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Full Length Research Paper

Antifungal activity of some actinomycetes isolated from Riyadh soil, Saudi Arabia: An evaluation for their ability to control *Alternaria* caused tomato blight in green house pot trial

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A collection of 105 strains of actinomycetes isolated from the soils of Riyadh, Saudi Arabia were evaluated for their ability to inhibit plant pathogenic *Fusarium oxysporum*, *Alternaria alternata*, *Aspergillus niger*, and *Aspergillus flavus in vitro*. About 61.90% isolates were able to inhibit the growth of *A. alternata*, followed by 61.8, 52.24 and 50.9% inhibiting *A. flavus*, *F. oxysporum* and *A. niger* respectively. Subsequently, these isolates were tested for their ability to control *Alternaria* blight on tomato plant (*Lycopersicon lycopersicum*) grown in sterilized peat moss soil. Broth and crude extract of all except, DS-8(32), DS-6(48) and DS-6(33) isolates of actinomycetes have shown effect on plant disease severity. Over all analysis of data showed that strain AS-2(29) was the most effective isolate. Broth and crude extract of this strain had shown maximum zone of inhibition against *F. oxysporum* (11 and 18 mm respectively) and *A. alternata* (15 and 17 mm respectively). Plants treated with this strain were healthy and green and only a few older leaves showed the blight symptom and growth was found at par with control plants. However, strain DS-6(33) has not shown any antifungal activity and plants succumbed to infection of *A. alternata,* whereas, broth of DS-8(24) and DS-1(23) showed strong antifungal activity against *A. alternata* (20 and 19 mm zone of inhibition). However, crude extract of these strains has not shown any antifungal activity and plants treated with these strains were healthy and only older leaves were blighted. Extract but not broth of DS-1(20) showed strong antifungal activity against *F. oxysporum* and *A. alternata* (15 and 16 mm respectively) and the growth of the plant was similar to that of the control plants in pot trial strains varied in their antifungal activity against one or both plant pathogenic fungi. Based on the cultural, morphological and physiological characteristics, most of the strains were identified as the different isolates of the genus *Streptomyces*. In the further study, *Streptomyces* species as biological control agents would be offered a much needed alternative to the use of synthetic agrochemicals.

Key words: Actinomycetes, screening antifungal activity, *A. alternata*, green house pot trial, scanning electron microscope, tomato blight disease.

INTRODUCTION

Fungal phytopathogens pose serious problems worldwide in the cultivation of economically important plants. *Fusarium oxysporum*, *Alternaria alternata*, *Aspergillus niger* and *Aspergillus flavus* are responsible for plant diseases such as alternaria blight, fusarium wilt and fruit rot. Chemical fungicides are extensively currently used in agriculture. However, excessive use of chemical fungicides in agriculture has led to deteriorating human health, environmental pollution and the development of pathogen resistance to fungicide. Because of the worsening problems in fungal disease control, a serious

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search is needed to identify alternative methods for plant protection, which are less dependent on chemicals and are more environmentally friendly. Microbial antagonists are widely used for the biocontrol of fungal plant diseases.

Actinomycetes are among the most widely distributed group of micro-organisms in nature. They are found abundantly in cultivated and uncultivated soils in various regions throughout the world (Goodfellow and Simpson, 1987; Goodfellow and Williams, 1983). Actinomycetes are an important group of filamentous, gram-positive bacteria producing antibiotics of agricultural and medicinal importance. Besides the enormous numbers of agroactive metabolites produced by actinomycetes (Tanaka and Omura, 1993), they also play an important role in agriculture as biocontrol agents. Antagonism against an extensive variety of plant pathogens has been reported (Bressan, 2003; Chamberlain and Crawford, 1999; Doumbou et al., 2002; Tahvonen and Avikainen, 1987; Yuan and Crawford, 1995). Many species of actinomycetes, particularly, those belonging to the genus *Streptomyces*, are well known as antifungal biocontrol agents that inhibit several plant pathogenic fungi (Errakhi et al., 2007; Joo, 2005; Xiao et al., 2002).

The antagonistic activity of actinomycetes to fungal pathogens is usually related to the production of antifungal compounds (Getha and Vikineswary, 2002; Ouhdouch et al., 2001; Trejo-Estrada et al., 1998) and extracellular hydrolytic enzymes (Mukherjee and Sen, 2006; Prapagdee, 2008; Valois et al., 1996). Chitinase and β-1,3-glucanase are considered to be important hydrolytic enzymes in the lysis of fungal cell walls, for example, cell walls of *F. oxysporum*, *Sclerotinia minor*, and *Sclerotium rolfsii* (El-Katatny et al., 2001; Singh et al.,1999).

The control of plant diseases is an urgent need for sustainable agriculture. The application of agrochemicals for this purpose is still an important method in agricultural practices, though it still has some problems, such as environmental pollution and the detrimental effects on non-target organisms. They produced the natural antibiotics within the microhabitat of the rhizosphere being less polluting and less stressful on indigenous microbes as compared with chemical fungicides. They also have the ability to colonize plant root surfaces protecting the plant from pressure of plant pathogens. These biological control agents compete for nutrients and space with plant pathogens; they also synthesize extracellular enzymes that attack the phytopathogenic fungal cell walls and have the ability to produce descant resistant spores to survive under water deficiency. All the properties exhibited by actinomycetes, especially, those that belonging to the genus, *Streptomyces* as biological control agents of fungal phytopathogens, not only give us a better understanding of their environmental and ecological benefits, but also in their impact as an attractive alternative for use in agriculture.

Though all over the world, actinomycetes has been reported as an important biocontrol agent, however, in the control of tomato plant pathogen, *A. alternata* has not been explored in details in the Kingdom of Saudi Arabia; thus, it will be the preliminary study of its kind in Saudi Arabia.

The study was carried out to evaluate the antifungal activity of some actinomycetes isolated from the soil of Saudi Arabia. So that actinomycetes showing antagonistic activity can be utilized in the development of alternative methods for plant protection, which can be less dependent on chemicals and more environmentally friendly.

MATERIALS AND METHODS

Fungal strains and culture conditions

The fungal pathogens *F. oxysporum*, *A. alternata*, *A. flavus* and *A. niger* were isolated and obtained from the Department of Botany and Microbiology, K.S.U. The procured fungi were grown on potato dextrose agar (PDA) plates and incubated at 28°C for 4 to 6 days. Stock cultures of test fungi were maintained on PDA slants and stored at 4°C.

Isolation of actinomycetes from the soil of Saudi Arabia

Soil samples were collected from different areas of Riyadh, Saudi Arabia and actinomycetes isolated by a modified standard dilution technique on different agar media (Ismet, 2003).

Cultural, morphological, physiological and biochemical characteristics

Cultural characteristics such as color of aerial mycelium, color of substrate mycelium and pigmentation of the selected actinomycete isolates were recorded according to Shirling and Gottlieb (1966) on yeast extract-soluble starch (YS) agar medium (yeast extract, 10 g; soluble starch, 10 g and agar, 18 g; pH, 7.3). Micromorphology of the actinomycete isolates were examined by slide culture method (Williams and Cross, 1971). The utilization pattern of carbon sources by the strains was carried out according to the methods of Gottlieb (1961) since it can be used as an aid for species determination (Pridham and Gottlieb, 1948). The ability of the isolates to produce different enzymes was examined by using standard methods (Holding and Collee, 1971). Tolerance of the strains to NaCl concentration was also evaluated (Tresner et al., 1968). The actinomycete strains were tested for their ability to produce H_2S, indole and melanin pigments (Holding and Collee, 1971).

Production of antifungal metabolites

The secondary metabolites produced by the actinomycete isolates were extracted by the method of Ismet (2003) and Ellaiah et al. (2005). Pure culture of the strains was transferred aseptically and individually into the seed fermentation medium (SGY broth, soluble starch, 10 g; glucose, 10 g; glycerol, 10 g; corn flour, 2.5 g; peptone, 5 g; yeast extract, 2 g; $CaCO_3$, 3.0 g and distilled water 1 L and pH, 7.3) (Ismet, 2003; Ismet et al., 2002; Vikineswary et al., 2003). After 72 h of incubation, the seed culture at a rate of 10% was inoculated into the production medium of the same composition as seed medium. The small scale fermentation in 250 ml flask was carried out at 28 ± 2°C for 1 week under agitation at

250 rpm. At every 24 h interval, the flasks were harvested and the biomass was separated from the broth. The dry weight of the biomass was recorded and expressed in mg/100 ml. The culture filtrates were extracted twice with methanol and the pooled solvent extracts were evaporated to dryness in a 40°C drying incubator to yield a crude residue. For the extraction of secondary metabolites, similar protocol was followed for all the isolates. The residue was then dissolved in sterile distilled water and the extracts thus, obtained were used for antifungal activity against the test fungi such as *A. alternata* and *F. oxysporum* by using agar well diffusion method (Cappuccino and Sherman, 2004). For the growth of test fungi and screening, Sabouraud Dextrose agar (SDA) medium was used. The methanolic extracts dissolved in sterile distilled water were added to each well using water as a negative control. The SDA plates were incubated at 30°C for 48 to 72 h and the diameter of the inhibition zones of the test fungi around each well was measured.

For the morphological and biochemical characterization, the actinomycete isolate were incubated on YS agar medium at 30°C for 7 days. The mycelium structure and arrangement of spore on the mycelium were examined under the scanning electron microscope. Different biochemical tests were performed for the identification of various actinomycetes isolates. For SEM, the agar blocks containing the organisms (cultivated on water agar medium for 21 days at 30°C) were fixed with the vapor of 1% osmium tetroxide, then used serial dehydration in ethanol series (50 to 100%) and dried samples were sputter coated with gold and viewed with scanning electron microscope (SEM, JEOL, JSM, 6060 LV) (modified method from Ismet, 2003). Scanning electron microscopic analysis was carried out in the Central Laboratory, King Saud University, Riyadh, Saudi Arabia.

Screening of actinomycetes isolated from the soil for the antagonistic activity against phytopathogenic fungi under *in-vitro* condition

All isolates were screened for their antagonism against test phytopathogenic fungi according to the modified cross plug method mentioned by Crawford et al. (1993); Ismet (2003) and Ismet et al. (2003, 2004).

Assessment of antifungal activity of metabolites of actinomycetes against phytopathogenic fungi

The antifungal activity of metabolites of actinomycetes against phytopathogenic fungi was evaluated by the standard agar well diffusion method (Ismet, 2003).

Preparation for extraction of bioactive secondary metabolites

The whole culture broth of actinomycetes was used and added a solvent methanol (1: 1) and incubated at room temperature in a shaker for 3 days. The mixture of broth and solvent were filtered and evaporated to dryness by using 40°C dryer incubator and then stored at 4°C for further analysis. The culture filtrates and crude extracts were collected from the whole culture broth of the selected strains and were tested antifungal activity against *F. oxysporum*, *A. alternata*. Extract of selected actinomycetes were evaluated for its antifungal activity by the modified agar well diffusion method (Ismet, 2003).

Determination of bioefficacy of selected actinomycetes against phytopathogenic fungi in pot condition

Bioefficacy of the selected actinomycetes was determined against phytopathogenic fungus in pot conditions and pot trial method as described by Perveen et al. (2007) with slight modification was used. Pots of 9 cm (diameter) were surface sterilized with 1% sodium hypochlorite and filled with 100 g mixture of autoclaved peat moss soil and sand (5: 1). Single seedling (about 28 days old) of tomato plant grown in sterilized soil was transplanted in each pot.

Four plates of *A. alternata* culture grown on PDA was scrapped and mixed in 300 ml sterilized distilled water. 10 ml from the stock of *A. alternata* was added in each pot and 5 ml of this was poured around the plant by removing the soil whereas, 5 ml was poured over the leaves of seedling. Later, 10 ml whole culture broth of actinomycetes isolates (incubated for 2 weeks) was poured on the soil and leaves in the same way as mentioned previously for fungus and was covered with the soil. For each treatment, three replicates were maintained. Plants were inoculated with 10 ml sterilized distil water and plants inoculated with only *A. alternata* served as uninoculated and inoculated controls respectively. Pots were arranged in glass house on a rack. Plants were watered with sterile water as required. Plants were observed daily to record symptoms and growth for over one month. After one month, plants were uprooted and plant height, plant fresh weight were recorded. All plants were evaluated for infection of *A. alternata* on a scale from 0 to 4 where 0 = symptom less, 1 = up to 25% plant infected (small lesions), 2 = 25 to 50% plant infected (large lesions/blighted) 3 = 50 to 75% plant blighted, 4 = 75 to100% plant blighted.

Statistical analysis

Experiments will be performed in triplicate. The arithmetic mean, standard deviation of the mean (SD) of control and experimental results will be calculated using the Student's t-test. $P < 0.05$ was considered statistically significant. ANOVA analysis was done with the SPSS statistics software.

RESULTS AND DISCUSSION

Our continuous search for new antifungal metabolites from the rhizosphere soil samples of Riyadh region led to the selective isolation of predominant actinomycete strains. A collection of 105 strains of actinomycetes were selected from soils in Riyadh, Saudi Arabia and were evaluated for their ability to inhibit plant pathogenic. *F. oxysporum*, *A. alternata*, *A. niger* and *A. flavus in vitro* are presented in Figures 1, 2 and 3. It was found that some isolates have the ability to inhibit the growth of one or all tested fungi. Out of the total, 61.90% isolates were able to inhibit the growth of *A. alternata*, followed by 61.8, 52.24 and 50.9% of *A. flavus*, *F. oxysporum* and *A. niger* respectively (Figure 1). Of all the tested fungi, *A. alternata* was highly sensitive to the most of the isolates used in preliminary screening using a cross plug assay (Figures 1 and 2). Aerial mycelium was white, light grey white to dark grey white for most of the potent strains while the colour of the substrate mycelium appeared brown to light cream yellow (Table 1). Rizk et al. (2007) reported grey and white colour series of actinomycetes as the dominant forms in the soil as compared to yellow, red, violet and green ones. Different colour series of actinomycetes were recorded in soil (Ndonde and Semu, 2000).

The metabolites collected from the strains and the data

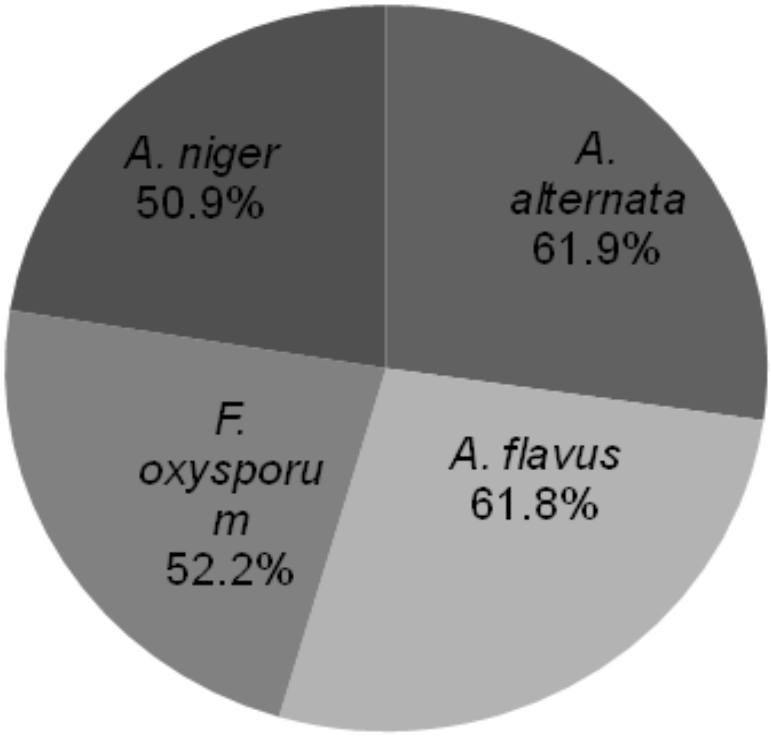

Figure 1. Screening of actinomycetes for antifungal activity *in vitro*, out of a total of 105, 61.90% strains were able to inhibit the growth of *A. alternata*, followed by 61.8, 52.24 and 50.9% of *A. flavus*, *F. oxysporum* and *A. niger* respectively.

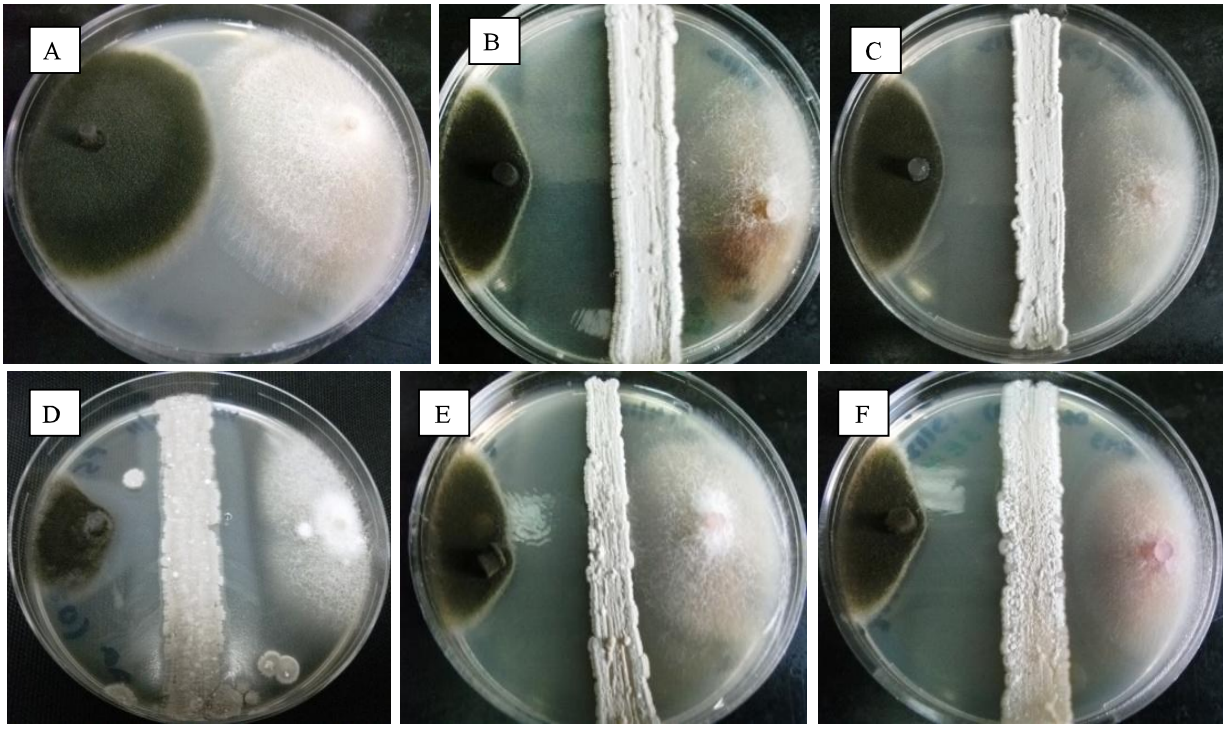

Figure 2. *F. oxysporum* using cross plug method incubated for 1 week at 30°C on Czapek-Dox agar medium. (A), control plate of *A. alternata* and *F. oxysporum*; (B), AS-2(29); (C), DS-1(23); (D), DS-1(20); (E), DS-8(24); and (F), DS-1 (22).

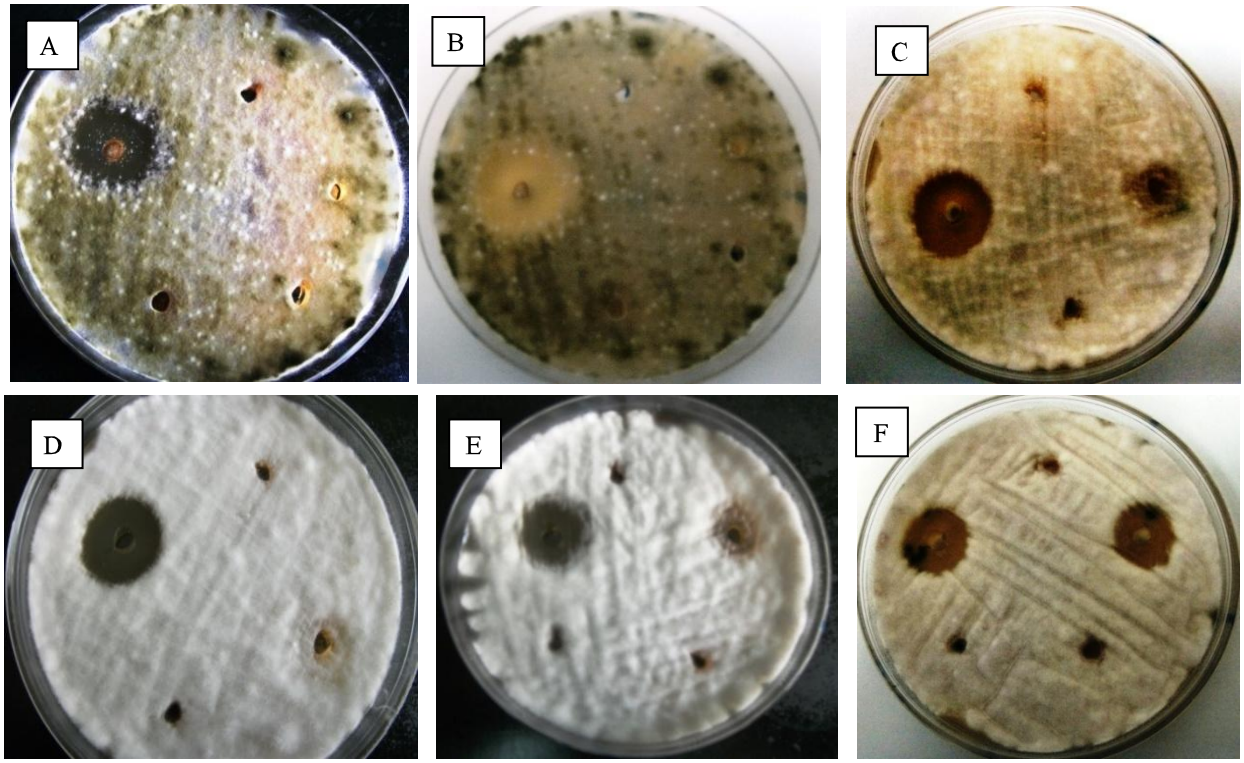

Figure 3. Secondary antagonism of actinomycetes against *A. alternata* (A, B, C) and *F. oxysporum* (D, E, F) using agar well diffusion method.

Table 1. Antifungal activity of culture filtrate and crude extract of actinomycetes against *A. alternata* and *F. oxysporum* under *in-vitro* condition using agar well diffusion method.

Isolates code no.	*A. alternata*		*F. oxysporum*	
	Broth	Extract	Broth	Extract
DS-1(23)	+++ (19 mm	-	±	-
AS-2(20)	±	-	+ (8 mm)	++(12 mm)
DS-6(51)	-	±	-	-
DS-1(20)	±	+++ (15 mm)	-	+++ (16 mm)
DS-8(32)	-	-	-	-
DS-8(24)	+++ (20 mm)	_	±	-
DS-1(22)	-	±	+ (10 mm)	+ (10 mm)
DS-8(18)	-	±	±	-
DS-6(5)	++	_	±	-
DS-6(48)	-	±	-	-
AS-2(29)	++ (14 mm)	+++ (17 mm)	++ (11 mm)	+++ (18 mm)
DS-6(33)	-	±	-	-

±, weak inhibition (< 8 mm); -, no inhibition.

of antifungal activity of broth and crude extract of twelve isolates having the greatest pathogen-inhibitory capabilities are presented in Table 1. It shows that broth and crude extract of all except DS-8(32), DS-6(48) and DS-6(33) isolates of actinomycetes have shown antifungal activity against one or both plant pathogenic fungi (Figure 3). Broth and crude extract of strain AS-2(29) has shown maximum zone of inhibition against *F. oxysporum* (11 and 18 mm respectively) and *A. alternata* (15 and 17 mm respectively) (Figure 3). Whereas, broth of DS-8(24) and DS-1(23) showed strong antifungal activity against *A. alternata* (20 and 19 mm zone of inhibition), however, crude

Table 2. Cultural, physiological, biochemical and morphological characteristics of the potent selected actinomycete strains.

Isolate code no.	Citrate utilization	Urease production	Casein hydrolysis	Milk peptonization and coagulation	Gelatin liquefaction	Lactose fermentation in kligler iron agar (KIA)	Glucose fermentation in KIA	H_2S production in KIA	Growth on 6% NaCl	Growth on 7% NaCl	Colony color as substrate mycelium color (substrate mycelium color)
DS-1(20)	+	+	+	+	-	-	-	-	+	+	Pale grey white (cream brown)
DS-1(21)	-	-	+	+	+	-	-	-	+	±	Grey white (red brown)
DS-6(33)	+	+	+	+	+	+	+	-	+	+	Dark grey white (dark brown)
DS-6(48)	-	+	+	+	+	-	-	-	+	+	Dark grey white (dark brown)
DS-8(23)	+	+	+	±	+	-	-	-	+	+	Grey (cream)
DS-8(24)	+	+	+	+	+	+	+	-	+	+	Grey white (grey brown)
DS-8(32)	+	+	+	±	+	-	-	-	+	+	Dark brown (grey)
AS-2(29)	-	+	+	±	+	+	+	-	+	+	Pale grey white (yellow)
KSU-2(20)	+	+	+	+	-	+	+	-	+	+	Pale grey (yellow)
DS-1(22)	+	+	+	+	+	-	-	-	+	+	Pale grey white (yellow)
DS-8(18)	+	+	±	±	-	-	-	-	+	+	Pale grey (grey brown)
DS-6(51)	+	+	+	+	+	-	-	-	+	+	Pale grey (red brown)
DS-6(5)	+	+	+	+	-	-	-	-	+	+	Pale grey (pale brown)

+, positive; -, negative.

crude extract of these strains have not shown any antifungal activity (Figure 3). Whereas, broth of DS-8(24) and DS-1(23) had shown strong antifungal activity against *A. alternata* (20 and 19 mm zone of inhibition), however, crude extract of these strains has not shown any antifungal activity (Figure 3). Based on the cultural, morphological and physiological characteristics, most of these strains were assigned to the family of Streptomycetaceae and the genus, *Streptomyces* (Pridham et al., 1958; Williams et al., 1983) (Table 2 and Figure 4). By far, streptomycetes are the most abundant culturable actinomycete (Lee and Hwang, 2002). The genus, *Streptomyces* belongs to the family Streptomycetaceae, a unique family of the suborder, Streptomycineae. Streptomycetes grow as mycelia filaments in soil; their mature colonies may contain two types of mycelia, the substrate (vegetative) mycelium, and the aerial mycelium. Each has a different biological role (Hopwood, 1999). Vegetative mycelia absorb nutrients and are composed of a dense and complex network of hyphae usually embedded in the soil or immobilize substrate. Once the cell culture becomes nutrient-limited, an aerial mycelium developed from the surface of the vegetative mycelium. The role of this type of mycelium is mainly reproductive; indeed, the aerial mycelium develops into spore chains at the matured stage in their life cycle (Hopwood, 1999). In this study, both, reproductive and aerial mycelium along with clearly sporulation of some mesophilic *Streptomyces* strains is shown in Figure 4.

The result of bioefficacy test of the selected twelve strains of actinomycetes on alternaria blight in pot condition presented in Table 3 shows that strains varied in their effect on plant disease severity. Plants treated with AS-2(29) strain were healthy, green, and only a few older leaves showed symptoms of blight and growth was found at par with the control plants (Figure 5). However, strain DS-6(33) has not shown any antifungal activity and plants succumbed to infection. Maximum plant height, weight and minimum disease index was observed in the plants treated with AS-2(29) which was 13.9 cm, 3.8 and 0.58 g respectively and it was significantly ($P<0.05$) higher than the inoculated control (Table 3). Plants treated with DS-1(23) did not show any significant ($P<0.05$) increase in plant weight (1.1 g) as compared to inoculated control (Table 3). Shirling and Gottlieb (1966) also stated that actinomycetes are an enormous reservoir for antibiotics and bioactive metabolites of which most of them many are excellent biocontrol agents for use in protecting plants against phytopathogens.

Streptomycetes, along with other bacterial strains belonging to the *Actinomycetales* have the ability to colonize plant root surfaces (Kortemaa et al., 1994; Tokala et al., 2002). Also, they have the capacity to synthesize extracellular enzymes that

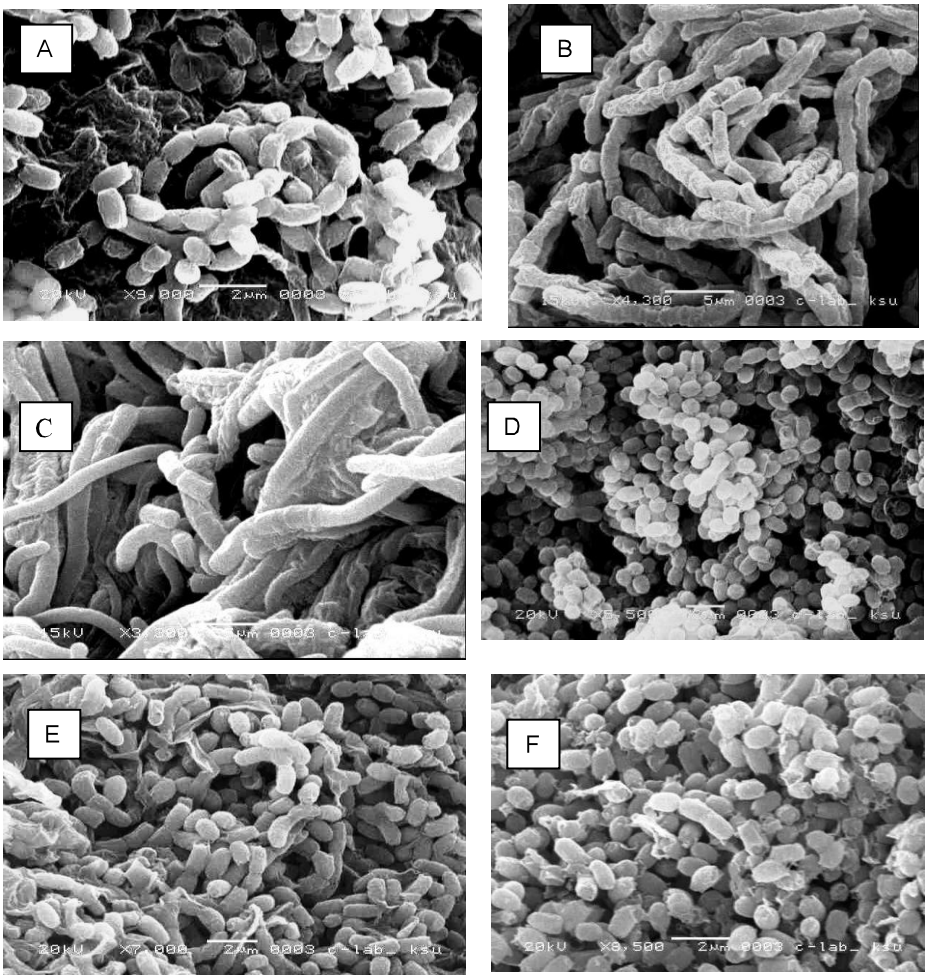

Figure 4. Scanning electron microscopic morphological structures of selected strains of potent actinomycetes (A), DS-8(18); (B) AS-2(18); (C), AS-2(16); (D), AS-2(29) (E), DS-8(32) and (F), DS-8(24). All the strains showed the spore chain morphology like as the genus *Streptomyces* under the family *Streptomycetaceae*.

Table 3. Effect of actinomycetes against *A. alternata* on tomato plants in pot condition.

Code	Plant height (cm)	Plant fresh weight (g)	Disease index (DI)
Uninoculated control	14.6	2.6	0
Inoculated control	6.6	0.9	3.8
DS-1(23)	8.5	1.1*	1.66
AS-2(20)	13.9	3.8	0.58
DS-6(51)	9.6	1.7	1.75
DS-1(20)	12.8	3.6	1.0
DS-8(32)	10.5	2.3	0.16
DS-8(24)	12.7	3.4	1.56
DS-1(22)	12.0	2.8	2.25
DS-8(18)	10.0	1.8	2.83
DS-6(5)	10.6	2.0	1.16
DS-6(48)	12.6	3.0	2.75
AS-2(29)	14	3.9	0.9
DS- 6(33)	d	d	d

*- non significant ($P<0.05$) as compare to inoculated control d- Plant died during study.

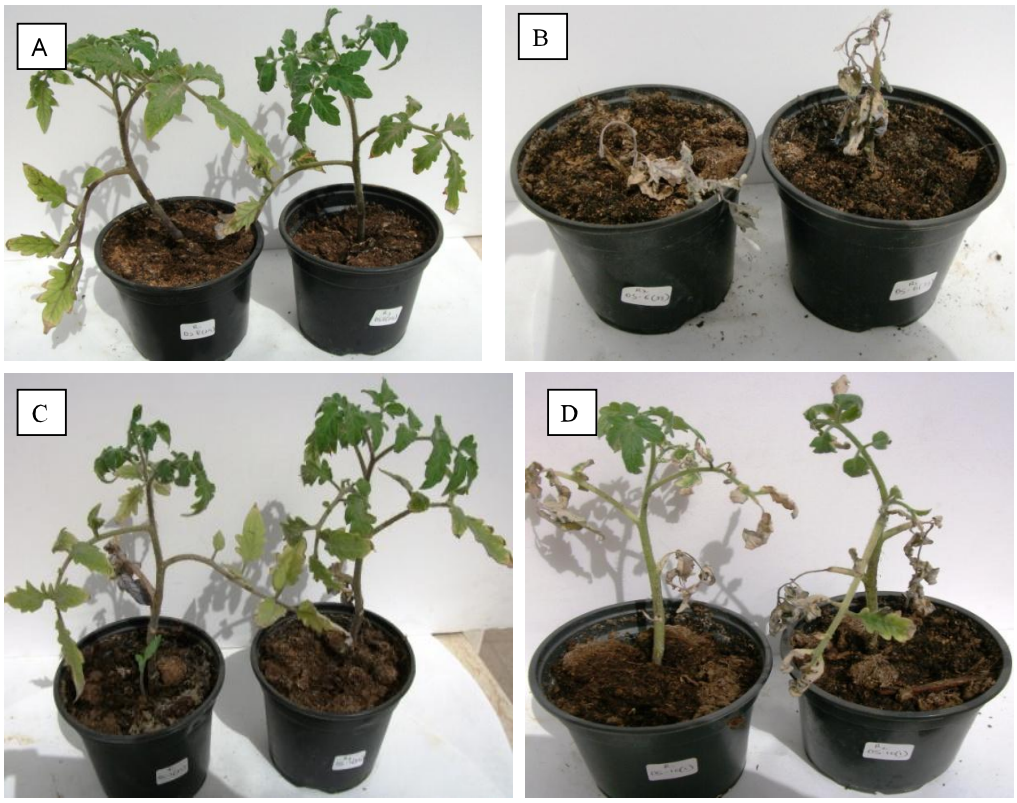

Figure 5. *In vivo* antagonism on green house tomato plant of the selected bioactive actinomycetes. (A), 0=symptom less, uninoculated control plant with sterile distilled water B: 4= 75 to 100% plant blighted, inoculated control plant with *A.* alternate only; (C),1= up to 25% plant infected (small lesions), inoculated plants with actinomycetes strain AS-2(29) and *A. alternata*; (D), 2 = 25 to 50% plant infected (large lesions/blighted Inoculated plants with actinomycetes strain DS-1(23) and *A. alternata*.

allow them to use recalcitrant organic compounds as energy sources and to degrade phytotoxin compounds (Goodfellow and Williams, 1983; Lewis and Starkey, 1969; McCarthy and Williams, 1992). Streptomycetes have the ability to produce iron chelating compounds and siderophores that starve pathogens for iron (Tokala et al., 2002).The ability to produce siderophores as a mechanism gives the biocontrol agent a competitive advantage in environments, such as rhizospheres where soluble iron is scarce (Mullen, 2004).

Although, thousands of antibiotics and bioactive metabolites have been described, these are thought to represent only a small fraction of the bioactive compounds produced by actinomycetes. In this study, we summarize the general characteristics and properties of actinomycetes as biocontrol agents. The mechanisms through which the biocontrol occurs *in vitro* and *in vivo* as well as, the impact of the phytopathogenic fungi in green house pot trial will be investigated in the near future.

Conclusion

The present study indicates the usefulness of whole culture broth and methanolic extract of the locally isolated actinomycetes especially, under the genus, *Streptomyces* for medicinal and biotechnological purposes. The further chemical investigation using GC-MS for the presence of antifungal metabolites might indicate its potentiality as a source of novel useful antifungal antibiotics for the local tomato seedlings and other plants in Saudi Arabia and worldwide.

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REFERENCES

Bressan W (2003). Biological control of maize seed pathogenic fungi by use of actinomycetes. Bio. Control, 48: 233-240.

Cappuccino JG, Sherman N (2004). Microbiology, a laboratory manual. Pearson Education, Inc., New Delhi p. 282.

Chamberlain K, Crawford DL (1999). *In vitro* and *in vivo* antagonism of pathogenic turfgrass fungi by *Streptomyces hygroscopicus* strains -

283. YCED9 and WYE53. J. Ind. Microbiol. Biotechnol., 23: 641-646.
Crawford DL, Lynch JM, Whipps JM, Ousley MA (1993). Isolation and characterization of actinomycete antagonists of a fungal root pathogen. Appl. Environ. Microbiol., 59: 3899-3905.
Doumbou CL, Hamby-Salove MK, Crawford DL, Beaulieu C (2002). Actinomycetes, promising tools to control plant diseases and to promote plant growth. Phytoprotection. 82: 85-102.
El-Katatny MH, Gudelj M, Robra KH, Elnaghy MA, Gübitz GM (2001). Characterization of a chitinase and an endo-β-1,3-glucanase from *Trichoderma harzianum* rifai T24 involved in control of the phytopathogen *Sclerotium rolfsii*. Appl. Microbiol. Biotechnol., 56: 137-43.
Ellaiah G, Adinarayana G, Saisha V, Vasu P (2005). An oligoglycosidic antibiotic from a newly isolated *Streptomyces albovinaceous*. Indian J. Microbiol., 45: 33-36.
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 (*Beta vulgaris* L.). World J. Microbiol. Biotechnol., 23: 1503-9.
Getha K, Vikineswary S (2002). Antagonistic effects of *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum* f. sp. *cubense* race 4: Indirect evidence for the role of antibiosis in the antagonistic process. J. Ind. Microbiol. Biotechnol., 28: 303-10.
Gottlieb D (1961). An evolution of criteria and procedures used in the description and characterization of *Streptomyces*, A co-operative study. Appl. Microbiol., 9: 55-60.
Goodfellow M, Williams ST (1983). Ecology of actinomycetes. Ann. Rev. Microbiol. 37: 189-216.
Goodfellow M, Simpson KE (1987). Ecology of streptomycetes. Frontiers Appl. Microbiol., 2: 97-125.
Holding AJ, Collee JG (1971). Routine Biochemical tests. Methods Microbiol., 6A: 1-31.
Hopwood DA (1999). Forty years of genetics with *Streptomyces*: from *in vivo* through *in vitro* to *in silico*. Microbiol., 145: 2183-2202.
Joo GJ (2005). Production of an anti-fungal substance for biological control of *Phytophthora capsici* causing phytophthora blight in red-peppers by *Streptomyces halstedii*. Biotechnol. Lett., 27: 201-5.
Ismet A (2003). Biological, molecular and chemical characterisation of a selected antagonistic *Micromonospora* sp. isolated from mangrove rhizosphere. Ph.D. thesis. Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia.
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.
Ismet A, Paramaswari S, Vikineswary S (2002). Diversity of *Micromonospora* in Malaysian mangrove rhizosphere soil. Malaysian J. Sci., 21: 51-59.
Kortemaa H, Rita H, Haahtela K, Smolander A (1994). Root coolonization ability of antagonistic *Streptomyces griseoviridis*. Plant Soil, 163: 77-83.
Lewis JA, Starkey RL (1969). Decomposition of plant tannins by some soil micro-organisms. Soil Sci., 107: 235-241.
Lee JY, Hwang BK (2002). Diversity of antifungal actinomycetes in varios vegetative soils of Korea. Can. J. Microbiol., 48: 407-417.
McCarthy AJ Williams ST (1992). Actinomycetes as agents of biodegration in the enviroment - a review. Gene, 115: 189-192.
Mullen MD (2004).Transformations of phosphorus and other elements. In *Principles and applications of soil microbiology* (ed. D. M. Sylvia, J. J. Fuhrmann, P. G. Hartel and D. A. Zuberer), pp. 369-386. Prentice Hall, New Jersey.
Mukherjee G, Sen SK (2006). Purification, Characterization, and antifungal activity of chitinase from *Streptomyces venezuelae* P_{10}. Curr Microbiol. 53: 265-9
Ndonde MJM, Semu E (2000). Preliminary characterization of Streptomyces species from Tanzanian soils and their antimicrobial potential against selected plant and animal pathogenic bacteria. World J. Microbiol. Biotechnol., 7: 595-599.
Ouhdouch Y, Barakate M, Finance C (2001). Actinomycetes of Moroccan habitats: Isolation and screening for antifungal activities. Eur. J. Soil Biol., 37: 69-74.
Perveen K, Haseeb A, Shukla PK (2007). Management of *Sclerotinia sclerotiorum* on *Mentha arvensis* cv. Gomti. J. Mycol. Plant Pathol., 37: 33-36.
Prapagdee B, Kuekulvong C, Mongkolsuk S (2008). Antifungal Potential of Extracellular Metabolites Produced by *Streptomyces hygroscopicus* against Phytopathogenic Fungi. Int. J. Biol. Sci., 4: 330-337.
Pridham TG, Gottlieb D (1948). The utilization of carbon compounds by some actinomycetales as an aid for species determination. J. Bacteriol., 56: 107-114.
Pridham TG, Hesseltine CW, Benedict RG (1958). A guide for the classification of Streptomyces according to selected groups. Appl. Microbiol. 6: 52-79.
Rizk M, Rahman TA, Metwally H (2007). Screening of antagonistic activityin different *Streptomyces* species against some pathogenic microorganisms. J. Biol. Sci., 7: 1418-1423.
Shirling EB, Gottlieb D (1966). Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol., 16: 313-340.
Singh PP, Shin YC, Park CS, Chung YR (1999). Biological control of *Fusarium* wilt of cucumber by chitinolytic bacteria. Phytopathology. 89: 92-9.
Tahvonen R, Avikainen H (1987). The biological control of seedborne *Alternaria brassicicola* of cruciferous plants with a powdery preparation of *Streptomyces* sp. J. Agric. Sci. Finland. 59: 199-208.
Tanaka Y. and Omura, S. 1993. Agroactive compounds of microbial origin. Ann. Rev. Microbiol., 47: 57-87.
Tokala RK, Strap JL, Jung CM, Crawford DL, Hamby Salove M, Deobald LA, Bailey JF, Morra MJ (2002). Novel plant-microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC-108 and the pea plant (Pisum sativum). Appl. Environ. Microbiol., 68: 2161-2171.
Tresner HD, Hayes JA, Backns EJ (1968). Differential tolerance of *Streptomyces* to sodium chloride as a taxonomic aid. Appl. Microbiol., 16: 1134-1136.
Valois D, Fayad K, Barasubiye T, Garon M, Dery C, Brzezinski R, Beaulieu C (1996). Glucanolytic actinomycetes antagonistic to *Phytophthora fragariae* var. *rubi*, the causal agent of raspberry root rot. Appl. Environ. Microbiol., 62: 1630-5.
Vikineswary S, Ismet A, Paramaswari S, Thong KL (2003). Rare actinomycetes in mangrove soil and leaf litter. J. Biosciences. 14: 105-109.
Williams ST, Cross T (1971). Isolation, purification, cultivation and preservation of actinomycetes. Methods Microbiol., 4: 295-334.
Williams ST, Good fellow M, Wellington EMH, Vickers JC, Alderson G, Sneath PHA, Sackin MJ, Mortimer AM (1983). A probability matrix for identification of *Streptomyces*. J. Gen. Microbiol., 129: 1815-1830.
Xiao K, Kinkel LL, Samac DA (2002). Biological control of *Phytophthora* root rots on alfalfa and soybean with *Streptomyces*. Biol. Control, 23: 285-95.
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.