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Biological Control of *Sclerotinia sclerotiorum* Using Indigenous Chitinolytic Actinomycetes in Jordan

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The white cottony stem rot pathogen *Sclerotinia sclerotiorum* was subjected to 70 different isolates of actinomycetes indigenous to Jordan as biological control agents. Forty of them demonstrated chitinase activity on crab shell chitin agar (CCA) media and they were segregated into three groups: 14 highly active, 12 moderately active, and 14 with low activity, with average clearing zones of (4.7-8.3), (3.7-4.3), and (2.3-3.3) mm surrounding colonies on CCA, respectively. Further, these isolates were able to inhibit radial mycelium growth of the pathogen and were categorized into three antagonistic groups: 13 strong, 13 moderate, and 14 weak antagonists, with antibiosis inhibition zones of (32.0-45.7), (22.7-31.3), and (3.7-22.3) mm, respectively. High levels of chitinase activity of the isolates Ma3 (8.3 mm), Ju1 (7.7 mm), and Sa8 (7.7 mm) with their antagonistic activity against mycelium growth of 45.7, 44.3, and 40.7 mm were observed, respectively. These isolates exhibited fungicidal activity against sclerotia of *S. sclerotiorum*. On the other hand, isolates Na5, Aj3, and Aj2 that produced no chitinase showed fungistatic effect only.

Keywords : Actinomycetes, Biological control, Chitinase, Sclerotia, *Sclerotinia sclerotiorum*

White cottony stem rot is caused by the fungus *Sclerotinia sclerotiorum* (Lib) de Bary. The fungus is world wide distributed and attacks a wide range of vegetables and field crops (Lumsden, 1979). The fungus also frequently causes serious and unpredictable yield losses to many economically important crops (Subbarao, 1998). The most obvious signs of the disease are the appearance of a white fluffy mycelial growth on the infected plant parts, which later develop dark sclerotia on the surface and inside of the infected plant parts (Osoofe et al., 2005). Sclerotia are resistant to heat, drought, and fungicides with high ability to remain viable in soil for many years (Purdy, 1979). These sclerotia can germinate carpogenically producing one to several cup-shaped apothecia which eject ascospores that become air-borne and serve as secondary inoculum for new infections. In addition, sclerotia can germinate by producing a mass of hyphae that also can cause infection (Purdy, 1979; Patterson and Grogan, 1985). More than 30 species of fungi and bacteria have been implicated by various workers as antagonists for *S. sclerotiorum* (Adams and Ayers, 1979). Chitinase-producing organisms could be used directly in biological control of microorganisms or indirectly using their purified protein or through gene manipulation (Gupta et al., 1995; Singh et al., 1999). Two chitinolytic bacteria (*Paenibacillus* spp. and *Streptomyces* spp.) were reported to suppress Fusarium wilt of cucumber (Singh, 1999). *Serratia marcescens* was also found to be an effective chitinolytic biocontrol agent against *Sclerotium rolfsii* through its chytinolytic culture filtrate (Ordentlich, 1988). *Streptomyces viridificans* was found to be a good chitinase producer, and its crude and purified enzyme has potential cell wall lysis of many fungal pathogens (Gupta, 1995). Several *Streptomyces* and non-*Streptomyces* bacteria are well known as producers of chitinase and exercising antagonistic potential against *Sclerotinia minor*, the pathogen of the basal drop of lettuce. Two of these isolates; *S. viridodiasticus* and *Mictomonospora carbonacea* are known to produce a high level of chitinase and thus significantly reduced the growth of *S. minor in vitro*, and reduced the incidence of the disease under controlled greenhouse conditions (El-Tarabily et al., 2000). The present research is aimed to isolate and characterize indigenous *Streptomycetes* capable of producing chitinase. Therefore, we screened antagonistic *Streptomyces* with chitinase activity by measuring mycelial growth and sclerotial viability of *S. sclerotiorum*.

Materials and Methods

Isolation of the pathogen. Cultures of *S. sclerotiorum* were obtained by plating on potato dextrose agar (PDA) sclerotia collected from the stem pith of infected cucumber (*Cucumis sativus* L, var. Loona) grown inside plastic-tunnels at Kuraymah in Jordan Valley. Pure cultures of this

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pathogen were maintained on PDA slants and stored at 4°C inside the refrigerator (El-Tarabily et al., 2000). The isolated pathogen was identified at the molecular level using specific primers following the procedures reported by Freeman et al. (2002) and Osoofe et al. (2005).

Pathogenicity test. Detached petioles of cucumber (*Cucumis sativus* L. var. Reema) and celery (*Apium graveolens* L.) were used to confirm the pathogenecity of the isolated *S. sclerotiorum.* This was done according to the method described by Boland and Smith (1991) with some modifications. Surface sterilized celery petioles were inoculated with mycelium plugs of 5 mm in diameter from the margin of an actively growing *S. sclerotiorum* culture over prick point injured spots. Inoculated petiole segments were placed 10 cm apart from each other over the surface of moist sterilized sands inside a plastic tray and the tray was covered with a plastic sheet. The tray was incubated at 25°C. Prick point injured but not inoculated petiole segments in addition to petiole segments, inoculated with sterile agar plugs served as controls. Pathogenicity result was checked after three days of incubation by observing water soaked lesions at and around the inoculation points.

Isolation of *Actinomycetes* spp. from soil. Soil samples were collected from fourteen different locations in Jordan. Each soil sample was taken up from 10 cm depth below soil surface after removing the 3 cm of top soil and placed inside polyethylene bag. The bags were tightly closed and stored at 4°C in the refrigerator (Saadoun and AL-Momani, 1997). In order to remove plant debris, each soil sample was air dried, mixed to ensure uniformity, and passed through a 2-mm pore size sieve (Retsch, Haan, Germany). Enrichment of soil samples for better actinomycetes isolation was made according to the procedure described by Saadoun and Gharaibeh (2002). Three replicates of 1 g of each soil sample were air-dried, sieved and then placed inside small crucibles. Those samples were subjected to drying and heat treatment inside an oven at 45°C for 12 hrs. After that, 0.1 g of $CaCO_3$ was added and thoroughly mixed with the soil sample. Enriched soils inside crucibles were incubated inside water bath at 27°C for 7 days prior to isolation. After such period of incubation, 1 g from each enriched soil sample prepared as described above was suspended in 99 ml of sterilized distilled water and incubated inside an orbital shaker incubator at 28°C with shaking at 140 rpm for 30 min. The soil suspensions were allowed to settle at room temperature and then used to prepare a series of further dilutions up to 10^{-4}. Aliquots of 0.1 ml from each dilution were spread on the surface of Starch-Casein Nitrate Agar (SCNA) media amended with cyclohexamide (50 μg/ml) using sterilized L-shaped glass rod. Inoculated plates incubated at 27°C for 10 days. Colonies of actinomycetes were carefully picked up by sterilized loop and streaked on fresh SCNA plates to obtain pure cultures. Pure actinomycetes isolates were maintained and stored on SCNA slants at 4°C inside a refrigerator.

Determination of chitinase activity of the Actinomycetes isolates. The isolated actinomycetes were screened for their chitinase activity. Three loop-full of 7-days old cultures of each isolate was transferred into sterile vials containing 2 ml of semi-solid SCNA (1g agar/L), and gently shaken for uniformity. Aliquot of 25 μl from each of the isolate suspension was placed as a single drop in the center of a colloidal chitin agar (CCA) plates (Hus and Lockwood 1975). For each isolate, three plates were used as replicates. Inoculated CCA plates were incubated at 28°C for 10 days. The size of the clear zone around each drop-colony (ΔY) was calculated in terms of the difference between the whole diameter of the clear zone including the diameter of the drop-colony (Y_2) and the diameter of the drop colony it self (Y_1), according the equation $\Delta Y=Y_2-Y_1$.

***In vitro* bioassay of the chitinase producing Actinomycetes against *S. sclerotiorum*.** Isolates with positive chitinase-activity were extensively screened for their antagonistic potential against the pathogen *S. sclerotiorum* as described by El-Tarabily (2000). Two kinds of media (CCA and SCNA) were used to test the potential of these isolates to antagonize *S. sclerotiorum in vitro.* Such isolates were streak-inoculated on one side of the CCA and SCNA plates and incubated at 28°C for 7 days. After such period of incubation, a disc (6 mm in diameter) of actively growing *S. sclerotiorum* was transferred onto the opposite side of the actinomycetes culture plates approximately 25 mm away from the bacterial growth. Plates were incubated at 25°C for 5 days. Control plates contained discs of *S. sclerotiorum* mycelial growth only. For each isolate, three plates were used as three replicates. The magnitude of the antagonistic activity for each isolate was evaluated in terms of the distance the fungus grew (X_1) toward the colony of the actinomycetes in contrast to the distance of the fungal growth when it is present alone (X_2), according to the equation of $\Delta X=X_2-X_1$. Positive chitinase producing actinomycetes were weighed for comparison and segregation according to their degree of antagonistic activity against *S. sclerotiorum.* The three most antagonistic actinomycetes isolates (Ma3, Ju1, and Sa8) were characterized morphologically following instructions given by the International *Streptomyces* Project (ISP) according to Shirling and Gottlieb (1964).

The capability of the chitinolytic isolates of Actinomycetes to inhibit sclerotia germination of *S. sclerotiorum*.

The three active isolates; Ma3, Ju1, and Sa8 including the non-chitinolytic control isolates Na5, AJ3, and AJ2 were evaluated for their potential to inhibit the sclerotia viability under laboratory conditions. These isolates were streak-inoculated on one side of the SCNA plates and incubated at 28°C. After twenty one days, surface sterilized sclerotia of *S. sclerotiorum* were placed on the opposite side of the actinomycetes culture within the plates. Plates were incubated at 25°C for 7 days. Control plates contained sclerotia of *S. sclerotiorum* only. For each treatment, ten plates were used as ten replicates and these were arranged using a completely randomised design (CRD). The viability of sclerotia in term of their germination was assessed using an arbitrary rating scale of 0 to 3 where; 0 = no germination, 1 = barely germinated (sclerotia giving rise to a very short hyphae on its structure only), 2 = slightly germinated (sclerotia gave a short hyphae around it), 3 = germinated (sclerotia giving dense fungal mycelia covering the whole plate).

In order to check the viability of sclerotia in the post exposure to actinomycetes, all tested and control sclerotia were transferred to a SCNA medium to determine if the antagonists have a fungicidal effect.

Data analysis. Data were statistically analyzed using analysis of variance (ANOVA) procedure in the MSTATC program (Michigan State University, MI, USA). Probability of significance was used to indicate significant difference among treatment means. LSD test (P=0.05) was used to compare means.

Results

Pathogenicity test. Water soaked lesions that become covered with dense white mycelium growth, developed at the inoculation points with *S. sclerotiorum* on cucumber and celery petioles three days after inoculation. Whereas petiole segments in the control treatments, prick point injured but not inoculated, and inoculated with only sterile agar plugs exhibited no disease symptoms and signs throughout the same period of inoculation.

Isolation of the Actinomycetes and their chitinase activity. Seventy different isolates of actinomycetes were isolated from fourteen different locations in northern Jordan (Table 1). Forty out of the 70 actinomycetes isolates demonstrated chitinase activity. These isolates produced clear zones on colloidal chitin media ranging from 2.0-8.3 mm. According to this chytinolytic activity, they were grouped into three groups: 14 isolates with high chitinase activity (4.7-8.0 mm), 12 isolates with moderate activity (3.7-4.3 mm) and, 14 isolates with low activity (2.3-3.3 mm of clear zone) (Table 2). Out of the 40 isolates, the most active isolates were; Ma3, Ju1, and Sa8, with a clearing zone of 8.3, 7.7, and 7.7 mm, respectively (Fig. 1).

Table 1. Sampling locations, geographical parameters and number of isolated actinomycetes

Location			Actinomycete isolates, abbreviated locations	Number of isolates
Name	Latitude	Longitude		
Aydun	32°19'N	36°10'E	Ay1, Ay2	2
Samad	32°28'N	35°50'E	Sa1-Sa8	8
Jumhah	32°33'N	35°47'E	Ju1-Ju4	4
Natifah	32°31'N	35°50'E	Na1-Na5	5
Jarash	32°17'N	35°54'E	Ja1-Ja9	9
Kafryuba	32°33'N	35°48'E	Ky1, Ky2	2
Kuraymah	32°16'N	35°36'E	Ku1-Ku5	5
Marw	32°37'N	35°53'E	Ma1-Ma4	4
Almazar	31°04'N	35°42'E	Mz1-Mz5	5
Ajlun	32°20'N	35°45'E	Aj1-Aj6	6
Al'al	32°38'N	35°54'E	Al1-Al3	3
Ham	32°31'N	35°49'E	Ha1-Ha4	4
Harima	32°38'N	35°53'E	Hm1-Hm9	9
Shek hussein	32°30'N	35°35'E	Sh1-Sh4	4
Total				70

***In vitro* effects of the chitinase producing Actinomycetes on the mycelial growth of *S. sclerotiorum*.** All of the fourty chytinolytic isolates showed antagonistic potential against the mycelial growth of *S. sclerotiorum, in vitro.* Such activity ranged from 3.7-45.7 mm of ΔX (the degree of antagonistic activity of each isolates). According to this antagonistic potential, isolates were grouped into three groups: 13 strong (Ant1), 13 moderate (Ant2), and 14 low (Ant3) antagonists inflecting reduction in the radial growth of the fungus hyphae equals to 32-45.6, 22.6-31.3, and 3.6-22.3 mm, respectively (Table 3) (Fig 2). The most active isolates, Ma3, Ju1, and Sa8, gave ΔX equals to 45.7, 44.3, and 40.7 mm respectively. These isolates were used in further biological control experiment.

Morphological characterization of the Actinomycetes isolates Ma3, Ju1, and Sa8. On Oat Meal Agar (OMA) medium, the isolates Ma3, Ju1, and Sa8 showed green color of their aerial mass growth. The substrate side of the mycelium (reverse side) of Ma3 and Sa8 exhibited distinctive pigmentation, whereas isolate Ju1 showed non-distinctive reverse side pigmentation. Unlike isolate Ju1, isolates Ma3 and Sa8 produced diffusible pigments. However, none of those three active isolates were considered to be a producer of melanin. The spore chain arrangement of the

Table 2. Chitinase activity of the 70 actinomycetes isolates collected from different locations in northern Jordan

Location	Degree of chitinase activity (Δy[b])			
	Group 1 High activity (ΔY=4.7-8.0 mm)	Group 2 Moderate activity (ΔY=3.7-4.3 mm)	Group 3 Low activity (ΔY=2.3-3.3 mm)	Group 4 No activity (ΔY=0.0 mm)
Ajlun	–[a]	Aj5	Aj6, Aj1	Aj2, Aj3, Aj4
Al'al	Al1, Al2	Al3	–	–
Aydun	–	–	–	Ay1, ay2
Ham	–	Ha2	Ha1	Ha3, Ha4
Harima	Hm8, Hm2, Hm5	–	Hm6, Hm1, Hm9	Hm3, Hm4, Hm7
Jarash	Ja7, Ja3	Ja8	Ja4	Ja1, Ja2, Ja5, Ja6, Ja9
Jumhah	Ju1, Ju4	–	Ju2	Ju3
Kuraymah	Ku4	Ku2	Ku3	Ku1, Ku5
Kafr yuba	–	–	Ky2	Ky1
Marw	Ma3	Ma4	Ma2	Ma1
Almazar	Mz5	Mz2, Mz3	Mz4	Mz1
Natifah	–	Na2	Na4	Na1, Na3, Na5
Samad	Sa8	Sa7, Sa6	Sa2	Sa1, Sa3, Sa4, Sa5
Shekh Hussein	Sh1	Sh2	–	Sh3, Sh4
Total	14 (20%)	12 (17.14%)	14 (20%)	30 (42.86%)

[a]indicates that no. isolate of that location fell in the respective group.
[b]ΔY indicates the size of the clear zone around each drop-colony of the actinomycetes isolates. ΔY value is the mean of three are replicates on colloidal chitin agar (CCA) plates representing the degree of chitinase activity.

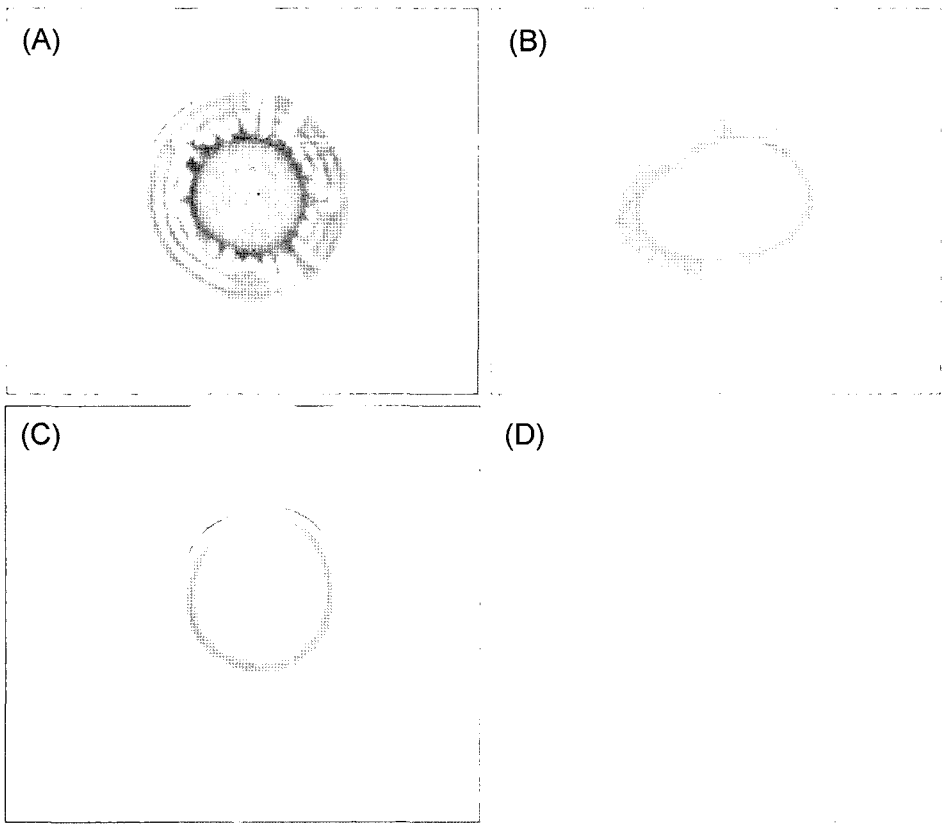

Fig. 1. Colony growth of the three actinomycetes isolates and a non-active isolate on CCA media showing a clear zone around colonies as an indicator of chitinolytic activity. A, isolate Ma3; B, Ju1; C, Sa8; D, the non active isolate Na5.

Table 3. Antagonistic activity of the 40 chytinolytic actinomycetes isolates against *S. sclerotiorum*

Location	Antagonistic activity (ΔX[a])		
	Group 1 High activity (ΔX=32.0-45.6 mm)	Group 2 Moderate activity (ΔX=22.6-31.3 mm)	Group 3 Low activity (ΔX=3.6-22.3 mm)
Ajlun	–[b]	Aj5	Aj6, Aj1
Al'al	Al1, Al2	–	Al3
Aydun	–	–	–
Ham	–	Ha2	Ha1
Harima	Hm8, Hm2, Hm5	–	Hm6, Hm1, Hm9
Jarash	Ja7, Ja3	Ja8, Ja4	–
Jumhah	Ju1, Ju4	–	Ju2
Kuraymah	–	Ku4, Ku2	Ku3
Kafr yuba	–	–	Ky2
Marw	Ma3, Ma4	Ma2	—
Almazar	Mz5	Mz2, Mz3	Mz4
Natifah	–	Na2	Na4
Samad	Sa8	Sa7, Sa6	Sa2
Shekh Hussein	–	Sh1	Sh2
Total	13 (32.5%)	13 (32.5%)	14 (35%)

[a]ΔX indicates the degree of antagonistic activity of each isolate of the actinomycetes isolates.
[b]indicates that no. isolate of that location fell in the promising group.
[c]ΔX values are means of three replicates of SCNA plates which indicates the degree of antagonistic activity of each isolates as represented by inhibition of linear growth on the mycelia of *S. clerotiorum*.

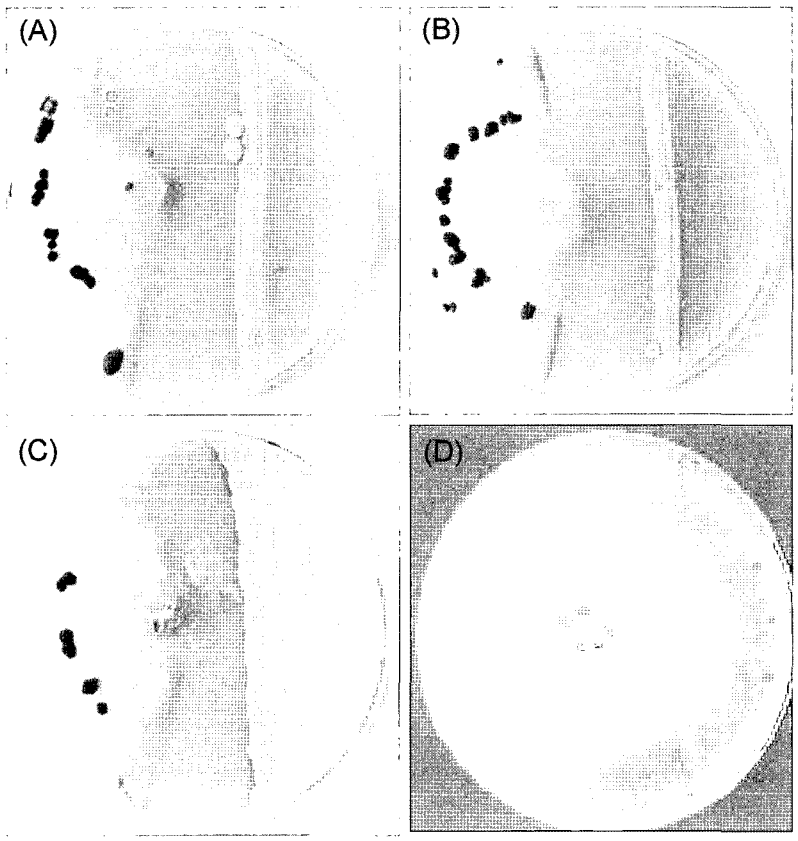

Fig. 2. Inhibition of *S. sclerotiorum* growth (grown on the left) by various actinomycetes isolates (grown on the right). A, Ma3 isolate; B, Ju1 isolate; C, Sa8 isolate; D, a culture plate of *S. sclerotiorum* alone as control.

Table 4. Morphological characterization of the actinomycetes isolates Ma3, Jul, and Sa8

Isolate	Morphological Characterization				
	Aerial mass color	Reverse side color	Diffusible pigments[a]	Melanin production	Spore chain morphology[b]
Ma3	Green	Distinctive	1	–	RF
Jul	Green	Non-Distinctive	0	–	RF
Sa8	Green	Distinctive	1	–	RA

[a]1, Diffusible pigment; 0, None.
[b]RF, Rectiflexibiles; RA, Retinaculum apertum.

Table 5. Effect of actinomycetes isolates on the sclerotia germination of *S. sclerotiorum.*

Sclerotia exposure to Actinomycetes isolates	Sclerotia Germination			
	During Exposure[a]	During Exposure (%)[b]	Post Exposure[a]	Post Exposure (%)[b]
No bacteria / control	3.0 a	100.0 a	3.0 a	100.0 a
Chitinolytic isolates				
Ma3	0.0 d	0.00 d	0.0 b	0.00 b
Jul	0.2 d	6.67 d	0.0 b	0.00 b
Sa8	0.1 d	3.33 d	0.0 b	0.00 b
Non-chitinolytic isolates				
Na5	0.7 c	32.33 c	3.0 a	100.0 a
Aj3	1.4 b	46.67 b	3.0 a	100.0 a
Aj2	1.3 b	43.33 b	3.0 a	100.0 a

[a]Sclerotia germination on a scale of 0-3, where 0=no germination. 1=slightly germination, sclerotia giving light mycelia on their structure. 2=Germination of the sclerotia giving fungal mycelia grow around their structure. 3=Germination of the sclerotia giving fungal mycelia grow dense, and covered the whole plate.
[b]Percentage data of sclerotia germination during and post exposure to actinomycetes isolates. Results are means of 10 plates as 10 replicates for each treatment. Values followed by the same letter are not significantly ($P < 0.05$) different according to LSD test.

isolates Ma3 and Jul showed Rectiflexibiles {RF} (that means it has both Rectus {R} and Flexibilus {F} spore chain form), whereas Sa8 showed Retinaculum-Apertum {RA} that means it has an open loop spore chain form (Table 4).

Effects of the Actinomycetes isolates on germination of *S. sclerotiorum* sclerotia. The three chitinolytic isolates (Ma3, Jul, and Sa8) as well as the none chitinolytic isolates (Na5, Aj3, Aj2) significantly inhibited sclerotia germination of *S. sclerotiorum* to different levels compared to the treatment containing the sclerotia alone. However, the chitinolytic actinomycetes isolates significantly inhibited the germination of *S. sclerotiorum* sclerotia more than non-chitinolytic isolates (Na5, Aj3, and Aj2) (Table 5; Fig. 3). The sclerotia of the control treatment and the sclerotia of treatment with the non-chitinolytic actinomycetes were germinated after they were transferred into SCNA medium, giving mycelia which coveted the plates. On the other hand, the sclerotia treated with the chitinolytic actinomycetes did not germinate after they were transferred into SCNA medium.

Discussion

The significance of their indigenous genetic resources has been long recognized by scientists dealing with living organisms. In one aspect of application, it is important to search novel and beneficial organisms for the sustainability of human life and their environment. Plant pathogens and their control were of great concern to human societies, specifically as they often resulted in wide burst in the use of pesticides. Hence, biological control of plant diseases has gained wider attention and have raised good hope and anticipation into a safe and environment friendly alternative to pesticides. White cottony stem rot disease of cucurbits caused by *S. sclerotiorum,* is a problem of increasing concern to farmers and growers in the Jordan Valley. Therefore, it becomes very timely to lunch intensive efforts in searching for promising biological control agents against this disease.

The present investigation evaluated the potential of actinomycetes isolated from different soils in Jordan to control white cottony stem rot disease. This study dealt with chitinase-producing actinomycetes as a promising

mechanism that could be utilized as biological control agents, because chitin is a major constituent of the *S. sclerotiorum* cell wall (Hus and Lockwood, 1975). Therefore, production of this enzyme was used as the main criterion for selection of potential biocontrol agents against this phytopathogen (El-Tarabily et al., 2000; Hus and Lockwood, 1975).

The significance of chitinase producing indigenous actinomycetes in the biological control process was demonstrated through several procedures to ensure their potential to inhibit mycelium growth and sclerotia germination of that pathogen, as well as to reduce the incidence and severity of this disease. Forty isolates out of the seventy indigenous actinomycetes isolates were chitinase producers and were able to inhibit the mycelial growth of the fungus on a SCNA medium. It was also demonstrated that isolates with high levels of chitinase production are more antagonistic to the fungus compared with the lower chitinase producers, indicating the effectiveness of chitinase production by those bio-control agents.

This finding confirms earlier findings by El-Tarabily et al. (2000) who reported such activity against *S. minor in vitro*, as well as those of Gupta et al. (1995) against several phyto-pathogenic fungi and Saadoun et al. (2000) against several food-associated fungi and molds. This confirms importance of the chitinase producing *Streptomyces* isolates as biocontrol agents, and also emphasizes the importance of indigenous *Streptomyces* spp. as biocontrol agents against this fungus and probably several other fungal phytopathogens.

In the present study, it was noted that the pathogen *S. sclerotiorum* was not able to grow on CCA medium because it present no available carbon source for this fungus. However, SCNA medium appears to be more suitable for screening of chitinase producer isolates in their antagonistic potential against *S. sclerotiorum* since both of the fungus and antagonistic bacteria were able to grow on this media. Such finding was necessary adjustment to what was reported by El-Tarabily et al. (2000) who used CCA medium only for such antagonistic assay.

High levels of chitinase activity were corresponded with high levels of antagonistic activity against mycelium growth as will as a fungicidal effect against sclerotia of the isolates Ma3, Ju1, and Sa8 *in vitro*. This confirms the effectiveness of chitinase as a fungicidal mechanism against sclerotia germination as well as against the mycelial growth of *S sclerotiorum*. The non-chitinase producers; Na5, Aj3, and Aj2, on the other hand, showed a fungistatic inhibition of sclerotia germination only. This can be explained by the fact that there are other inhibitory mechanisms that may be produced by those non-chitinolytic isolates and may have been involved in the inhibition of sclerotia germination with no fungicidal effect. Hence, the non-chitinase producers and weak chitinase producers should not be overlooked as some may be good biological control agents against some other plant pathogenic fungi. The present investigation emphasizes the potential of chitinase producing microorganisms as promising biocontrol agents of fungal plant pathogens with chitinous cell wall. Biotechnology work and gene transfer could open the door into transgenic organisms more effective and practical to be used in plant disease control. Field trials are now in progress to further evaluate this potential.

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References

Adams, P. and Ayers, W. 1979. Ecology of *Sclerotinia* species. *Phytopathology* 69:895-898.

Boland, G. and Smith, E. 1991. Variation in cultural morphology and virulence among protoplast regenerated isolates of *Sclerotinia sclerotiorum*. *Phytopathology* 81:766-770.

El-Tarabily, K., Soliman, M., Nassar, A., Al-Hassani, H., Sivasithamparam, K., McKenna, F. and Hardy, GE StJ. 2000. Biological control of *Sclerotinia minor* using a chitinolytic bacterium and actinomycetes. *Plant Pathol.* 49:573-583.

Freeman, J., Ward, E., Carmen, C. and Alastair, M. 2002. A polymerase chain reaction (PCR) assay for the detection of inoculum of *Sclerotinia sclerotiorum*. *Eur. J. Plant Pathol.* 108:877-886.

Gupta, R., Saxena, R., Chaturvedi, P. and Virdi, J. 1995. Chitinase production by *Streptomyces viridificans*: its potential in fungal cell wall lysis. *J. Appl. Bact.* 78:378-383.

Hus, S. and Lockwood, J. 1975. Powder chitin agar as a selective medium for enumeration of actinomycetes in water and soil. *Appl. Microbiol.* 29:422-426.

Lumsden, R. 1979. Histology and physiology of pathogenesis in plant diseases caused by *Sclerotinia* spp. *Phytopathology* 69: 890-895.

Ordentlich, A., Elad, Y. and Chet, I. 1988. The role of chitinase of *Serratia marcescens* in biological control of *Sclerotium rolfsii*. *Phytopathology* 78:84-88.

Osofee, H., Hameed, K. and Mahasneh, A. 2005. Relatedness among indigenous members of *Sclerotinia sclerotiorum* by mycelial compatibility and RAPD analysis in the Jordan Valley. *Plant Pathol. J.* 21:106-107.

Patterson, C. and Grogan, R. 1985. Differences in epidemiology and control of lettuce drop caused by *Sclerotinia minor* and

Sclerotinia sclerotiorum. Plant Dis. 69:766-770.

Purdy, L. 1979. *Sclerotinia sclerotiorum*: History, diseases and Symptomology, host range, geographic distribution, and impact. *Phytopathology* 69:875-880.

Saadoun, I. and AL-Momani, F. 1997. *Streptomyces* from Jordan soils active against *Agrobacterium tumefaciens*. *Actinomycetes* 8:30-36.

Saadoun, I. and Gharaibeh, R. 2002. The *Streptomyces* flora of Jordan and its potential as a source of antibiotics active against antibiotic resistant Gram-negative bacteria. *World J. Microbial Biotech.* 18:465-470.

Saadoun, I., Hameed, K., AL-Momani, F., Malkawi, H., Meqdam, M. and Mohammad, M. 2000. Characterization and analysis of antifungal activity of soil *Streptomycetes* isolated from north Jordan. *Egypt. J. Microbiol.* 35:463-471.

Shirling, E. B. and Gottlieb, D. 1964. Methods for characterization of *Streptomyces* species. *Inter. J. Syst. Bacteriol.* 16:313-340.

Singh, P., Shin, Y., Park, C. and Chung, Y. 1999. Biological control of Fusarium wilt of cucumber by chitinolytic bacteria. *Phytopathology* 89:92-99.

Subbarao, K. 1998. Progress toward integrated management of lettuce drop. *Plant Dis.* 82:1068-1078.