the streptomycete. Phytotoxic metabolites from harvested biocontrol agents can be diluted by washing, centrifugation or ultrafiltration [35]. When strain g10 was added as soil amendments, neither growth-inhibiting nor growth-stimulating effects were observed in banana plantlets. Some *Streptomyces* alter plant growth through

biological control of disease and the action of growth-stimulating substances [18].

The evidence presented here suggests a possible biological control for wilt of banana. According to Marois [22], it may be possible to establish antagonistic microbial communities that would increase the buffering capacity of soils against subsequent Foc invasion in areas where the pathogen has not yet occurred. However, this approach may not be suitable in soils where the pathogen is already established. In such cases, it would be better to protect the infection sites rather than alter the entire soil microbial community. The host plant provides the habitat and nutrients necessary for the control agent to become established at the infection site. Although Foc is a soil-borne pathogen and can spread through irrigation water and infected planting stocks, once the infection of plant roots has taken place, the fungus invades the vascular system internally and escapes from external pressures. Since strain g10 colonizes soil/rhizosphere externally to plant tissues, the critical step in achieving active disease control would be to target the pathogen before it gains entry into the corm tissues. Therefore, strain g10 must be introduced to root systems well in advance of Foc infestation. This procedure is directly applicable to the tissue-culture system, wherein strain g10 could be applied to banana plantlets before moving them to the field. To our knowledge this is the first report showing the possibility of applying fungus-antagonistic streptomycetes to tissue-cultured banana plantlets as an alternative approach to control *Fusarium* wilt.

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