academicJournals

Vol. 8(48), pp. 6241-6249, 12 December, 2013
DOI: 10.5897/AJAR2013.7082
ISSN 1991-637X ©2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Inhibitory effects of *Acremonium* sp. on Fusarium wilt in bananas

Liu Yuelian* and Lu Qingfang

Agricultural College, Guangdong Ocean University, Zhanjiang, Guangdong 524088, China.

Accepted 5 September, 2013

An endophytic fungus, strain Q34, isolated from *Kandelia candel* (L.) Druce showed strong *in vitro* antagonistic activity toward *Fusarium oxysporum* f. sp. *cubense* (foc) race 4. The crude extract of the strain inhibited the growth of foc race 4 and caused conidial deformation. In gas chromatography-mass spectrometry (GC-MS) analysis, the crude extract showed 18 main peaks. Three substances from the main peaks showed strong inhibitory activity against the growth of foc race 4. Moreover, the strain Q34 reduced the incidence of Fusarium wilt in banana plantlets under greenhouse conditions and in the field. This strain was identified as *Acremonium* sp. on the basis of morphology. These results suggest that strain Q34 is potentially useful for the biological control of foc race 4.

Key words: *Fusarium oxysporum* f. sp. *cubense* race 4, *Acremonium* sp., biological control, Fusarium wilt.

INTRODUCTION

Widespread infection of banana (*Musa* spp.) by *Fusarium oxysporum* f. sp. *cubense* (E. F. Smith) Snyder and Hansen (foc) is increasingly affecting the production of this important fruit crop in tropical and subtropical regions. Among the 4 races of this species, foc race 4 is a particularly virulent pathogen that infects banana plants in all growth stages, from seedlings to fruit-bearing crops, often causing serious economic loss (Ploetz, 2006; Wu et al., 2010). Following visual detection of the infection, the most common method of controlling foc race 4 is removal of the infected banana plants and quarantine of the infested areas.

Currently, there are no effective methods to chemically control Fusarium wilt. For example, carbendazim, the most effective fungicide against other *F. oxysporum* strains, has been found to have relatively lower efficacy against foc race 4 (Buddenhagen, 2009; Cao et al., 2004; Ploetz, 2006; Wu et al., 2010; Sun et al., 2011). Moreover, its effectiveness against susceptible strains is often not as adequate as desired by growers, especially when the opportunity for early detection is missed and the infection is already widespread. To manage foc race 4 infection, researchers are increasingly focusing on biological control. For example, *Pseudomonas aeruginosa* FP10 reduces the vascular discoloration resulting from foc infection (Ayyadurai et al., 2006), and *Streptomyces noursei* Da07210 shows strong antagonism toward foc race 4 in plate bioassays (Wu et al., 2009). Moreover, *Pseudomonas* (Altinok et al., 2013), *Bacillus subtilis* (Sun et al., 2011), *Streptomyces* (Cao et al., 2005), Nonpathogenic *F. oxysporum* and *Pseudomonas fluorescens* (Belgrove et al., 2011), and bacteria in the rhizosphere (Li et al., 2012), were reported to have inhibitory effects against Fusarium wilt, encouraging researchers to continue searching for more effective strains.

In the current study, antagonistic strains were isolated from a variety of terrestrial environments and from marine

*Corresponding author. E-mail: mushwoman@126.com. Tel: +86 1372 9129985. Fax: +86 0759 2383303.

environments Mangrove plants growing in specia saltwater environments, have potential of producing novel metabolite. This situation occurs to mangrove-derived endophytes too because of their co-evolution (Guo 2001; Wen et al., 2010). Here, we used *K. candel* (L.) Druce as endophytic fungal host, which grows in mangrove forests and is free from foc race 4 infection. To investigate endophytes derived from *K. candel*, a comprehensive isolation and screening program was initiated.

MATERIALS AND METHODS

Endophytic microbes and pathogenic fungi

Endophytic fungal strains were isolated from disease-free *K. candel* in Zhanjiang (Guangdong, China) using the modified method described by Hyde and Soytong (2008). Hyphal tips were transferred to new potato dextrose agar (PDA) dishes, and the dishes were incubated at 28°C for 7 days.

The phytopathogenic fungus foc race 4 was obtained from a stored culture from South China Agricultural University.

Screening of antagonistic strains

Pathogenic fungi were inoculated on PDA plates for 7 days at 28°C. The antagonistic activity of each endophytic fungal strain was determined by using the dual plate culture technique, with three replicates for each strain. Then, the following formula was used to assess the inhibition activity (Si et al., 2005):

Rate of inhibition (%) = [(average diameter of the control pathogen colony − average diameter of the test pathogen colony)/average diameter of the control pathogen colony] × 100

Identification of antagonistic strains

Identification of antagonistic strains to foc race 4 based on colony characteristics and morphological characteristics were performed under a light microscope (Olympus) referencing (Kirk et al., 2001).

Extraction of inhibitory compounds

The endophytic fungal strain showing antagonistic activity was grown in potato dextrose broth at 28°C with shaking at 120 rpm for 10 days. Next, 20 L of the culture was filtered and centrifuged at 8 kr min^{-1} for 10 min at 4°C. The supernatant was extracted twice with an equal volume of ethyl ethanoate, and the ethyl ethanoate layer was subsequently evaporated under reduced pressure in a rotary evaporator to obtain the crude extract of this strain.

Inhibition assay using the crude extract

The hyphal extension-inhibition assay described by Roberts and Selitrennikoff (1986) was used for this experiment. In brief, agar plugs (5-mm diameter) of foc race 4 were obtained from a freshly growing colony (5 days) and placed at the center of PDA plates containing various concentrations (100, 50, 25, 12.5, and 6.25 µg mL^{-1}) of the crude extract; ethyl ethanoate was used as the control. After incubation at 28°C for 5 days, diameters of the fungal colonies were measured, and the fungal conidia were observed under a microscope (100 × magnification) to determine abnormal morphology at 10 days after plating. The percentage of inhibition of mycelial growth was calculated using the aforementioned formula.

Gas chromatography-mass spectrometry analysis of the crude extract

The crude extract was dissolved in 1 mL of ethyl ethanoate (1:9, v/v). A high-performance gas chromatography-mass spectroscopy (GC-MS) system (GC6890-MS5973) with a mass-selective detector and electron impact ionization was used to analyze the crude extract. The following operating conditions were used: DB25 ms (30 m × 0.32 mm, 0.25-µm film thickness), carrier-gas flow rate of 1.0 mol min^{-1}, and split ratio of 50:1. The GC oven temperature was programmed from 40°C (5 min) to 280°C (5°C min^{-1}). The electron impact ionization conditions included 70 eV ion energy and a 50–650 amu mass range in full-scan acquisition mode. Compounds of the crude extract were identified using the Wiley mass spectral library and verified against reference compounds (Zhang et al., 2008).

Identification of the inhibitory compounds of the crude extract

Inhibitory compounds were identified, with a similarity index greater than 850, from a database search based on a comparison of the mass spectrum of the substance with GC-MS system databanks (Wiley 138 and NBS 75k Library). Each sample was tested twice. For each detected candidate inhibitory compound, antifungal activity was confirmed using the same pure commercial compound (analytical grade) instead of the crude extract.

The effects of the commercial compounds (100 µg mL^{-1}) on the mycelial growth of foc race 4 were evaluated in a hyphal extension-inhibition assay, and compounds with an inhibition rate of 100% in this assay were then selected for determining the IC_{50} by the FAO method (Georgopoulos and Dekker, 1982). Next, the effects of different concentrations of these compounds on the mycelial growth of foc race 4 were assessed by hyphal extension-inhibition assays. The compounds were dissolved in 99% methyl N-(1H-benzimidazol-2-yl)carbamate and were used as negative and positive controls, respectively.

Biological control assay under greenhouse conditions

Strain Q34 and foc race 4 were prepared as described above. The effectiveness of strain Q34 against foc race 4 on the plantlets of a Cavendish banana ('Yueke No. 1') was evaluated using the procedure described by Subramaniam et al. (2006) with minor modifications. Banana plantlets were grown in pathogen-free tissue culture until 3–4 fully expanded leaves had grown and healthy roots had formed. To facilitate infection, the roots were slightly bruised by removing the root ball. Next, plantlets were divided into six treatment groups, with three plants in each group and three replicates per plant. Plantlet roots were then treated for 30 min with (i) the fermented liquid of strain Q34, (ii) fermentation filtrate, (iii) medium only, (iv) methyl N-(1H-benzimidazol-2-yl)carbamate, (v) sterilized water (foc race 4 was later administered to i to v, as described below), and (vi) sterilized water (foc race 4 was not added, as described below). Plantlets were then placed in 250-mL plastic cups of sterile soil, with holes in the bottom of the cups. Three plantlets were planted in each cup. All plantlets were inoculated with 1 mL conidial suspension (10^5 mL^{-1}) of foc race 4 near the region between the stems and roots, except for those treated with (vi). All cups were watered to saturation and placed in a greenhouse (28–32°C). The percent disease incidence was calculated based on the number of plantlets with chlorotic leaves for 25 days including whole 54 plantlets. Disease incidence (%) =

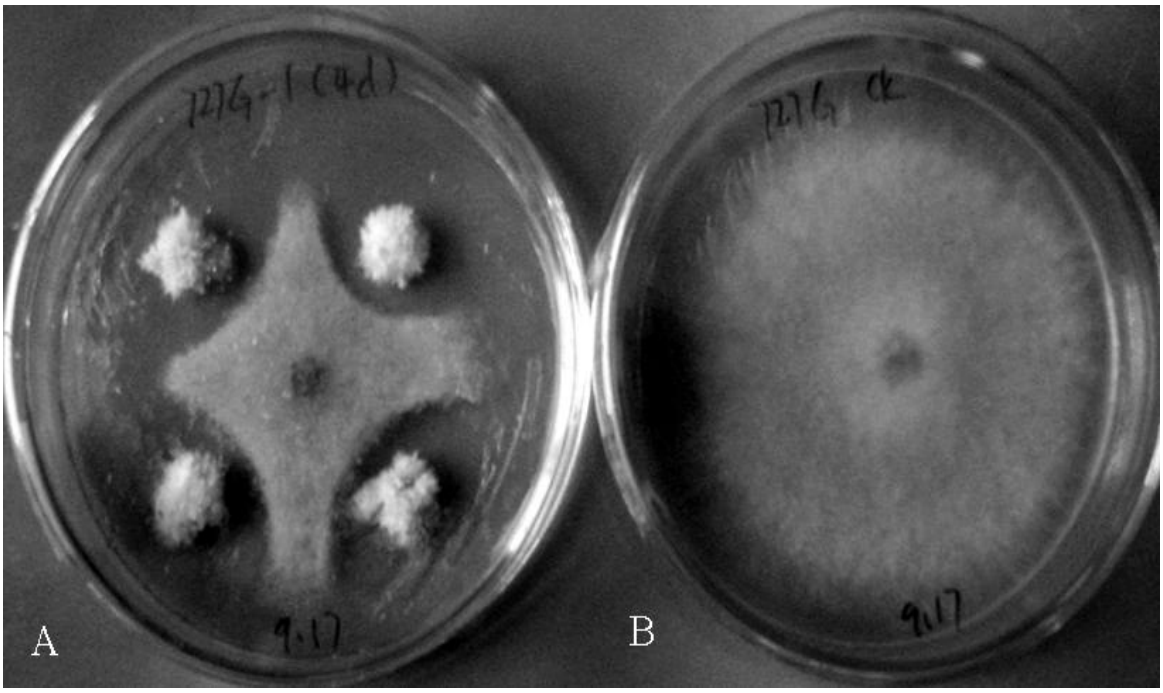

Figure 1. Q34 against Foc race 4 (4d) - A: Treatment; B: Control.

(number of plantlets with discolored leaves/total number of plantlets) × 100 (Subramaniam et al., 2006).

Biological control assay in field conditions

Field experiment was conducted at Zhanjiang, Guangdong province, China, latitude: 21°10'; longitude: 110°30 '; rainfall: 1100 mm; mean temperature: 23.2°C; RH: 80–95%. The crop was grown in the rainfed condition (soil pH: 5.4, soil type: clay, organic matter: 25.5%, P: 1.75 gkg^{-1}, N: 0.90 gkg^{-1}, K: 7.57 gkg^{-1}.The experiment was designed as follow: (A) the fermented liquid of strain Q34, (B); methyl N-(1H-benzimidazol-2-yl)carbamate, (C) sterilized water, (D). sterilized water. A,B and C were inoculated with conidial suspension (10^5 mL^{-1}) of Foc race 4, but not D. 100 mL conidial suspension, or 100 mL both the fermented liquid of strain Q34 (10 days), or 100 mL methyl N-(1H-benzimidazol-2-yl)carbamate (30 μgmL^{-1}) were dripped into each banana plantlet, near the region between the stems and the roots. Uniform tissue-cultured banana plantlets cv. "Yueke No. 1" with more than 4 new leaves and of 18 to 20 cm height was used in the experiments. In each treatment, there were 9 plants and 4 m × 2 m spacing was adopted. Disease severity was measured in the 30th, 50th, 70th and 90th day respectively. The disease incidence was calculated as the same as the assay in greenhouse.

Statistical analysis

Statistical analysis was conducted with the Statistical Analysis System (SAS Institute, Inc., Cary, NC, USA). LSD was performed using ANOVA in SAS in order to assess differences in mycelial growth rates and pathogenicities at a significance level of $p \leq 0.05$. IC_{50} values were analyzed by ANOVA performed with the General Linear Model (GLM) of SAS.

RESULTS

Isolates and antagonistic activity

In total, 125 endophytic fungal strains were obtained from *K. candel*: 65, 53, and 7 strains were obtained from the stem, leaves, and roots, respectively. One strain isolated from the leaves of *K. candel* exhibited the strongest activity toward foc race 4, with an average inhibition rate of 72.83% ($P \leq 0.05$; Figure 1). This strain was coded as Q34.

Strain Q34 was identified as *Acremonium* sp. (Figure 2), a fungus that is found in decomposed banana stud (Krishnaveni et al., 2009) and in banana cortex (Pocassangre et al., 2000), but not found in banana in China. Q34 colonies grew slowly and attained a diameter of 17 mm after 14 days of incubation at 25°C on PDA. At first, colonies appeared white; however, their color changed over time from pale, to yellow, and finally to brown. The conidiophores of Q34 were indistinct from the colorless hyphae of the vegetative mycelium. The conidiogenous cells arose from a single hypha: they were solitary, branchless, slender, hyaline-like, thin-walled, 10 to 20 µm long, 1.8 to 2.5 µm wide, and 0.5 to 1.0 µm wide at the apex. Conidia occurred in long chains; were hyaline-like, fusiform, and aseptate; and measured 3.6 to 4.8 × 0.9 to 1.4 µm. These morphological characteristics of strain Q34 were similar to those of *Acremonium* (J.C. Gilman and E.V. Abbott) W. Gams (1975). Strain Q34 is now stored in the Laboratory of the Agricultural College of

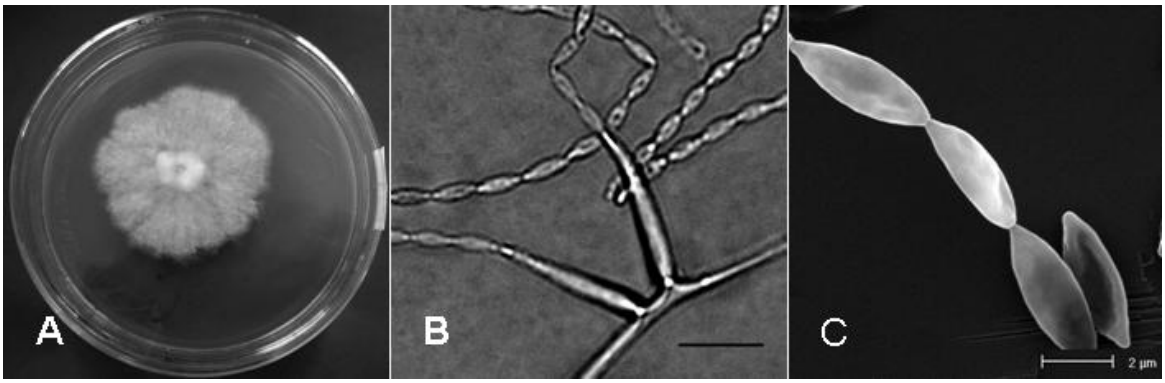

Figure 2. Morphology of Q34 - **A**: Colony; **B**: Conidiophores; **C**: Conidia Bar in **B** = 10 μm, Bar in **C** = 2 μm.

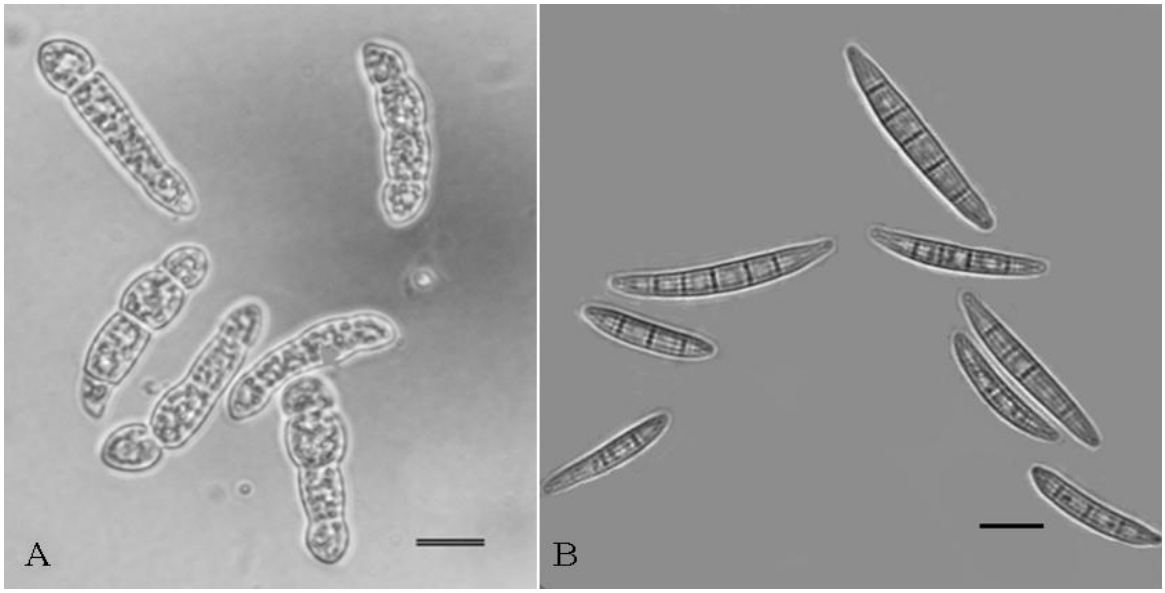

Figure 3. Effect of the crude extract of strain Q34 on conidia of Foc race 4 (5d) **A**: Crude extract; **B**: Sterilized water.

Guangdong Ocean University.

Effects of the crude extract on the growth of foc race 4

In the presence of the crude extract of strain Q34, the mycelial growth of foc race 4 was significantly inhibited (IC_{50}, 40.00 μg mL^{-1}; regression equation, $Y = 24.84 - 0.63X$; $R^2 = 0.87839$). Microscopic observation revealed that treatment with the extract caused distorted conidial growth of foc race 4 (Figure 3). After 4 h, the conidia appeared deformed and were unable to germinate.

Characteristics of the compounds derived from the crude extract

Next, we sought to identify the components of the crude extract from strain Q34 using GC-MS analysis. GC-MS analysis revealed 18 compounds, as represented by major peaks, in the crude extract (Figure 4). Ten of the compounds with similarity indices of greater than 850 were sterilants, drug intermediates, or unknowns: 99% 2-phenylethanol; 100% 1,2,3,4-tetrahydronaphthalene; 95% 1,2,4,5-tetramethylbenzene; 100% 2,3-dihydro-4,7,dimethyl-1H-indene; 100% 1,1′-biphenyl; 100% benzyl benzene; 100% 9H-fluorene; 100% anthracene; 100% octadecane; and 100% icosane. Pretest results showed that 2-phenylethanol (peak 1), 2,3-dihydro-4,7,dimethyl-1H-indene (peak 4), and 1,1′-biphenyl (peak 5) displayed inhibition rates of 100% for the mycelial growth of foc race 4 (Figure 5).

Taken together, these results suggested that 3 compounds from strain Q34 were primarily responsible for inhibiting mycelial growth. Further studies showed that 2-phenylethanol significantly inhibited the mycelial

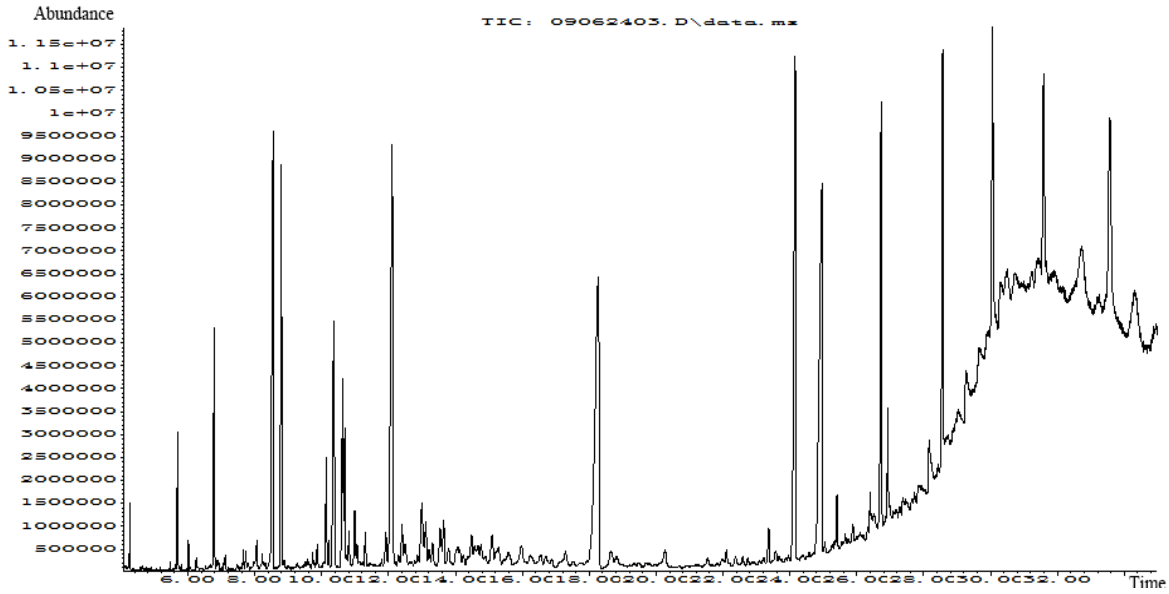

Figure 4. Abundance ion chromatography of chemical constituents in extract substances of Q34.

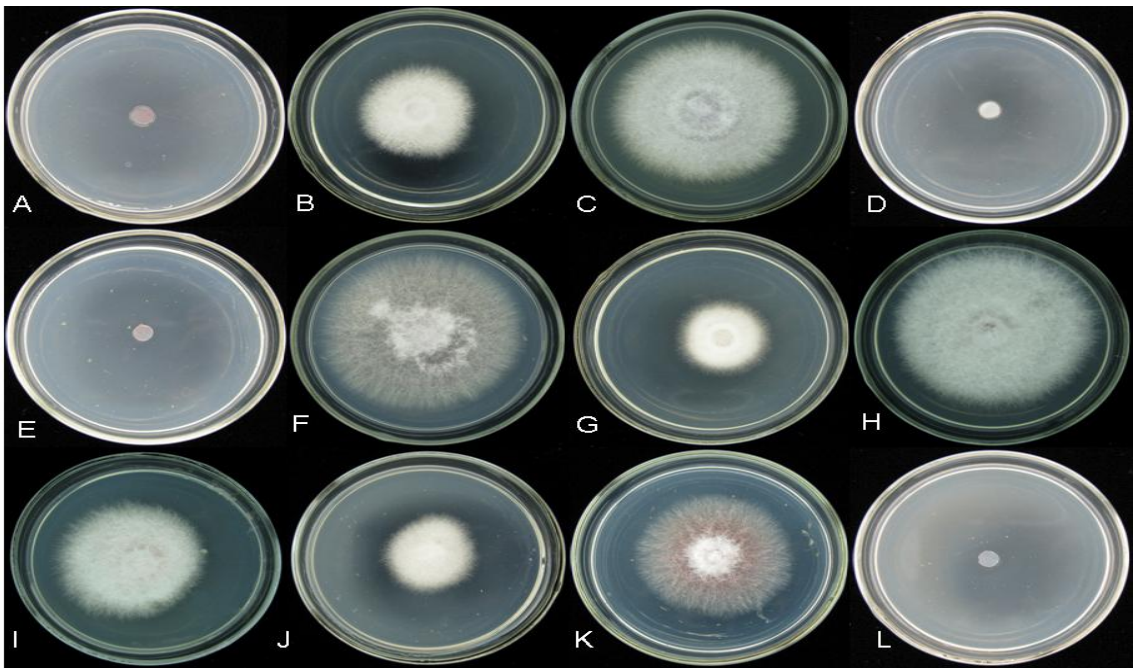

Figure 5. Effects of compounds at concentration 50 μgmL^{-1} on mycelia growth of Foc race 4 (after 5 days). A: phenylethyl alcohol; B: 1,2,3,4-etrahydronaphthalene; C: 1,2,4,5-tetramethylbenzene; D: 2,3-dihydro-4,7, dimethyl-1H-indene; E: biphenyl; F: diphenylmethane; G: fluorene; H: anthracene; I: octadecane; J: eicosane; K: ethyl acetate; L: methyl N-(1H-benzimidazol-2-yl)carbamate.

growth of foc race 4 (IC_{50}, 23.03 μg mL^{-1}) and was more effective than methyl N-(1H-benzimidazol-2-yl)carbamate (IC_{50}, 29.69 μg mL^{-1}). With an IC_{50} value of 26.74 μg mL^{-1}, 1,1′-biphenyl was the second most effective compound and was also more effective than methyl N-(1H-benzimidazol-2-yl)carbamate. In contrast, 2,3-dihydro-4,7,dimethyl-1H-indene was inferior to methyl N-(1H-benzimidazol-2-yl)carbamate (IC_{50}, 40.36 μg mL^{-1}; Table 1). Moreover, 2-phenylethanol and 1,1′-biphenyl had short retention times (5.701 and 9.869 min,

Table 1. Effects of 4 compounds on mycelia growth of Foc race 4 (after 5 days).

Candidate of compounds	Concentration (μgmL^{-1})	Inhibition of micelial growth (%)	Regression equation	R^2	IC50 (μgmL^{-1})
Phenylethyl alcohol (1)	50	85.87±7.07[a]	y = 14.01 + 1.56 x	0.91044	23.03
	25	63.47±3.12[b]			
	12.5	39.93±2.04[c]			
	6.25	25.77±3.53[d]			
	3.125	16.37±3.12[d]			
1H-Indene,2,3-dihydro-4,7-dimethyl (4)	50	63.49±3.12[a]	y = 3.91 + 1.14x	0.97637	40.36
	25	28.15±1.18[b]			
	12.5	15.20±3.53[c]			
	6.25	12.84±1.18[c]			
	3.125	10.48±3.12[c]			
Biphenyl (5)	50	77.63±1.18[a]	y = 11.59 + 1.43x	0.90640	26.74
	25	56.24±2.36[b]			
	12.5	39.93±2.04[c]			
	6.25	15.19±2.04[d]			
	3.125	8.13±2.04[e]			
Methyl N-(1H-benzimidazol-2-yl)carbamate	50	77.62±1.18[a]	y = 0.67+ 1.66x	0.91518	29.69
	25	58.78±1.18[b]			
	12.5	14.02±2.36[c]			
	6.25	9.31±1.18[cd]			
	3.125	4.59±2.04[d]			

*: (P ≤ 0.05).

respectively) and more than 95% similarity to the standard. Therefore it was found to be three composts with antagonistic activity against foc.

Effects of strain Q34 on foc race 4 in banana plantlets grown under greenhouse conditions

Next, we wanted to determine whether treatment with strain Q34 extract could inhibit infection of foc race 4 in banana plants. As shown in Figures 6 and **7**, significant differences between the various treatments were observed. The fermented liquid and fermentation filtrate of strain Q34 inhibited infection by and growth of foc race 4 on banana plantlets (disease incidence, 31.81 and 43.60%, respectively; $P \leq 0.05$; Figure 6). Plantlets treated with the fermented liquid showed disease symptoms on day 12 after inoculation, whereas those treated with medium only showed wilting on day 6 (Figure 7). In the latter case, the disease developed rapidly (disease incidence, 61.67%; $P \leq \leq 0.05$).

Effects of fermented liquid of strain Q34 on Foc race 4 in banana plantlets in field

The practical application of strain Q34 was tested to reveal that the strain Q34 controlled the disease by Foc race 4 in the field. The disease of the treatment (A), (B) and (C) were shown on the 30th day. Later, the disease incidence of the treatment (A) showed a peak in the 50th day, but declined in the 70th and 90th days, while the treatment (B) and (C)'s increased (Figures 8 and 9). These results showed that the fermented liquid of strain Q34 possessed inhibition on the development of Foc race 4.

DISCUSSION

In the current study, we sought to identify components isolated from *K. candel* that would inhibit the occurrence of Fusarium wilt in banana plants. We found that strain Q34, an endophytic fungal strain isolated from *K. candel*, had significant inhibitory activity toward foc race 4. In fact, Q34 showed stronger antagonistic activity toward foc race 4 than did cardenza, with an average inhibition rate of 72.83%. The crude extract of this strain was effective at inhibiting the growth of foc race 4 and caused distorted conidial growth. GC-MS analysis of the crude extract of Q34 revealed more than 1 compound with inhibitory activity against this pathogenic fungus. Moreover, in the biological control assay, the fermented liquid and fermentation filtrate of strain Q34 significantly inhibited

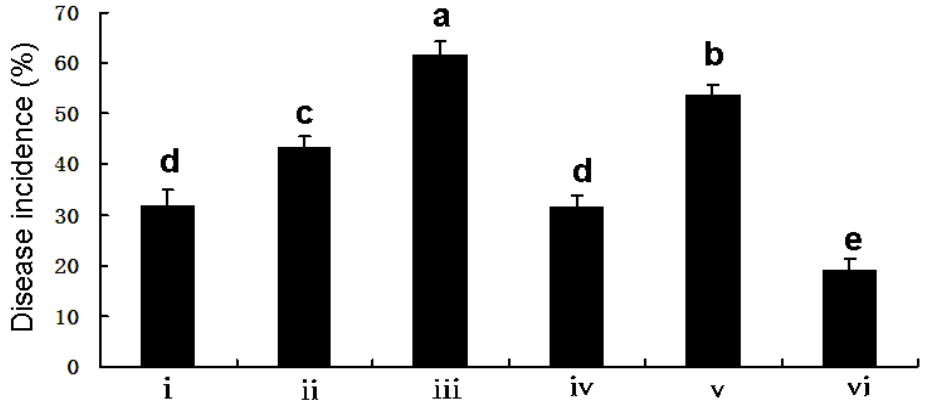

Figure 6. Disease incidence of different treatments on banana in the field (25 days). i: Fermented liquid of Strain Q34; ii: Fermentation filtrate; iii: Medium only; iv: Methyl N-(1H-benzimidazol-2-yl)carbamate; v: Sterilized water; vi: Sterilized water without Foc race 4.

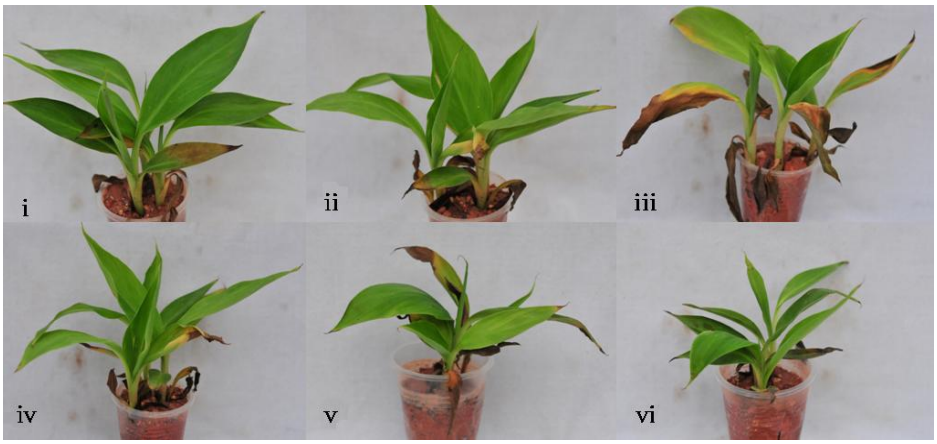

Figure 7. Disease incidence of different treatments on banana in the field (25 days). i: Fermented liquid of Strain Q34; ii: Fermentation filtrate; iii: Medium only; iv: Methyl N-(1H-benzimidazol-2-yl)carbamate; v: Sterilized water; vi: Sterilized water without Foc race 4.

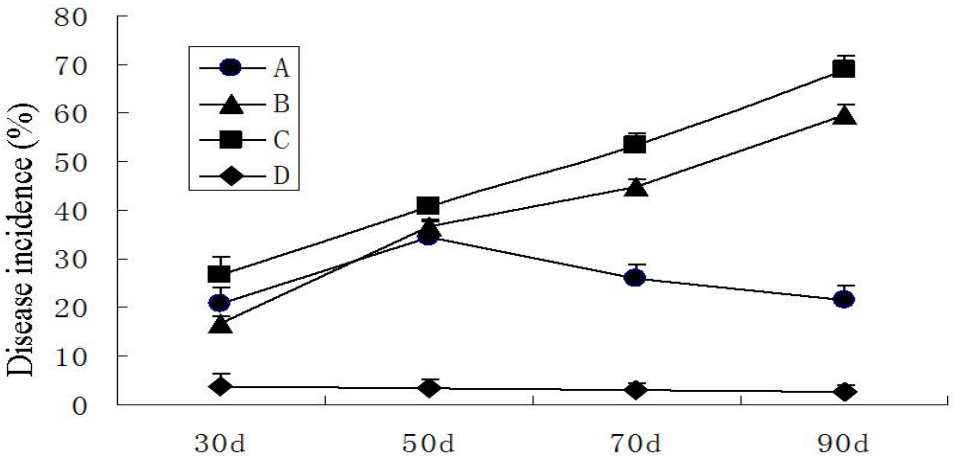

Figure 8. Disease incidence of different treatments on banana in the field (90 days). A: Fermented liquid of Strain Q34; B: Methyl N-(1H-benzimidazol-2-yl)carbamate; C: Sterilized water; D: Sterilized water without Foc race 4.

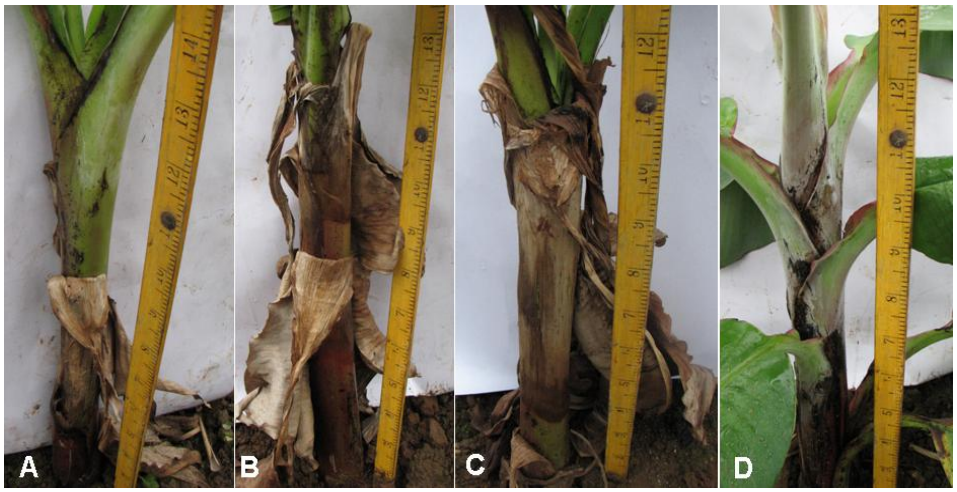

Figure 9. Effects of strain Q34 on Foc race 4 in banana plants grow in the field (90 days). A: Fermented liquid of Strain Q34; B: Methyl N-(1H-benzimidazol-2-yl)carbamate; C: Sterilized water; D: Sterilized water without Foc race 4.

infection by and growth of foc race 4 on banana plantlets. There appeared to be several compounds produced by strain Q34 that resulted in the inhibition of foc race 4.

In our experiment, we wounded the roots of plants by removing the root ball, allowing young plants to be easily infected by foc race 4. This method, combined with planting three banana plants per cup, which would cause nutrient depletion, resulted in the early appearance of symptoms of infection. Therefore, this method could be used for rapid screening of antagonistic strains inhibiting Fusarium wilt in an *in vivo* bioassay.

In the current study, we described novel structures of secondary metabolites produced by some endophytic fungi that have activity against foc, and this is becoming the focus of a new approaches for biological control (Backman and Sikora, 2008; Mejia et al., 2008). Previous studies have shown that endophytic fungi isolated from *K. candel* have antifungal activity. For example, a marine endophytic fungus (No. 1893) isolated from the dropper of *K. candel* produces two metabolites that exhibit significant activity against *Heliothis armigera* and *Sinergasilus* spp. (Chen et al., 2003). *Talaromyces* sp. ZH-154, another endophytic fungus isolated from the stem bark of the same host, contains 7-epiaustdiol and 8-*O*-methylepiaustdiol, which have been evaluated for their antimicrobial activities (Liu et al., 2010). In our study, only 10 of 18 compounds were tested; the anti-foc race 4 activities of the remaining 8 compounds are not known. In fact, these additional 8 compounds may represent novel antimicrobials. Indeed, with these novel resources from the mangrove, new species, new genes, and new compounds may be identified, allowing us to develop better tools with which to control foc race 4 infections.

In our study, Q34 was identified as *Acremonium* sp., some species of which are human pathogens. Previous reports have shown that the endophytic fungus *A. implicatum*, which forms beneficial symbiotic associations with *Brachiaria* spp., exhibits activity against *Drechslera* spp., species that cause leaf spots (Kelemu et al., 2001). From our study, we found that *Acremonium* sp. Q34 is similar to *A. implicatum* in morphology. While *A. implicatum* has not been implicated as a human pathogen (Gams, 1975), the potential for Q34 to behave as a human pathogen should be considered before its application as a biological control agent.

In the present study, we found that *Acremonium* strain Q34 inhibited the growth of foc race 4. This strain contains at least three compounds with inhibitory effects were found in the crude extract of Acremonium spp. Q34, which may explain its effectiveness against this pathogenic fungus. Antagonism involving a single substance is incomplete or weak, while a combination of two or more substances may enhance antagonism. To inhibit the disease process, a mixture of various fungicides, fungicides plus an antifungal strain, or a combination of two or more antifungal strains has been used in some studies (Chaves et al., 2009; Singh, 2010).

Antifungal substances have different modes of action. One mode may involve stimulation of some plant resistance mechanisms by antagonistic fungi, leading to activation of the corresponding signal transduction pathway. Another possible mode of action is antagonism between fungi, resulting in the inhibition of pathogen growth. Although we noted that strain Q34 inhibited the growth of foc race 4, the underlying mechanism is still unknown. Furthermore, *Acremonium* sp. Q34 could be colonized in banana with a long-term antifungal effect, while *Acremonium* sp. was found as endophytic fungi in banana cortex (Pocassangre et al., 2000). Further studies on these issues are in progress.

ACKNOWLEDGMENTS

We thank Prof. Jiang Zide (South China Agricultural University, Guangzhou, China) for the use of laboratory space and helpful suggestions. We also thank Dr Han Qiao Hu for writing assistance, associate professor Chang Hui Ye for help with statistical analyses, and the Editage Online Team for providing language help. This study was supported by the Science and Technology Project of Guangdong Province (No. 2012A020200009).

Abbreviations: **foc,** *Fusarium oxysporum* f. sp. *cubense*; **GC-MS,** gas chromatography-mass spectrometry; **IC_{50},** median inhibitory concentration; **PDA,** potato dextrose agar; **FAO,** Food and Agricultural Organization.

REFERENCES

Altinok HH, Dikilitas M (2013). Potential of Pseudomonas and Bacillus isolates as biocontrol agents against Fusarium wil of eggplant. Biotechnol. Biotechnol. Equip. 27(4):3952-3958.

Ayyadurai N, Naik PR, Rao MS, Kumar RS, Samrat SK, Manohar M, Sakthivel N (2006). Isolation and characterization of a novel banana rhizosphere bacterium as fungal antagonist and microbial adjuvant in micropropagation of banana. J. Appl. Microbiol. 100:926-937.

Backman PA, Sikora RA (2008). Endophytes: an emerging tool for biological control. Biol. Control 46:1-3.

Belgrove A, Steinberg C, Viljoen A (2011). Evaluation of Nonpathogenic *Fusarium oxysporum* and *Pseudomonas fluorescens* for Panama DiseaseControl. Plant Dis. 95(8):951-959.

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

Cao LX, Qiu ZQ, Dai X, Tan HM, Lin YC, Zhou SN (2004). Isolation of endophytic actinomycetes from roots and leaves of banana (Musaacuminata) plants and their activities against *Fusarium oxysporum* f. sp. *cubense*. World J. Microbiol. Biotechnol. 20:501-504.

Cao LX, Qiu ZQ, You JL, Tan HM, Zhou SN (2005). Isolation and characterization of endophytic streptomycetes antagonists of fusarium wilt pathogen from surface-sterilized banana roots. FEMS Microbiol. Lett. 247:147-152.

Chaves NP, Pocasangre LE, Elango F, Rosales FE, Sikora R (2009). Combining endophytic fungi and bacteria for the biocontrol of *Radopholus similis* (Cobb) Thorne and for effects on plant growth. Sci. Hortic. 122:472-478.

Chen G, Lin Y, Lu W, Vrijmoed LLP, Gareth Jones EB (2003). Two new metabolites of a marine endophytic fungus (No. 1893) from an estuarine mangrove on the South China Sea coast. Tetrahedron 59:4907-4909.

Gams W (1975). *Cephalosporium*-like hyphomycetes: some tropical species. Trans. Br. Mycol. Soc. 64:389-404.

Georgopoulos SG, Dekker J (1982). Detection and measurement of fungicide resistance general principles. FAO Plant Prot. Bull. 30:39-42.

Guo LD (2001).Advances of researches on endophytic fungi. Mycosystrma 20(1):148-152.

Hyde KD, Soytong K (2008). The fungal endophyte dilemma. Fungal Divers. 3:163-173.

Kelemu S, White JF, Muñoz F, Takayama Y (2001). An endophyte of the tropical forage grass *Brachiaria brizantha*: Isolating, identifying, and characterizing the fungus, and determining its antimycotic properties. Can. J. Microbiol. 47:55-62.

Kirk PM, Cannon PF, David JC, Stalpers JA (2001). Ainsworth and Bisby's dictionary of the fungi.9th ed. CAB International, Egham. 1-655.

Krishnaveni R, Rathod V, Thakur MS, Neelgund YF, (2009). Transformation of L-tyrosine to L-dopa by a novel fungus, *Acremonium rutilum*, under submerged fermentation. Curr. Microbiol. 58:122-128.

Li P, Ma L, Feng YL, Mo MH, Yang FX, Dai HF, Zhao YX (2012). Diversity and chemotaxis of soil bacteria with antifungal activity against Fusarium wilt of banana. J. Ind. Microbiol. Biot. 39(10):1495-1505.

Liu F, Cai XL, Yang H, Xia XK, Guo ZY, Yuan J, Li MF, She ZG, Lin YC (2010). The bioactive metabolites of the mangrove endophytic fungus *Talaromyces* sp. ZH-154 isolated from *Kandelia candel* (L.) Druce. Planta Med. 776:185-186.

Mejia LC, Rojas EI, Maynard Z, Van Bael S, Arnold AE, Hebbar P, Samuels GJ, Robbins N, Herre EA (2008). Endophytic fungi as biocontrol agents of Theobroma cacao pathogens. Biol. Control 46:4-14.

Ploetz RC (2006). Fusarium wilt of banana is caused by several pathogens referred to as *Fusarium oxysporum* f. sp. *cubense*. Phytopathology 96:653-656.

Roberts WK, Selitrennikoff CP (1986). Isolation and partial characterization of two antifungal proteins from barley. Biochim. Biophys. Acta 880:161-170.

Si M, Xue Q, Yu B, Yuan H, Cai Y, Lai H, Chen Z (2005). Study on the antagonistic function of thirty-six strains' antagonistic actinomycete to four kinds of pathogenic fungi. J. Northwest. Sci-Tech. Univ. Agric. For. (Nat. Sci. Ed.) 33:55-58.

Singh AK (2010). Integrated management of *Cercospora* leaf spot of urd bean (*Vigna mungo*). J. Mycol. Plant Pathol. 40:595-596.

Subramaniam S, Maziah M, Sariah M, Puad MP, Xavier R (2006). Bioassay method for testing Fusarium wilt disease tolerance in transgenic banana. Sci. Hortic. 108:378-389.

Sun JB, Peng M, Wang YG, Zhao PJ, Xia QY (2011). Isolation and characterization of antagonistic bacteria against fusarium wilt and induction of defense related enzymes in banana. Afr. J. Microbiol. Res. 5(5):509-515.

Wen L, Chen G, She ZG, Yan CY, Cai JN, Mu L (2010). Two new paeciloxocins from a mangrove endophytic fungus *Paecilomyces* sp. Russ. Chem. Bull. 59:1656-1659.

Wu X, Huang H, Chen G, Sun Q, Peng J, Zhu J, Bao S (2009). A novel antibiotic produced by *Streptomyces noursei* Da07210. Antonie van Leeuwenhoek 96:109-112.

Wu YL, Yi GJ, Peng XX (2010). Rapid screening of *Musa* species for resistance to Fusarium wilt in an *in vitro* bioassay. Eur. J. Plant Pathol. 128:409-415.

Zhang CL, Zheng BQ, Lao JP, Mao LJ, Chen SY, Kubicek CP, Lin FC (2008). Clavatol and patulin formation as the antagonistic principle of *Aspergillus clavatonanicus*, an endophytic fungus of *Taxus mairei*. Appl. Microbiol. Biotechnol. 78:833-840.