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Full Length Research Paper

Studies of actinomycetes for biological control of *Colletotrichum musae* pathogen during post harvest anthracnose of banana

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For the studies and preliminary screening, eighty actinomycetes isolates were selected as antagonists against *Colletotrichum musae*, causal agents of anthracnose of banana. Actinomycetes isolates were cultured with *C. musae* (dual culture) to determine their *in vitro* antagonistic ability. Seventeen strains that strongly inhibited hyphal growth of the pathogen were selected as potent antagonists. Green healthy fruits were treated with the test pathogen and potent antagonistic actinomycetes to determine their effective inhibition of pathogen. The selected actinobacterial isolates significantly reduced severity of anthracnose on artificially inoculated banana in storage conditions for one week incubation at about 30°C. Bioactive and potent isolates CF-1(1), Dir-10(10), Dir-10(4) and Dir-10(3) reduced the size of the lesion as compared to control. These streptomycetes isolates exhibited disease inhibition of 77 to 85% in the artificially inoculated banana fruits and maximum disease inhibition of 85.87% in banana was exhibited by the potent actinobacterial isolate Dir-10(10).

Key words: Actinomycetes**,** scanning electron microscopy (SEM), mycelial deformation, post harvest disease, biological control, banana anthracnose, *Colletotrichum musae*.

INTRODUCTION

Saudi Arabia is one of the largest fruit consuming markets, huge amounts of fruits are imported from different parts of the world. Bananas (*Musa* spp.) are imported in Saudi Arabia from tropical countries and are considered as most popular fruits (Abd-Elsalam et al., 2010). However, post harvest decay of fruits causes tremendous losses. It is estimated that 20 to 25% of harvested fruits are decayed by pathogens during post harvest handling even in developed countries (Droby, 2006; Zhu, 2006). However, in developing countries, post harvest losses are often more severe due to inadequate storage and transportation facilities (Rashad et al., 2011). Fungal fruit infections may occur during the growing season, harvesting, handling, transport, post-harvest storage and marketing conditions, or after purchasing by the consumer.

Fruits due to their low pH, high moisture content and nutritional composition are very susceptible to attack by pathogenic fungi which in addition to causing rots may also make them unfit for consumption by producing mycotoxins (Stinson et al., 1980; Philips,1984; Singh and Sharma, 2007). Microorganisms associated with post harvest spoilage of fruits have engaged the attention of mycologists for many years (Okigbo, 2001).

Colletotrichum musae (Berk and Curt) causes anthracnose in banana fruit and is confined to mature fruits (Waller, 1992). It is a widely distributed disease causing significant damage to crops in tropical,

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subtropical and temperate regions. Infection on banana usually starts when conidia contaminate the banana fruits during the month after flowering (Chillet et al., 2000). These conidia quickly germinate and form appresoria which are quiescent structures of the pathogen. As the banana ripens the appresoria germinate and forms infected hyphae that colonize the peel and penetrate into the fruit pulp (Chillet et al., 2007) leading to the development of anthracnose. The shipping of banana fruits as bunches and with subsequent ripening at high temperature may magnify the anthracnose problem (Meredith, 1960).

Post harvest losses have been reduced mainly through post harvest fungicides (Eckert and Ogawa., 1985, 1998) and to a lesser degree, through post harvest management practices to reduce inoculums. Normally, fungicides are prime means of controlling post harvest diseases (Eckert et al., 1994). However use of chemical fungicides to control post harvest rots and deterioration has been limited due to their potential carcinogenicity, teratogenicity, environmental pollution, effects on food and human health (Unnikrishnan and Nath, 2002; Mari et al., 2003). Thus alternative methods to control post harvest diseases, particularly those that are environmentally safe are urgently needed (Conway, 1982; Conway et al., 1991; Sugar et al., 1997; Wilson et al., 1997).

In view of this, the application of biological control agents (BCAs) seems to be one of the promising approaches. Biocontrol involves the use of naturally occurring non pathogenic microorganisms that are able to reduce the activity of plant pathogens and thereby suppress diseases. Antagonistic microorganisms can compete with the pathogen for nutrients, inhibit pathogen multiplication by secreting antibiotics or toxins, or reduce pathogen population through hyper parasitisms (Svetlana et al., 2010).

Many species of actinomycetes, particularly those belonging to the genus *Streptomyces* are well known as antifungal biocontrol agents that inhibit several plant pathogenic fungi (Anitha and Rebeeth, 2009). *Streptomyces* is the largest antibiotic producing genus in the microbial world discovered so far. The number of antimicrobial compounds reported from the species of this genus per year has increased almost exponentially for about two decades. Recent reports show that this group of microorganisms still remains an important source of antibiotic (Watve et al., 2001). Biological control of fungal plant diseases using *Streptomyces* spp. has been studied by many investigators in the last three decades (Hwang et al., 2001; Jeong et al., 2004; Prapagdee et al., 2008; Oskay, 2009; Degtyareva et al., 2009). However, there is still considerable interest in finding more efficient strains, which differ considerably with respect to their biocontrol effectiveness. The aim of the present study was to isolate antagonistic actinomycetes from Saudi Arabian soil and to investigate their *in vitro* and *in vivo* antagonistic activity against post-harvest pathogen *C. musae* (Berk and Curt).

MATERIALS AND METHODS

Isolation of fungal pathogens

Banana fruits with typical disease symptoms were collected from different markets of Riyadh, Saudi Arabia. Infected areas of the fruits were cleaned with sterile distilled water, cut into small pieces (3 to 5 mm in diameter) and each piece was treated in 1% sodium hypochlorite for 1 min and washed in three series of sterile distilled water. The washed tissues were transferred to the surface of Potato Dextrose Agar (PDA) medium using sterile forceps. Plates were then incubated at 25 ± 1°C and the fungal isolates obtained were purified by transferring single spore to a fresh PDA plate. Conidial morphology and measurements were determined microscopically after incubation on PDA plates at 25 ± 1°C for 10 to 12 days. Identification of the fungal isolates was done according to appropriate taxonomic key and description (Sutton, 1980, 1992; Mordue, 1980).

Pathogenicity assay

Pathogenicity test was carried on healthy green banana fruits. Fruits were surface sterilized with 70% ethanol, and three wounds were made on each fruit with sterilized scalpel. In each wound a mycelia plug 3 mm from a 7days old culture was inoculated. Uncultured pure PDA plug served as a control. Three replicates were made for each treatment. Inoculated fruits were covered with plastic wrap, and incubated at 25 ± 1°C and observed for 1 to 2 weeks.

Isolation of soil borne actinomycetes

Soil from different regions of Saudi Arabia was collected and subjected to isolation and purification of actinomycetes were performed according to Ismet et al., 2002; 2004; 2012a,b.

Identification of actinomycetes

Identification and characterization was performed by using cultural, physiological, biochemical and morphological analysis (Ismet et al., 2002; 2004; 2012a, b). Morphological analysis was done using light and scanning electron microscopy (Figure 1).

Assessment of antifungal activity of metabolites of actinomycetes against pathogenic fungi *in vitro*

All isolates were screened for their antagonism against test pathogenic fungi according to the modified cross plug method mentioned by Crawford et al. (1993). The antifungal activity of metabolites of actinomycetes against pathogenic fungi was evaluated by the modified agar well diffusion method (Ismet et al., 2004; 2012a, b).

Preparation for extraction of bioactive secondary metabolites

Freshly grown whole culture broth of actinomycetes and extraction solvent, methanol (1:1) was added to a flask and shaken at room temperature in a shaker for 3 days. The liquid extracts were then filtered and evaporated to dryness by using 40°C dryer incubator and then stored at 4°C for further analysis.

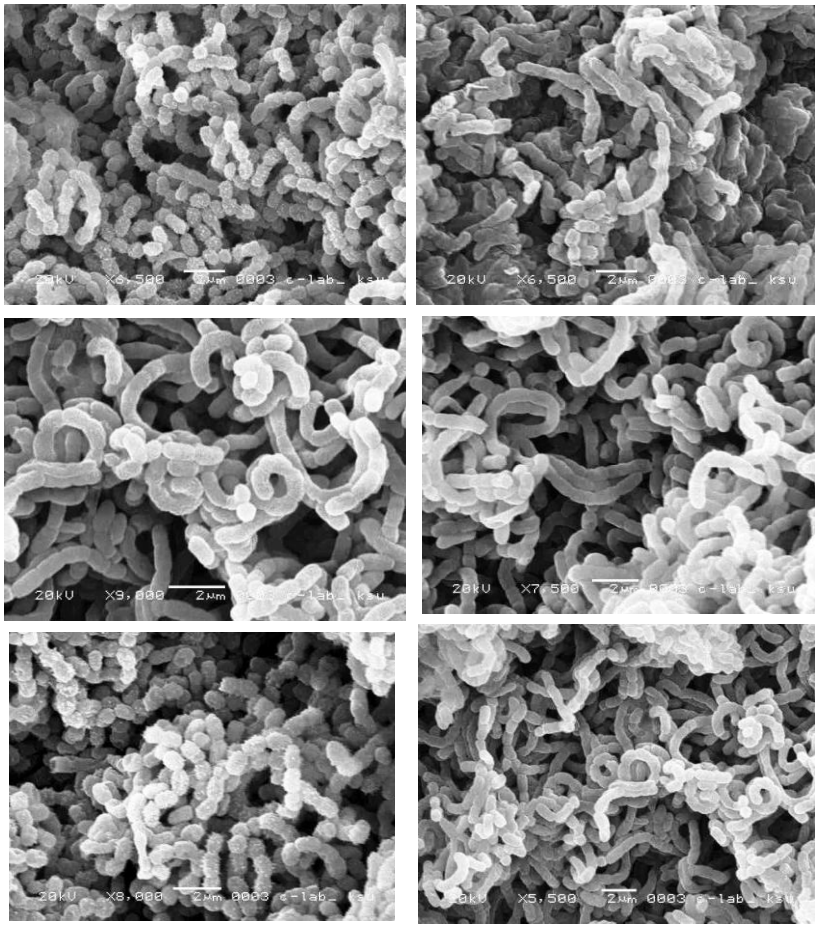

Figure 1. Scanning electron micrographs of the different selected potent *Streptomyces* isolates grown on water agar medium incubated for 2 weeks at 30°C. Spiral, curly, short and long chain morphology of the isolates identified as streptomycetes under the genus *Streptomyces*.

Screening for the antagonistic activity of isolated actinomycetes against post harvest fruit pathogens

All the isolates were screened for their *in vitro* antagonism against *C. musae,* according to the modified cross-plug method of Soares et al. (2007) and Yuan and Crawford (1995). Pathogenic fungi freshly grown on PDA plates and actinomycetes grown on yeast extract-soluble starch (YS) agar plates (Ismet et al., 2012a, b) were used in this experiment. Actinomycetes were inoculated in the center of the YS agar plates and incubated at 30 ± 1°C for 7 days. This was done to allow the bioactive actinomycetes culture to be established on the agar surface and to sporulate prior to inoculation of plates with pathogenic fungi. In order to investigate the inhibitory effect of actinomycetes on the fungal growth, mycelium plug of 5.0 mm diameter from 7 days old actively grown colony of each fungus was cut with a sterile cork borer and transferred to the plate on each side of the actinomycetes colony which is in the center (approximately 3 cm from the central line). Plates were re-incubated at 30 ± 1°C. Inhibition zones (mm), which were determined as the shortest distance between the pathogenic fungi and actinomycetes, were measured after 7 days of incubation. Plates inoculated with fungal pathogens alone without actinomycetes served as control treatment and three replicates were maintained for each cross-plug experiment.

***In vivo* antifungal activity of extracellular metabolites in the cell free culture filtrates**

Source of fruit materials

Bananas were collected from local markets and used the same day for *in vivo* assays. All the fruits were free of rots and wounds and as much as possible homogenous in maturity.

Preparation of fungal spore suspension and whole culture filtrates of actinomycetes

Fungal spore suspensions of *C. musae* were prepared from 14 days old cultures on PDA plates. Conidia from the surface of agar plate were scrapped with wet cotton swab and re-suspended in sterilized distilled water containing 5% Tween 80. The suspension concentration was determined by using a Neubauer haemocytometer and adjusted to 1×10^6 spores per ml.

***In vivo* assay**

All the fruits used were surface disinfected with 5% sodium

Table 1. Antifungal activity of the isolated actinomycetes under the genus *Streptomyces* against *C. musae.*

Antifungal activity*	*Colletotrichum musae*	Number of isolates (%) among eighty soil-borne actinomycetes possessing antifungal activity
+++	14**	17.50
++	11	13.75
+	17	21.25
-	38	47.50

*Antifungal activity was determined by the inhibition zone. **Each isolate was tested using three replications. The rating of antifungal activity was modified from those of El-Tarabily et al. (2000). +++ = strong inhibition zone ≥ 20 mm; ++ = moderate inhibition.

hypochlorite solution for 3 min, washed thrice with sterilized distilled water and then air dried at room temperature. Surface sterilized fruits were wounded with a sterilized cork borer (5 mm diameter and 5 mm depth from the surface) and using surgical blade. Three wounds were made per fruit and the wounds were inoculated with both 50 µl extract of whole culture filtrates of antagonistic actinomycetes and 50 µl of conidial suspension of fungal pathogens individually. The inoculated fruits were placed on plastic trays and enclosed in clean plastic bags to maintain high humidity and incubated at room temperature (27 ± 1°C) for 2 weeks. After 7 days of storage the severity (%) of disease was observed and recorded by measuring their lesion diameters. Conidial suspensions of *C. musae* alone served as positive control and culture broth as negative control.

Statistical analysis

The means and standard deviation of inhibition levels, radial growth and lesion diameters were calculated. Data was analyzed by one way analysis of variance (ANOVA). Significant differences ($P \leq 0.05$) between the means were determined by the DUNCANS multiple range tests.

RESULTS AND DISCUSSION

Identification of the isolated fungi from post harvest diseased fruits

Identification was done according to the fungal colony and conidial morphology. Pathogen isolated from anthracnose lesions of banana, collected from different markets of Riyadh was identified as *C. musae* (on PDA plate it formed white aerial mycelium which turned pinkish orange in color with age. Several dark orange structures developed abundantly on the incubated culture surface after 10 to 12 days, which were mostly acervuli including dark orange conidia. The pathogen isolated from banana was morphologically similar to the description of banana pathogen *C. musae* (Sutton, 1980; Price, 1995). The colony characters and microscopic data is in accordance with the description provided by Sutton and Waterson (1970) and Mordue (1980). The isolate was identified as *C. musae.* Moreover, appressorium characteristics of the isolates were almost like those of *C. musae* characteristics, including colony shape on PDA and conidial color confirmed that the pathogen is *C. musae*. Our findings in the present study are in agreement with the results of Abd-Elsalam et al. (2010) and Lim et al. (2002).

Pathogenicity test

Inoculated banana fruits showed typical anthracnose symptoms after 5 to 7 days of incubation. Lesions were black necrotic, circular and sunken, and these lesions showed white mycelia growth and produced orange colored conidial masses later. On banana the pathogen showed cultural characteristics that is, lesion colour, and growth texture, which was similar to the descriptions reported by Sutton (1980), Price (1995) and Photita et al. (2001). Based on the pathogenicity observed after inoculation of healthy banana fruits with mycelial plugs through wounds on the banana fruits, and comparing the results obtained with the typical symptoms of anthracnose disease by *C. musae*, it is clear that our isolate is able to cause anthracnose disease. Infections stimulate ripening of fruits, and lesions elongate with ripening. On ripening fruits, sunken brown spots develop with orange acervuli (Sutton and Waterston, 1970; Stover, 1987; Lim et al., 2002). *C. musae* and *C. gloeosporioides* have been found as endophytes in banana, but these fungi also cause anthracnose of banana fruits (Photita et al., 2001). Similar symptoms were observed on performing pathogenicity test on banana (Abd-Elsalam et al., 2010; Lim et al., 2002).

Antifungal activity of antagonist actinomycetes isolated from soil

A total of eighty isolates of actinomycetes were selected from soils of Riyadh, Saudi Arabia. The percentage of isolated actinomycetes with antifungal activity against *C. musae* was 52.50%. Of these only 14 isolates exhibited strong antagonistic activity against *C. musae* (Table 1) (Figures 2, 3 and 4). Scanning electron microscopic studies have determined the mycelial deformation of the inoculated pathogenic fungi as thickened and bulbous structures shown in Figure 4.

Our findings indicate that potent antagonists inhibited

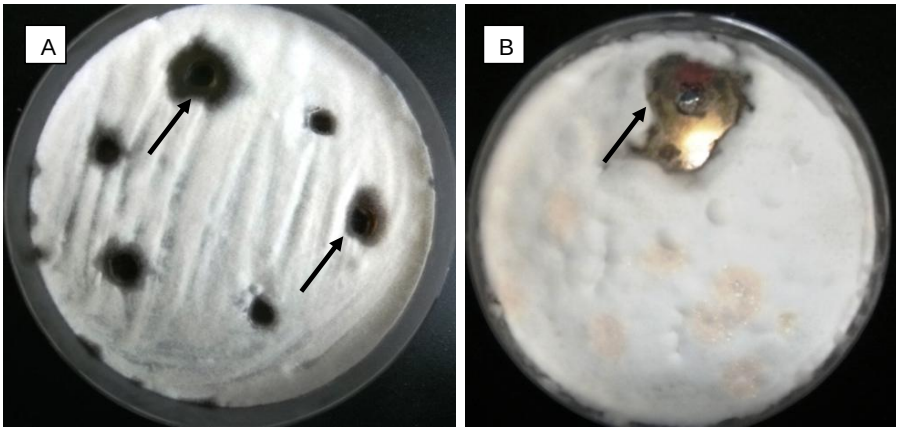

Figure 2. Secondary screening of bioactive streptomycetes isolates against fruit pathogenic fungi *C. musae* (A and B) using agar well diffusion technique at 30°C for 7 days.

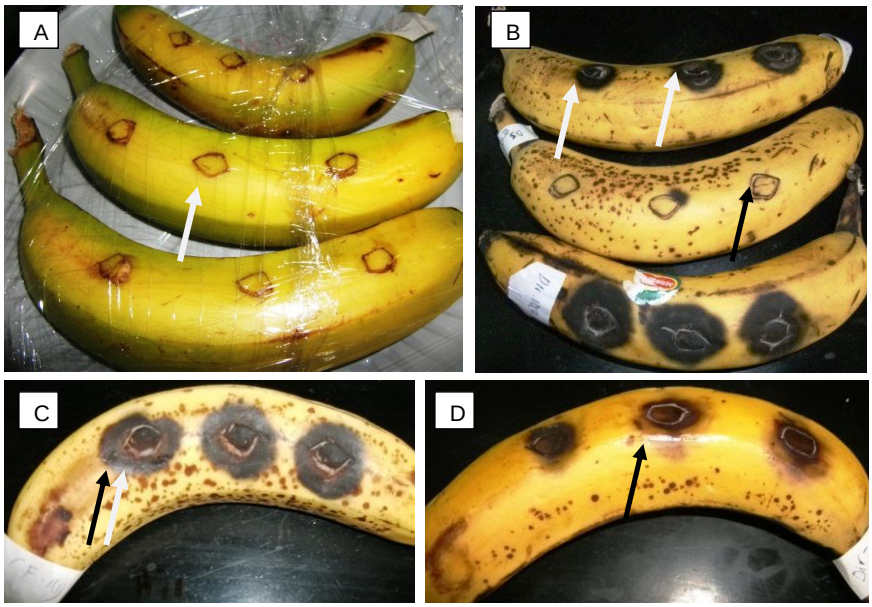

Figure 3. Banana fruits treated with together the cell suspension of antagonistic bacterium streptomycetes isolates CF-1(1), DIR-10(10) and CF-1(4) and pathogenic fungus *C. musae* (A, B and D), and treated with only pathogenic fungus *C. musae* (C) under storage conditions. After 7 days. Anthracnose of banana symptoms were reduced significantly and are shown in Table 2.

the growth of test pathogens by releasing extracellular diffusible metabolites that inhibited the hyphal growth of *C musae.* However actinomycetes isolates showed varying degree of antifungal activity. Several researchers have already reported similar antifungal activity of actinomycetes against fungal pathogens. Taechowisan et al. (2009) isolated 39 strains of actinomycetes from soil, out of which only 6 inhibited the growth of *C. musae* and only one isolate of *Streptomyces* showed strong antagonism toward *C. musae.* Prapagade et al. (2008) isolated 146 strains of indigenous actinomycetes from rhizosphere soil, however, only 10 strains exhibited antifungal activity against *C. gloeosporioides* and *S. rolfsiii*. Bharti et al. (2010) obtained 316 actinomycetes strains from different soils, of which 31% exhibited anti-fungal activity against important phytopathogens. Many species of actinomycetes, particularly those belonging to the genus *Streptomyces,* are well known as antifungal

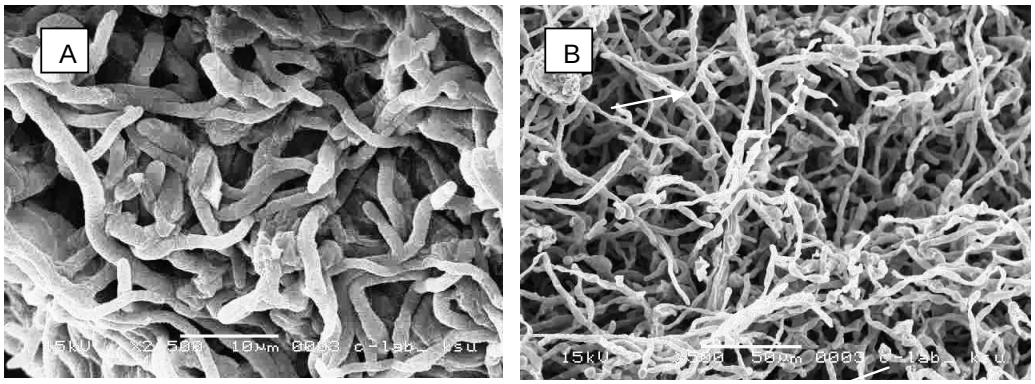

Figure 4. Antagonism of crude extract of potent *Streptomyces* sp. strain CF-1(1) to *C. musae* on PDA agar medium. Micrographs of control fungi (A); *In vitro* plate assay after 7 days incubation (source of fungal-mycelium samples for scanning electron microscopic studies were taken from the inhibition part) (B); Hyphal ends of *C. musae* (B); Hyphae showing thickened and bulbous structures (arrow) at the edges of the inhibited fungal colonies in the agar well diffusion method (B). On the control plate, however, fungal mycelium showed regular, radial growth (A).

biocontrol agents that inhibit several plant pathogenic fungi (Errakhi et al., 2007; Khamna et al., 2009). The antagonistic activity of *Streptomyces* to fungal pathogens is usually related to the production of antifungal compounds (Fguira et al., 2005; Atta, 2009) and/or extracellular hydrolytic enzymes (Mukherjee and Sen, 2006; Prapagdee et al., 2008). It was observed that crude extracts of antagonistic isolates caused hyphal swelling and distortation in PDA plates. Similar results were observed by Svetlantana et al. (2010) reported the fungal malformation might be due to antibiotic metabolites.

Effect of promising antagonists for controlling the development anthracnose of banana

The disease incidence on all antagonist treated banana fruits was significantly lower than those on the control fruit ($P < 0.05$) (Table 2). Of the seventeen most promising antagonists selected from the *in vitro* experiments, tested on banana fruits, isolate DIR-10(10), CF-1(1) and DIR-10(3) showed disease inhibition of 85.87, 81.63 and 80.20%, respectively. Other isolates were less effective but exhibited reduced lesion diameter as compared to the control 2.36 cm. However isolate CF-1(4) showed maximum lesion diameter of 14.60 mm and minimum diseases inhibition of 37.85% as compared to the other isolates (Figure 3 and Table 2).

The reduction in lesion diameter by isolates DIR-10(10), CF-1(1) and DIR-10(3), with respect to control clearly indicates their strong antagonistic ability against the anthracnose pathogen *C. musae*. Similar results of disease inhibition produced by *Streptomyces* sp. R03 metabolite against lemon pathogens has been reported earlier by Maldonado et al. (2010). Taechowisan et al. (2005), also reported that secondary metabolites from endophytic *Streptomyces aureofaciens* CMUAc130 had strong antifungal effects against *C. musae* and *Fusarium oxysporum.*

In the present study, the *in vitro* antagonistic assay showed promising results, for the use of *Streptomyces* as antifungal agents against phytopathogenic fungi. Similar results have been reported previously in actinomycetes screening (Taechowisan et al., 2009; Al-Askar et al., 2011). The reduction in diameter of the lesion and disease inhibition on fruits in the *in vivo* assay by isolates CF-1(1), DIR-10(10), DIR-10(3), indicate their role as strong antifungal agents against *C. musae.* The use of specific microbial agents has brought remarkable success in the control of plant pathogens (Okigbo and Ikediugnu, 2000, 2001). Many actinomycetes especially, genus *Streptomyces* exihibit strong antifungal activity aganist phytopathogens (Maldonado et al., 2010; Al-Askar et al., 2011; Svetlana et al., 2010). However screening is an important and critical step in the development of BCAs. The success of all subsequent stages depends on the ability of a screening procedure to identify an appropriate candidate (Svetlana et al., 2010). Several recent studies have shown that antagonistic microorganisms from the genus *Streptomyces*, *Bacillus*, *Trichoderma* and *Gliocladium* spp. can help limit fungal pathogen damage in various fruits. *Bacillus subtilis* have proved to be antagonists against the causal agent of anthracnose, blossom rot and crown rot of banana like *Burkholderia cepacia*, *Trichoderma harzianum* (Devi and Arumugam, 2005; Costa and Erabadupitiya, 2005). Post harvest treatment of mango fruit with *B. subtilis* decreases anthracnose development (Chuang and Ann, 1997). Our results support these findings by showing that our streptomycetes isolates restrict the *in vitro* and *in vivo* growth and conidial germination of *C. musae*, the most common and economically important pathogen of banana

Table 2. Efficacy of promising antagonistic actinomycetes for controlling anthracnose of banana fruit.

Treatment	Average diameter of lesion (mm)*	Disease inhibitions (%)**
Negative control	0	100
CF-1(7)	10.00	56.21
CF-2(3)	11.00	53.38
CF-1(4)	14.60	37.85
CF-3(3)	10.00	57.62
CF-1(27)	11.60	50.56
CF-1(1)	4.50	81.63
DIR-10(8)	6.30	73.16
DIR-10(4)	5.30	77.39
DIR-10(10)	3.30	85.87
DIR-10(7)	12.60	46.32
DIR-10(3)	4.60	80.20
DIR-7(27)	10.30	56.20
DIR-10(9)	12.30	47.73
DIR-10(6)	11.30	51.97
DIR-10(1)	8.30	64.68
DS-6(32)	7.30	68.92
DS-8(46)	13.60	42.08
Positive control	23.60	0.00

*, All values are means of three replicates. **, All means in a column are significant at (P<0.05). The means and standard deviation of inhibition levels, radial growth and lesion diameters were calculated. Data was analyzed by one way analysis of variance (ANOVA). Significant differences (P ≤ 0.05) between the means were determined by the DUNCANS multiple range tests.

(Figures 1, 2, 3 and 4).

The present work demonstrated the *in vitro* and *in vivo* potential of these selected actinomycetes to control the most important fruit pathogenic fungi that cause anthracnose on banana. However, further studies are necessary to evaluate the effect of these potential biocontrol agents in greenhouse and field conditions and also to purify and characterize the secondary metabolites produced by these actinomycetes.

Conclusion

The purpose of this work was to prove locally isolated actinomycetes which tentatively identified under the genus *Streptomyces* and their potential to reduce or eliminate some of the main banana post harvest anthracnose disease. Further studies are needed in order to determinate the purity and the nature of *Streptomyces* species metabolites and their clear mechanism of action; this biological agent could be an alternative to the synthetic fungicides used in packing house.

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REFERENCES

Abd-Elsalam KA, Roshdy S, Amin OE, Rabani M (2010). First morphogenetic identification of the fungal pathogen *Colletotrichum musae* (*Phyllachoraceae*) from imported bananas in Saudi Arabia. Gen. Mol. Res., 9(4): 2335-2342.

Al-Askar AA, Abdul Khair WM Rashad YM (2011). *In vitro* antifungal activity of *Streptomyces spororaveus* RDS28 against some phytopathogenic fungi. Afr. J. Agr. Res., 6(12): 2835-2842.

Anitha A, Rebeeth M (2009). *In vitro* Antifungal Activity of *Streptomyces griseus* Against Phytopathogenic Fungi of Tomato Field. Acad. J. Plant Sci., 2(2): 119-123.

Atta HM (2009). An Antifungal Agent Produced by *Streptomyces olivaceiscleroticus*, AZ-SH514. World Appl. Sci. J., 6(11): 1495-1505.

Chillet M, Hubert O, Lapeyre Lde, Bellaire de (2007). Relationship between physiological age, ripening and susceptibility to banana to wound anthracnose. Crop Protect., 26: 1078-1082.

Chillet M, Lapeyre Lde, Bellaire de, Dorel M, Joas J, Dubois C, Marchal J, Perrier X, (2000). Evidence for variation in susceptibility of bananas to wound anthracnose due to *Colletotrichum musae* and the influence of edaphic conditions. Sci. Hortic., 86: 33-47.

Chuang TY, Ann PJ (1997). Biological control of mango anthracnose. Plant Protect. Bull. Taipei., 39: 227-240.

Conway WS (1982). Effect of post harvest calcium treatment on decay of delicious apple. Plant Dis., 66: 402-3.

Conway WSC, Sams E, Abbott J, Bruten BD (1991). Post harvest treatment of apple fruit provides broad spectrum protection against post harvest pathogens. Plant Dis., 75: 620-622.

Costa DMde, Erabadupitiya HRUT (2005). An integrated method to control postharvest diseases of banana using member of the *Burkholderia cepacia* complex. Postharvest Biol. Technol., 36(1): 31-39.

Crawford DL, Lynch JM, Whipps JM, Ousley M.A (1993). Isolation and characterization of actinomycete antagonists of a fungal root pathogen. Appl. Environ. Microbiol., 59: 3899-3905.
Degtyareva EA, Vinogradova KA, Aleksandrova AV, Filonenka VA, Kozhevin PA (2009). Soil actinomycetes as potential biofungicides. Moskow Univ. Soil Sci. Bull., 64: 73-77.
Devi AN, Arumugam T (2005). Studies on the shelf life and quality of Rasthali banana as affected by postharvest treatments. Orissa J. Hortic., 33(2): 3-6.
Droby S (2006). Improving quality and safety of fresh fruits and vegetables after harvest and the use of biological control and natural materials. Acta Hortic., 709: 45-51.
Eckert JW, Ogawa JM (1985). The chemical control of post harvest diseases: Sub tropical and tropical fruits. Annu. Rev. Phytopathol., 23: 421-54.
Eckert JW, Ogawa JM (1998). The chemical control of postharvest diseases: deciduous fruits, berries, vegetables and root/tuber crops. Ann. Rev. Phytopathol., 26: 433-469.
Eckert JW, Sievert JR, Ratnayake M (1994). Reduction of imazalil effectiveness against citrus green mold in California packinghouses by resistant biotypes of *Penicillium digitatum*. Plant Dis., 78: 791-794
Errakhi R, Bouteau F, Lebrihi A, Barakate M (2007). Evidences of biological control capacities of *Streptomyces* spp. against *Sclerotium rolfsii* responsible for damping-off disease in sugar beet (*Betavulgaris* L.). World J. Microbiol. Biotech., 23: 1503-1509.
Fguira LF, Fotso S, Ameur-Mehdi RB, Mellouli L, Laatsch H (2005). Purification and structure elucidation of antifungal and antibacterial activities of newly isolated *Streptomyces* sp. strain US80. Res. Microbiol., 156: 341-347.
Hwang BK, Lim SW, Kim BS, Lee JY, Moon SS (2001). Isolation and *in vivo* and *in vitro* antifungal activity of phenylacetic acid and sodium phenylacetate from *Streptomyces humidus*. Appl. Environ. Microbiol., 67: 3739-3745.
Ismet A, Bukhari NA, Aref NM, Shinwari MMA, Bakir MA (2012a). Antiviral activities of streptomycetes against tobacco mosaic virus (TMV) in Datura plant: Evaluation of different organic compounds in their metabolites. Afr. J. Biotechnol., 11(8): 2130-2138.
Ismet A, Bukhari NA, Wijayanti DR, Bakir MA (2012b). Proteolytic activity of alkaliphilic, salt-tolerant actinomycetes from various regions in Saudi Arabia. Afr. J. Biotechnol., 11(16): 3849-3857.
Ismet A, Paramaswari S, Vikineswary S (2002). Diversity of *Micromonospora* in Malaysian mangrove rhizosphere soil. Malaysian J. Sci., 21: 51-59.
Ismet A, Vikineswary S, Paramaswari S, Wong WH, Ward A, Seki T, Fiedler HP, Goodfellow M (2004). Production and chemical characterization of antifungal metabolites from *Micromonospora* sp. M39 isolated from mangrove rhizosphere soil. World J. Microbiol. Biotechnol., 20: 523-528.
Jeong D, Park K, Kim SH, Kim KR, Choi SW, Kim JT, Choi KH, Kim JH (2004). Identification of *Streptomyces* sp. producing antibiotics against phytopathogenic fungi, and its structure. J. Microbial. Biotechnol., 14: 212-215.
Khamna S, Yokota A, Peberdy JF, Lumyong S (2009). Antifungal activity of *Streptomyces* spp. isolated from rhizosphere of Thai medicinal plants. Int. J. Integr. Biol., 6(3): 143-147.
Lim J, Lim TH, Cha B (2002). Isolation and identification of *Colletotrichum musae* from imported bananas. Plant Pathol. J., 18: 161-164.
Maldonado MC, Orosco CE, Gordillo MA, Navarro AR (2010). *In vivo* and *in vitro* antagonism of *Streptomyces* sp. RO3 against *Penicillium digitatum* and *Geotrichum candidum*. Afr. J. Microbiol. Res., 4(22): 2451-2456.
Mari MP, Bertolini, Pratella GC (2003). Nonconventional methods for the control of post harvest pear diseases. J. Appl. Microbiol., 94: 761-766.
Meredith DS (1960). Studies on *Gloeosporium musarum* Cke. & Mass. causing storage rots of Jamaican bananas. I. Anthracnose and its chemical control. Annu. Appl. Biol., 48: 279-290.
Moss MO (2002). Mycotoxins review. 1. *Aspergillus* and *Penicillium*. Mycologist, 16: 116-119.
Mukherjee G, Sen SK (2006). Purification, Characterization, and antifungal activity of chitinase from *Streptomyces venezuelae* P10. Curr. Microbiol., 53: 265-9.
Okigbo RN (2001). Occurence, Pathogenicity and Survival of *Macrophoma mangiferae* in leaves, branches and stems of mango (*Mangifera indica* L.). Plant Protect. Sci., 37: 138-144.
Okigbo RN, Ikediugwa FEO (2000). Studies on biological control of post harvest rot of yams (*Dioscorea* spp.) with *Trichoderma viride*. J. Phytopathol., 148: 351–355.
Okigbo RN, Ikediugwa FEO (2001). Biological control of tuber surface mycoflora of yams (*Dioscorea rotundata*). Tropical Sci., 41: 85–89.
Oskay M (2009). Antfungal and antibacterial compounds from *Streptomyces* strains. Afr. J. Biotechnol., 8: 3007-3017.
Philips DJ (1984). Mycotoxins as post harvest problem. In post harvest pathology of fruits and vegetables: Post harvest losses in perishale crops. (Ed): H.E. Moline, Agricultural experimental station, University of California, Berkeley Publication, N.E, pp. 50-54.
Photita W, Lumyong P, Hyde KD (2001). Endophytic fungi of wild banana (*Musa acuminata*) at Doi Suthep Pui National Park, Thailand. Mycol. Res., 105: 1508-1513.
Prapagdee B, Kuekulvong C, Mongkolsuk S (2008). Antifungal Potential of Extracellular Metabolites Produced by *Streptomyces hygroscopicus* against Phytopathogenic Fungi. Intl. J. Boil. Sci., 4(5): 330-337.
Price NS (1995). The origin and development of banana and plantain cultivation. In: *Bananas and Plantains* (ed. S. Gowen). Chapman and Hall. London, UK, pp. 317-381.
Rashad R Al-Hindi, Ahmed R Al-Najada, Saleh AM (2011). Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. Afr. J. Microbiol. Res., 5(4): 443-448.
Soares ACF, Sousa CDS, Garrido MDS, Perez JO (2007). Production of streptomycete inoculum in sterilized rice, Sci. Agric. (Piracicaba, Braz.), v.64, n.6, pp. 641-644.
Stinson EE, Bills SF, Osman J, Siciliano MJ, Ceponis, Heisler EG (1980). Mycotoxins production by *Alternaria* species grown on apples, tomatoes and blue berries. J. Agric. Food Chem., 28: 960-963.
Stover RH (1987). Diseases and Disorders. In: Bananas (Stover RH and Simonds NW, eds.). Longman, New York, pp. 281-323
Sugar DTL, Righettic EE, Sanchez, Khemira E (1997). Management of nitrogen and calcium in pear trees for enhancement of resistance of post harvest decay. Hortic. Technol., 2: 382-387.
Sutton BC (1980). The Coelomycetes. Common Wealth Mycological Institute, Kew. pp. 696
Sutton BC (1992). The Genus *Glomerella andits* Anamorph *Colletotrichum*. In: *Colletotrichum* Biology, Pathology and Control (Bailey JA and Jeger MJ, eds). CAB International, Wallingford, U.K, pp. 1-26.
Sutton BC, Waterston JM (1970). *Colletotrichum musae*. CMI Description of Pathogenic Fungi and Bacteria, pp. 222.
Svetlana Z, Stojanović S, Ivanović Z, Gavrilović V, Tatjana P, Jelica B (2010). Screening of antagonistic activity of microorganisms against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. Arch. Biol. Sci. Belgrade, 62(3): 611-623.
Unnikrishnan V, Nath BS (2002). Hazardous chemical in foods. Ind. J. Dairy Biosci., 11: 155-158.
Waller JM (1992). *Colletotrichum* Disease of Perennial and other cash Crops. In: *Colletotrichum:* Biology, Pathology and Control, Bailey, JA and MJ Jeger (Eds). CAB International, Wallingford, U.K, pp. 167-186.
Watve MG, Tickoo R, Jog MM, Bhole BD (2001). How many antibiotics are produced by the genus Streptomyces? Arch. Microbiol., 176: 386-390.
Wilson CL, Solar JM, EL-Ghaouth A, Wisniewski ME (1997). Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinera*. Plant Dis., 81: 204-210.
Yuan WM, Crawford DL (1995). Characterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal root and seed rots. Appl. Environ. Microbiol., 61: 3119-3128.
Zhu SJ (2006). Non-chemical approaches to decay control in postharvest fruit. In: Noureddine B., Norio, S. (Eds.), Advances in Postharvest Technologies for Horticultural Crops. Research Signpost, Trivandrum, India, pp. 297-373.