African Journal of Biotechnology Vol. 10(30), pp. 5785-5794, 27 June, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2017
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Biological control of *Sclerotinia sclerotiorum* (oilseed rape isolate) by an effective antagonist *Streptomyces*

A. Baharlouei[1], G. R. Sharifi-Sirchi[2]* and G. H. Shahidi Bonjar[3]

[1]Gradutate student of Department of Agricultural Biotechnology, College of Agricultural Sciences, Shahid Bahonar University of Kerman, Iran.

[2]Department of Agricultural Biotechnology, College of Agricultural Sciences, Shahid Bahonar University of Kerman, Iran.

[3]Department of Plant Pathology, College of Agricultural Sciences, Shahid Bahonar University of Kerman, Iran.

Accepted 2 May, 2011

***Sclerotinia sclerotiorum* (Lib) De Bary, the causal agent of stem rot of oilseed rape, is one of the most important phytopathogens. In order to find appropriate biocontrol agents, antagonistic and especially chitinolytical activities of 110 soil actinomycetes were examined. Among assayed isolates, *Streptomyces* sp. isolate 422 significantly reduced ($P \leq 0.01$) the incidence of disease. Biological and physiological analysis of this isolate shown that, its active metabolite had polar nature, long shelf life and fungicidal activity features. Moreover, a partial chitinase (600 bp) gene was cloned and sequenced. This study has tried to introduce an effective biocontrol agent against *S. sclerotiorum* with a broad spectrum of antagonistic activity against phytopathogens.**

Key words: Biocontrol, *Brassica napus* L. ssp. *Oleifera*, *Sclerotinia sclerotiorum*, streptomycetes.

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) De Bary is a necrotrophic fungal pathogen with broad ecological distribution (Abawi and Grogan, 1979). Three important characters; wide host range (64 families, 225 genera and 361 species of plants), high potential virulence and long time survival of the sclerotia in an unfavorable environmental conditions introduces it as one of the most destructive phytopathogens throughout the world (Boland and Hall, 1994; Purdy, 1979). Losses of this fungus on oilseed rape (*Brassica napus* L.), sunflower (*Helianthus annuus* L.) and soybean (*Glycine max* (L.) Merr.) was estimated to be more than U.S. $ 60×10^6 (Lu, 2003). On oilseed rape, stem lesions develops from the soil line or axils of branches or leaves. It can be extended to girdle the stem and eventually to kill the plant (Nyvall, 1979). Because of significant economic damages on important crops, attempt to control this pathogen has received considerable attention.

Due to the absence of resistant varieties in the stated crops, developing appearance of resistance in *S. sclerotiorum*'s population to commonly used fungicides is increasing public concern regarding environmental contamination with fungicidal residues. Control of sclerotinia diseases were interested as an acting substitute to other controlling methods. Biocontrol via antagonists such as *Epicoccum purpurascens* (Zhou and Reeleder, 1989) and *Bacillus cereus* (Huang et al., 1993) against this pathogen was reported previously. The Gram-positive bacteria in the genus *Streptomyces* have potentially proper biological control agents against soil-borne plant fungi. Production and secretion of hydrolytic enzymes such as chitinase is a major factor in their antagonistic activity (Trejo-Estrada et al., 1998). Chitinases hydrolyze the β, 1-4 linkages in chitin chain. Chitin, an insoluble polymer of N-acetylglucosamine, is the second most abundant biopolymer in nature which is mainly found in arthropod exoskeletons, fungal cell walls and nematode egg shells (Merzendorfer and Zimoch, 2003).

This study investigated the biocontrol of *S. sclerotiorum* by *Streptomyces* isolates based on chitinolytic activity and characterize the morphological, physiological and

*Corresponding author. E-mail: sharifi-sirchi@mail.uk.ac.ir. Tel: 0098-341-3202638. Fax: 0098-341-3222043.

molecular features of the most effective biocontrol agent.

MATERIALS AND METHODS

Fungi, bacterial strain and vector

Escherichia coli, XL1blue and plasmid pTZ57R/T were provided from Cinagen Co., Iran and Fermentas Co., Germany, as bacterial host strain and vector in cloning experiments, respectively. Pure culture of *S. sclerotiorum* (oilseed rape isolate) obtained from Dr. N. Safaie, Department of plant phytopathology, University of Tarbiat Modares, Iran.

Culture media

Casein glycerol agar (CGA) was prepared from basic ingredients as described by Kuster and Williams (1964) and used as streptomycetes growth medium. Excluding the agar from CGA, casein glycerol (CG) medium was applied for streptomycetes submerged cultures.

The minimal chitin agar medium (MCA) (colloidal chitin 0.4%, KH_2PO_4 1.72 mM, K_2HPO_4 5.14 mM, $MgSO_4 \cdot 7H_2O$ 2mM, $FeSO_4 \cdot 7H_2O$ 0.035 mM, $ZnSO_4 \cdot 7H_2O$ 3.4 µM, $MnCl_2 \cdot 4H_2O$ 5 µM and agar 1.8%, pH was adjusted to 8 to 8.5) was used to evaluate chitinase activity of *Streptomyces* strains as had been described by Hsu and Lockwood (1975). The fungus was grown on potato dextrose agar (PDA, Difco) at 25°C.

Greenhouse pathogenicity test

To prove the pathogenicity of the fungus, 6 week-old oilseed rape *plants (Brassica napus* L. ssp. *oleifera* cv. Opera) were inoculated with a 5 mm diameter colonized PDA disk by *S. sclerotiorum* (oilseed rape isolate) which was placed in the wounds made in the basal stem with a sterile scalpel. Control plants were treated with the agar disk that did not contain mycelium. Then, plants were covered with a plastic bag for 72 h to maintain high humidity. After that, the bags were removed and the plants were maintained in a growth chamber at 25°C with a 12 h photoperiod and 75% relative humidity. Pathogenicity tests were repeated three times. The fungus was reisolated from all inoculated plants, confirming Koch's postulates (Tziros et al., 2008).

Soil sampling and isolation of actinomycetes

Soil samples were randomly collected from grasslands, orchards and vegetable fields in different places of Kerman province, Iran. Soil samples were taken from a depth of 10 to 20 cm below the soil surface using an open-end soil borer (20 cm in depth, 2.5 cm in diameter) as described by Lee and Hwang (2002). After air-drying at room temperature for 7 to 10 days, soil samples were sieved with a 0.8 mm mesh and preserved in polyethylene bags at room temperature.

Afterwards, soil samples (10 g) were brought up to final volume of 100 ml by adding sterile distilled water. The mixtures were shaken vigorously for 1 h and then, allowed to settle for 1 h. The mixture was diluted to 10^{-2}- 10^{-6} and 1 ml of aliquots of 10^{-3}- 10^{-6} soil dilutions added to 20 ml autoclaved CGA at 50°C. Plates were incubated at 28°C for up to 20 days. From day 7, Actinomycetes colonies were isolated and grown as pure cultures on CGA, for one week at 28°C and then, stored at 4°C.

In vitro antifungal assays

Agar disk method

For screening of streptomycetes with antifungal activity, each isolate was smeared on CGA medium as a single streak. From well-grown streaks, one disk (6 mm diameter) of each isolate was transferred to PDA plates, having fresh lawn cultures of fungus. Plates were then, incubated at 28°C for 4 to 6 days (Acar and Goldstein, 1996; Dhingra and Sinclair, 1995).

Dual culture bioassay

Mycelia disc plugs (6 mm diameter) from the peripheral region of 4 to 6 day old culture of *S. sclerotiorum* were placed in the center of the fresh PDA plates and incubated at 28°C for 48 h. Afterwards, one disc (6 mm diameter) of a 5 to 7 day old culture of *Streptomyces* isolates were placed at 3 cm of the center inocula of the pathogen and as control, sterile agar discs were used at similar positions in all plates. Antifungal activity was quantitatively measured using Radial Mycelia Growth (RMG) reduction calculated in relation to growth of the controls as:

$$(C - T) \div C \times 100 = \% \text{ Inhibition of RMG}$$

While, C is the RMG of the pathogen towards control disc plug and T is RMG of the pathogen towards the disc plug of *Streptomyces* isolates. The experiment was performed three times with five replicate PDA plates (Vincent, 1947).

Well diffusion method

In order to evaluate the antifungal activity of aqueous samples, wells (6×4 mm, 2 cm apart) were punctured by sterile cork borer in fresh lawn cultures at 30 mm distance from plugs of *S. sclerotiorum*. Specific concentrations of dimethyl sulfoxide were prepared in: methanol (1/1: v/v) solvent (DM solvent) and then, put it into each well. Plates were incubated at 24°C for 4 to 6 and 14 days in lawn culture method and dual culture method, respectively. Disk-plug bioactivity was determined by means of inhibitory zones (mm) from three replications in comparison with control which included DM solvent without test compounds (Aghighi et al., 2004).

Chloroform assay

Spore suspensions (approximately 10^8 spores ml^{-1}) of individual actinomycete isolates were prepared and dotted (10^{-6} L per spot) onto 15 ml starch casein agar plates, five dots per plate. Afterwards, plates were incubated at 28°C for 3 days. To kill dotted isolates and inactivate secreted enzymatic components, uncovered Petri plates were inverted over 4 ml of chloroform in a watch glass for 1 h. Then, watch glasses were removed and the plates were aerated in a fume hood for 30 min to permit evaporation of chloroform. Then, plates were overlaid with 10 ml of 1% water agar, inoculated with 100 µl of *S. sclerotiorum* spore suspensions (approximately 10^8 spores ml^{-1}) spread uniformly over the surface of the agar and incubated at 28°C for 3 days. Growth inhibition zone of each dotted isolate was measured in millimeters from the edge of the dotted colony to the edge of the cleared zone (Davelos et al., 2004).

Preparation of crude extract

Streptomyces isolates were grown in CG medium on shaker

incubator at 30°C with 130 rpm, to obtain maximum antifungal activity time, aseptically small aliquots of culture media were taken every 24 h and the activity was evaluated by well diffusion-method. To prepare crude extract, spores and mycelia were excluded from the culture by filtration at maximum activity time. The clarified sap was dried, pulverized and kept refrigerated before use (Aghighi et al., 2004).

Determination of minimum inhibitory concentrations (MIC)

To measure the MIC values, two-fold serial dilutions of 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781 and 0.390 $mgml^{-1}$ of crude extract were prepared in DMSO: MeOH solvent and assayed by well diffusion-method (Aghighi et al., 2004).

Solubility studies of active crude in organic solvents

To evaluate the relative polarity of the active component of the crude, 2 ml of different solvent systems containing H_2O, methanol, acetone and chloroform were added to 20 mg pulverized-crude samples separately and vortexed for 20 min. Each sample was then centrifuged at 3000 rpm for 15 min. Supernatants and pellets were separated, dried under reduced air at 50°C and assayed at concentration of 10 mg/ml by well diffusion-method (Ebrahimi et al., 2009).

Determination of shelf life or stability of active crude

Active crude solution with concentration 5 mg ml^{-1} were prepared in distilled water and stability of the active crude in soluble state were tested against *S. sclerotiorum* using agar diffusion-method at 15 days intervals as long as the activity persisted (Ebrahimi et al., 2009)

Detection of fungicidal and/or fungistatic activity

Small blocks of *S. sclerotiorum* mycelium was transferred near to inhibition zones (1 mm^3) of Streptomyces isolate 422 into fresh PDA plates and incubated for 7 days at 28°C. During incubation, growth or lack of growth of the fungus was investigated both visually and microscopically. Rejuvenation of growth was representative of fungistatic and lack of growth represented fungicidal properties of the antagonist (Shafii et al., 2005).

Greenhouse studies

Seeds of oilseed rape *(Brassica napus* L. ssp. *oleifera* cv. Opera) were sown in sterilized soil in pots. The pots were kept in a greenhouse (25°C) and watered as required. Tests were performed on a complete randomized plan with the repetition of ten pots for each treatment. Four treatments included as follows: (A) Streptomyces isolate no. 422, (B) *S. sclerotiorum* (C) *S. sclerotiorum* plus *Streptomyces* isolate no. 422 and (D) control. To prepare inocula, *Streptomyces* isolate no. 422 and *S. sclerotiorum* was grown on CGA and PDA media, respectively. All seedling stems were wounded by a sterile razor. However, for avoidance of wound dryness in control treatment, they received sterile wet cotton pads. Similarly, other treatments received a 2 cm^2 of the media mat of the well grown pathogen and the antagonist at the end covered with parafilm. All treated plants were covered with the transparent plastic bags for 48 h to prevent accidental dryness and then, provide high relative humidity for onset of pathogen/antagonist activities. Symptoms recorded 10 days after inoculation at 25°C. Bio control assay was performed based on the percentage of the health plants to stem rot plants.

Preparation of colloidal chitin

Twenty grams of chitin powder (Sigma Chemicals Co, USA,) was mixed with 200 ml HCl 37% (Merck, Germany) stirred for 15 h and then, centrifuged at 5000 rpm for 5 min. The resulting precipitate was washed with deionized water until the pH reached 5.0 and then neutralized by adding of 6 N NaOH. The suspension was centrifuged and washed with deionized water for desalting. And again, the resulted precipitate was suspended with deionized water to attain concentration of 1% (Sadegui et al., 2006).

Chitinase assay

Plain disks of *Streptomyces* isolates colony were inoculated to MCA plates and incubated at 28°C for two weeks. Then, chitinase activity was evaluated by observing the clear zones representative of enzymatic digestion of chitin in the vicinity of colonies of *Streptomyces* isolates (Sadegui et al., 2006).

DNA extraction

Streptomyces isolate 422 was grown for 6 days in submerged culture of CG medium in shaker incubator at 28°C and 130 rpm for 6 days. The colonies were harvested using bench centrifugation at 10000 rpm for 5 min and genomic DNA was isolated by CTAB method as described by Rogers and Bendich (Rogers and Bendich, 1988).

Polymerase chain reaction (PCR)

Four sets of primers consists of C1HB (5´-ccttgacaggtccagacca-3´ and 5´- tcagcagctcaggtt- 3); C2HB (5´–ccggccggccacga caacgc - 3´ and 5- ´gtcccagttggtcgacca- 3); C3HB(5´- cgaggcccagttcaacca-3`and 5- tcagcagtacaggtt- 3) and C4HB (5-´ctgatacttgcgctcgccg- 3´ and 5´- aggccgctgtcgatcgcgc- 3) were designed based on the known sequences from the family 18 and 19 chitinase genes of *Strepyomyces* spp. It used the FPCR package software (Microsoft visual studio 6.0, visual Basic 6.0 SP6 Company) (http://visual-basic-6-sp6.en.malavida.com/d3364-free-download-windows). The primers were synthesized in Isogene Company, Netherlands. A PCR was run which utilized melting temperature of 94°C for 5 min, following 35 cycles of 94°C for 1 min, 55°C for 45 s, 72°C for 1 min and final extension at 72°C for 5 min followed by the last cycle. Electrophoresis of samples in 1% agarose gel and photography of resolved bands performed as described by Sambrook and Russell (2001).

RNA extraction and cDNA synthesis

Isolation of RNA was performed by SDS and hot phenol method (Kelemen et al. 1996)). And also, chitinase cDNA was synthesized by Revert Aid™ first strand cDNA synthesis kit and specific primers (Fermentas, Germany).

Cloning of DNA fragment

The amplified fragment was purified with an AccuPrep® PCR purification kit (Takapouzist, Iran), ligated into the pTZ57R/T vector using InsT/A Clone™ PCR cloning kit (Fermentas, Germany).

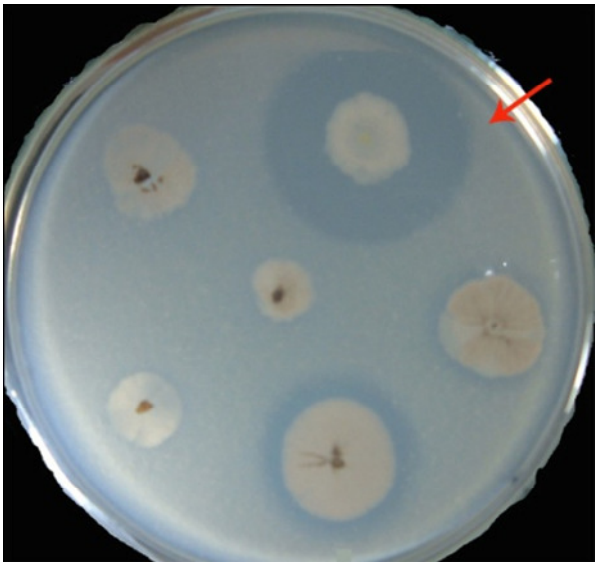

Figure 1. Chitinase activity of *Streptomyces* isolates on minimal chitin-agar media, 8 to 10 days after incubation at 28°C. Clockwise from top: *Streptomyces* isolates of 422, 398, *S. plicatus* strain 101, 387, 382 and 379 at center.

Recombinant plasmids were inserted into competent *E. coli* XL1Blue and then, bacteria were grown at 37°C in Luria-Bertani (LB) broth. For agar medium, Luria-Bertani broth was solidified with 1.5% (w/v) agar. Screening was performed based on blue-white selection method as described in Sambrook and Russell (2001) and ampicillin resistance. In addition, transformed colonies were confirmed by colony PCR and enzyme digestion. The recombinant plasmids were extracted by using AccuPrep® plasmid extraction kit (Bioneer, Korea). The plasmids digestion was performed with *Pst*1 and *Eco*R1 restriction enzymes at 37°C for 1 h.

DNA sequencing

Recombinant plasmids were sequenced in both directions by extending M13 reverse and forward primers, by Automatic DNA Sequencer (Macrogene, Korea). The sequence data were analyzed using the Chromas version 1.41 software. Nucleotide and deduced amino acid sequence of DNA fragment blasted by nucleotide and protein blast programs in the NCBI databank and deduced amino acid sequences aligned with related sequences from other isolates of *Streptomyces* by DNAMAN software package (Lynnon Biosoft, Quebec, Canada) (Kumar et al., 2001).

Statistical analyses

Antagonistic effects of *Streptomyces* isolates against *S. sclerotiorum* were analyzed based on completely randomized design (CRD) with three replications by MSTATC software. The data were transformed as Arcsine x to make the variances homogeneous and means were compared by Duncan's multiple range test at the 0.01 level at confidence.

Nucleotide sequence accession number

The GenBank accession number for partial chitinase gene of *Streptomyces* isolates No. 422 was recorded as GQ255896.

RESULTS

Actinomycete isolation and chitinase activity

One hundred and ten (110) actinomyces, were isolated from Kerman province, southeast of Iran, from which 18 isolates showed high level of chitinolytic activity in minimal chitin-agar medium (Figure 1).

Antifungal bioassays

From the high level chitinolytic isolates, 6 isolates showed strong antifungal activity against *S. sclerotiorum*. Variance analysis of antagonistic effects of *Streptomyces* isolates indicated significant difference at the 0.01 level of confidence. Also, Duncan's multiple range test showed that the *Streptomyces* isolates No. 422 had highest antagonistic activity. Therefore, the work continued on the mentioned isolate as the best biocontrol agent (Figure 2).

Chloroform assay

All of *Streptomyces* isolates having strong antifungal activity did not show this character after exposure to chloroform.

Antifungal activity of submerged culture

Activity contrary to post seeding time in submerged media cultures of *Streptomyces* isolate No. 422 is illustrated (Figure 3). Also, test result of bioactivity of *Streptomyces* isolate No 422 in well diffusion method on lawn culture of *S. sclerotiorum* at the peak day is showed in Figure 4. Maximum antifungal activity was obtained at 11 days after post seeding. Based on this result, crude extract were prepared at the maximum activity day.

Determination of MIC

In well diffusion-method, MIC of the crude was determined as 0.781 mg mL^{-1} against *S. sclerotiorum.*

Solubility of active crude in organic solvents

Solubility results of crude extract of *Streptomyces* isolate No 422 are shown in Table 1. As the results show, the active principle (s) has apparently a polar nature since activity is recoverable only in H_2O supernatants and pellets of tested organic solvent.

Shelf life or stability of active crude

Stability of the active crude in distilled water at room tem-

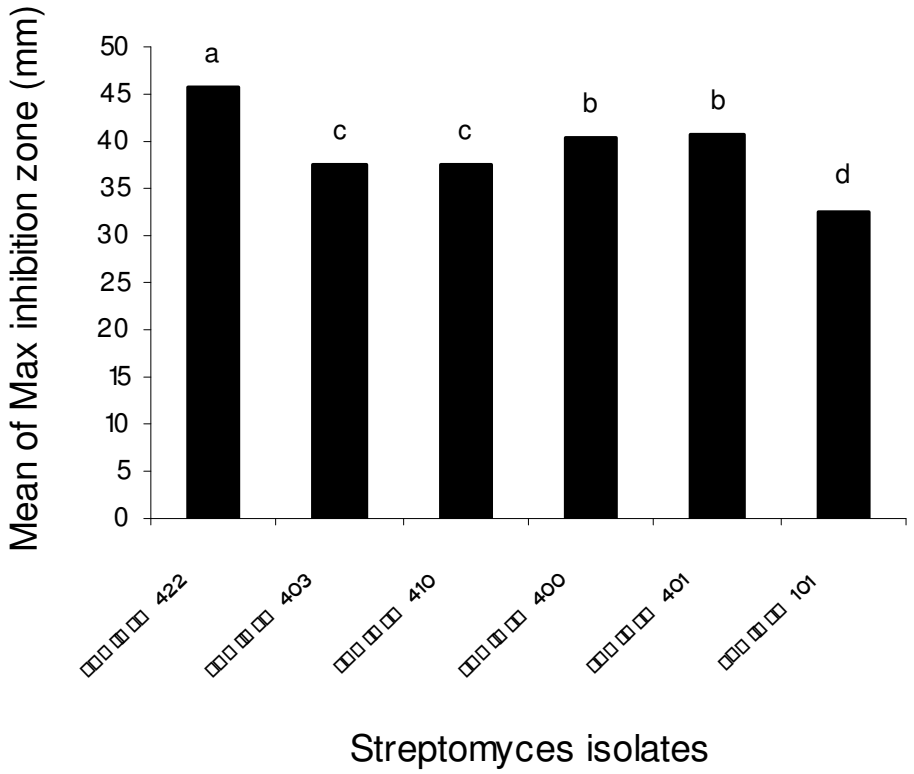

Figure 2. Variance analysis of antagonistic effects of *Streptomyces* isolates against *Sclerotinia sclerotiorum*. Means within columns followed by different letters (a, b, c, d) have significant difference (P = 0.01) according to Duncan's multiple-range test. Results indicated that *Streptomyces* isolates No. 422 had highest antagonistic activity.

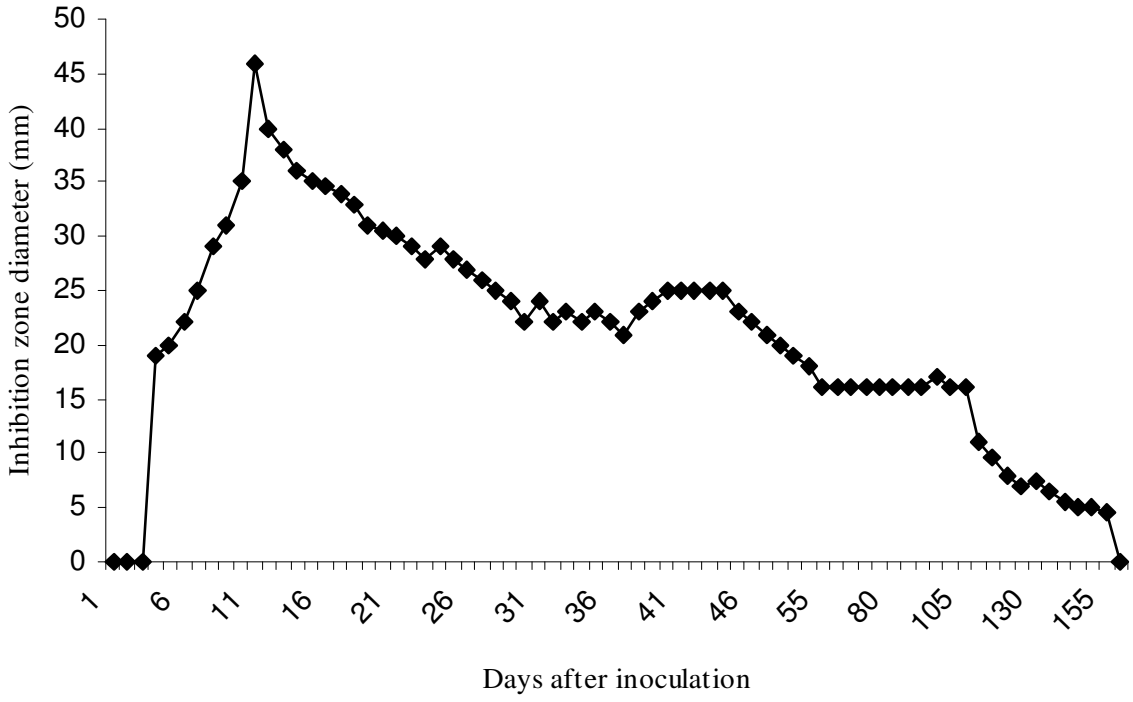

Figure 3. Activity versus post seeding time in submerged cultures of *Streptomyces* isolate No. 422 monitored by well diffusion-method against *S. sclerotiorum*.

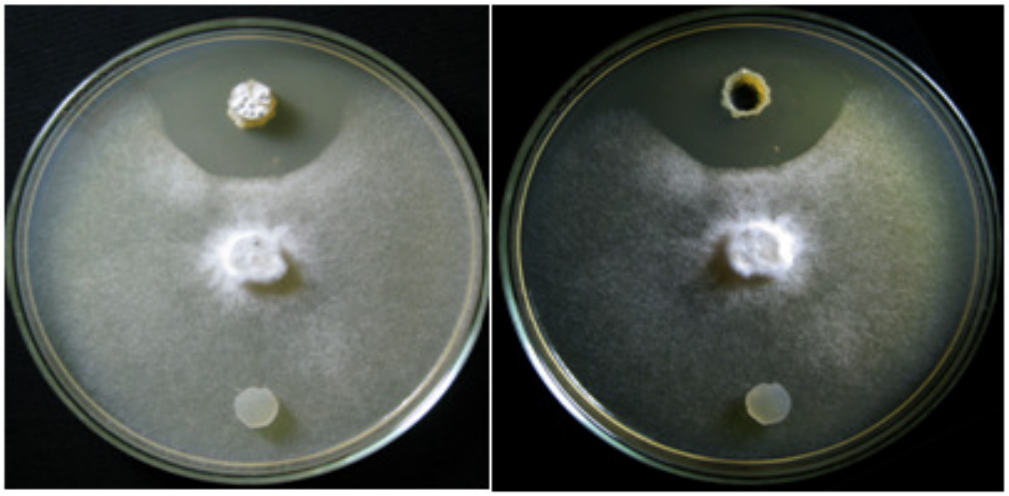

Figure 4. Antifungal activity of selected *Streptomyces* isolates using (A) dual culture bioassay and (B) Well diffusion method. In both figures: Center, agar plug of *Sclerotinia sclerotiorum* with radial growth; top, *Streptomyces* isolate No.422 ; bottom plain agar plug as control; showing inhibitory effect against mycelial growth of *S. sclerotiorum*.

Table 1. Bioassay results of solubility tests of the antifungal principle (s) of *Streptomyces* isolate No. 422 against *S. sclerotiorum* in fractions of different solvents indicated by well diffusion-method at 20 mg mL^{-1} of dry crude.

Solvent	Fraction	Activity	Inhibition zone
H_2O	S	+	21
	P	+	23
Methanol	S	-	-
	P	+	21
Chloroform	S	-	-
	P	-	-
Acetone	S	-	-
	P	+	22

S, Supernatant; P, pellet; +, soluble; -, insoluble.

perature (12 to 30°C) was 220 days; it was assayed by well diffusion-method against *S. sclerotiorum*.

Fungicidal and/or fungistatic activity

Transferred *S. sclerotiorum* mycelium blocks from inhibition zones did not regrow in fresh PDA plates which was indicative of fungicide activity of *Streptomyces* isolate 422.

Scanning electron microscope studies

Scanning electron micrograph of mycelia and spores of *Streptomyces* isolate No. 422 is shown in Figure 5.

Chitinase activity

After 8 to 10 days of incubation of the inoculated minimal chitin-agar media at 28°C, clear zones observed around *Streptomyces* isolate colonies were representative of chitinase activity of this strain.

Greenhouse studies

Symptoms as stem rot and wilt appeared 4 to 6 days after inoculation in seedlings inoculated with *S. sclerotiorum* (P), while other treatments including streptomyces isolate No. 422 (A), *S. sclerotiorum* plus *Streptomyces* isolate No. 422 (A+P) and control, which was not treated by pathogen (C) did not develop signs of the disease

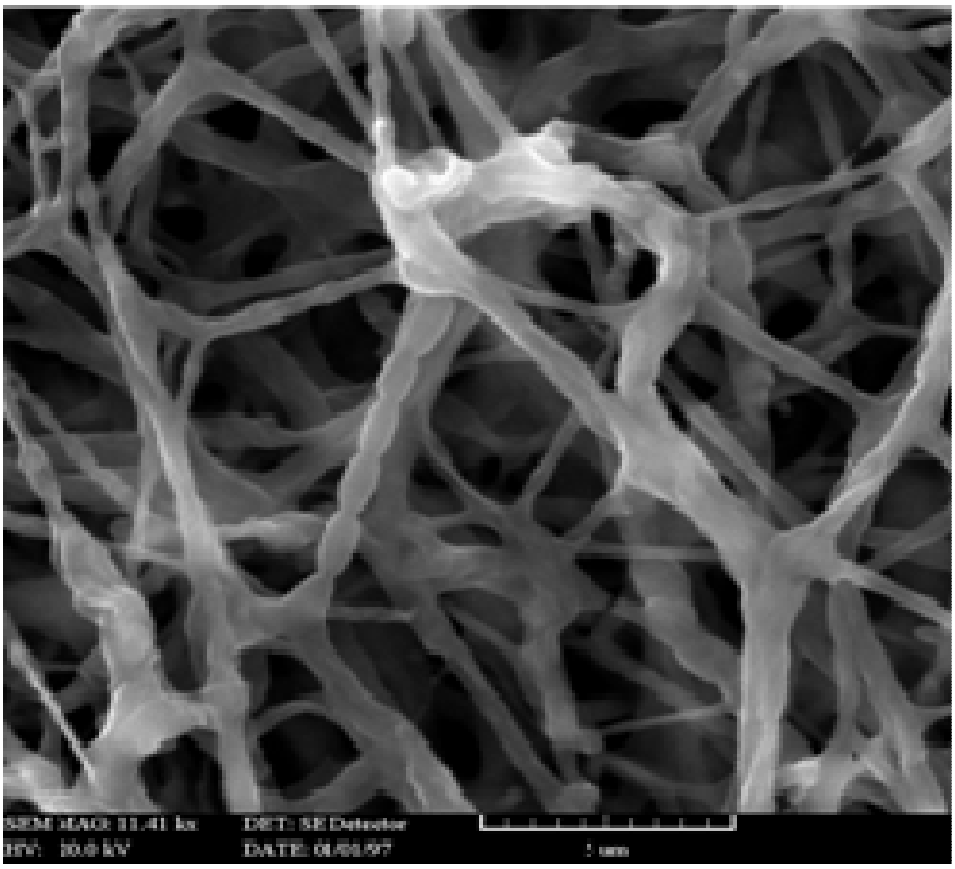

Figure 5. Scanning electron micrograph of mycelia of *Streptomyces* isolates No. 422.

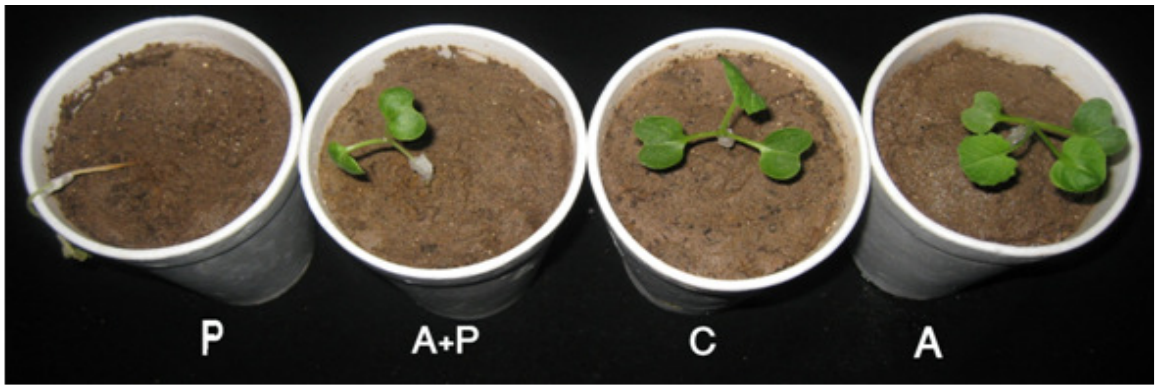

Figure 6. *In vivo* greenhouse results in oilseed rape seedlings (P): In plants inoculated with the pathogene (*S. sclerotiorum*) alone and (A+P), Plants inoculated with both *S. sclerotiorum* and the antagonist *Streptomyces* isolate 422, (C), untreated control plants. (A), Plants inoculated with *Streptomyces* isolate 422 alone.

(Figure 6).

Isolation, cloning and sequencing of partial chitinase gene

The amplified PCR fragments of 600 bp was obtained by using forward and reverse primers of C3HB, while cDNA from *Streptomyces* isolate No. 422 was used as the template. The amplified PCR fragment was cloned and sequenced from both sides. The fragment consists of 600 nucleotide encoding 200 amino acid and a theoretical isoelectric point of 6.46 and also, molecular weight of 21636.9 Da with atomic composition formula as $C_{964}H_{1423}$

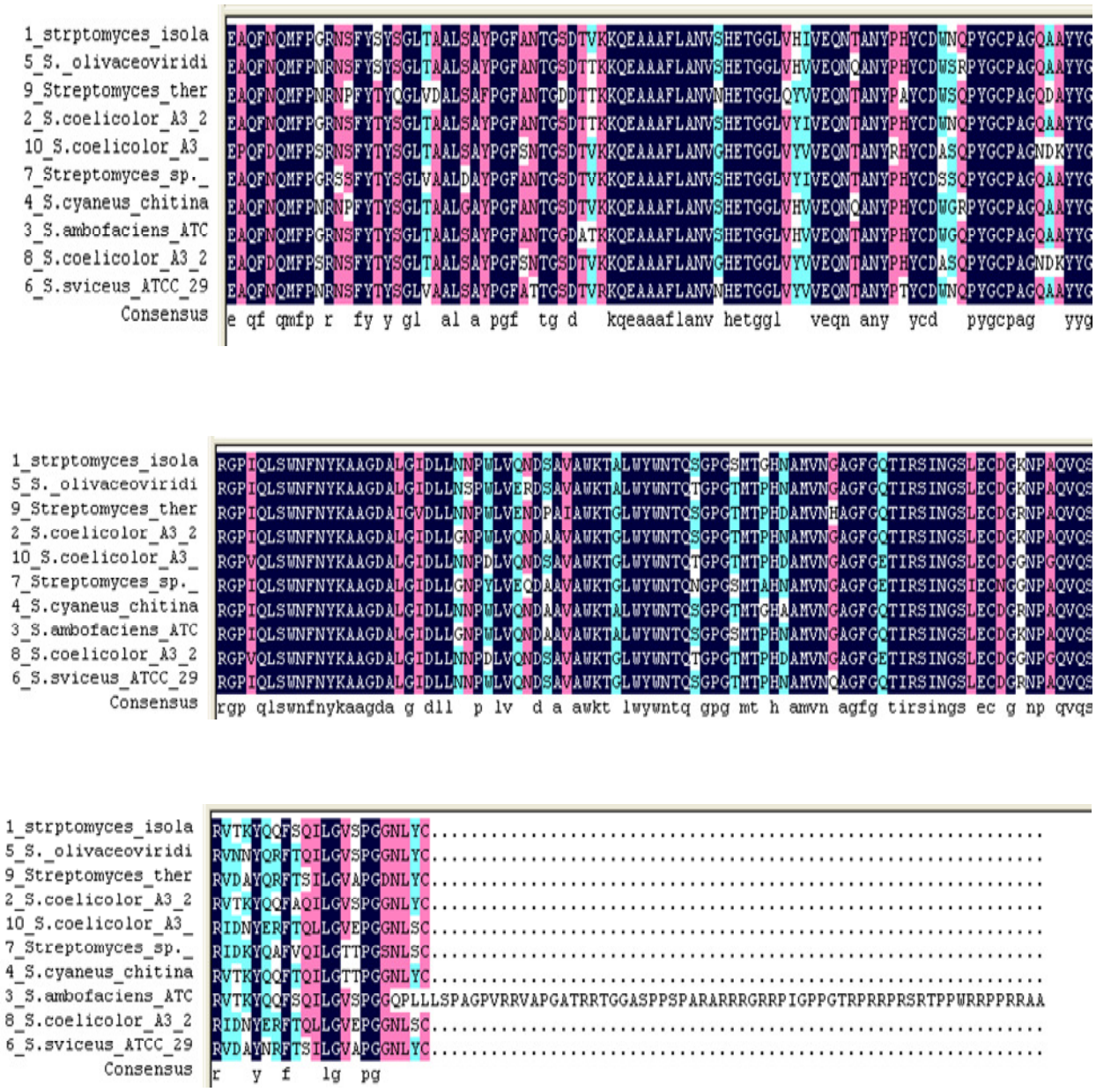

Figure 7. Alignment of the amino acid sequence of partial chitinase gene from *Streptomyces* isolate 422 with other amino acid sequences of families of 18 and 19 chitinases. The sequences indicate: 1: *Strptomyces* isolate No. 422, partial C-terminal chitinase gene; 2: *S. coelicolor* A3(2), ChiF; 3: *S. ambofaciens* ATCC 23877, Chitinase; 4: *S. cyaneus*, Chi A; 5: *S. olivaceoviridis*, Chi 30; 6: *S. treptomyces sviceus* ATCC 29083, Chitinase 7: *S. sp.* J-13-3, Chitinase; 8: *S. coelicolor* A3(2), Chitinase; 9: *S. thermoviolaceus*, Chi25 and 10: *S. coelicolor* A3(2), ChiG.

$N_{265}O_{293}S_7$. The instability index (II) of deduced amino acid sequence computed to be 37.29 which is classified as stable product and has more than 10 h half life (http://www.expasy.ch/cgi-bin/protparam). Deduced amino acid sequence showed high homology with Chi F from *Sclerotinia coelicolor* A3(2) (95.5%), Chitinase from *Sclerotinia ambofaciens* ATCC 23877 (93.5%), Chi A from *Sclerotinia cyaneus* (92.5%), Chi 30 from *Sclerotinia olivaceoviridis* (92%), Chitinase from *Sclerotinia sviceus* ATCC 29083 (89.5%), Chitinase from *S. sp.* J-13-3 (84.5%), Chitinase from *S. coelicolor* A3(2) (86.5%), Chi25 from *Sclerotinia thermoviolaceus* (83%) and ChiG from *S. coelicolor* A3(2) (83.5%) as indicated in Figure 7.

DISCUSSION

Much concerns over the impact of chemical fungicide of soil-born fungus (*S. sclerotiorum*) on the environment like their last and chemical residues, have led to increased

interest in biological control of this pathogen. Previously, several biocontrol agents such as mycoparasitic fungi and parasitic fungus *Coniothyrium minitans* have been presented. The last one is commercially available as contans (Vrije et al., 2001). However, few studies have been done to explore a bacterial biocontrol agent against *S. sclerotiorum* especially the causal agent of canola stem rot. Streptomycetes are Gram-positive bacteria which have high potential to secrete a number of degradative enzymes and antibiotics in control of many pathogens (Mc Carthy and Williams, 1992). Assays for antagonistic activity of *Streptomyces* isolates No. 422 revealed that, it was able to inhibit mycelial growth of *S. sclerotiorum* significantly. The potentiality of antagonistic bacteria to control plant diseases has been demonstrated in several reports. However, the efficiency of biocontrol agents can be improved through understanding the effective components. The main components in antifungal activity are chitinase enzymes which are involved in the lysis of fungal cell walls (Fernando et al., 2006). This isolate showed high chitinolytic activity in selective minimal chitin agar medium. Furthermore, this isolate did not retain its antifungal activity after exposing to chloroform. Therefore, antifungal activity is not because of antibiotic characteristic of this isolate. Chitinase enzymes can probably be one of the causal agents in this behavior. Furthermore, one chitinase gene was purified and cloned from the cDNA of the mentioned isolate which confirmed the chitinase expression of this isolate. Shapira and his colleagues showed that, the expression of cloned chitinase gene in *E. coli* could control the diseases caused by *Sclerotium rolfsii* in bean and cotton (Shapira et al., 1989). Also, chitinase C of *Streptomyces griseus* HUT6037 inhibited *in vitro* growth of *Tricoderma reesei* (ITOH et al., 2003). Several reported experiments on *in vitro* studies have documented satisfactory results in using of *Streptomyces* against some pathogens (Vrije et al., 2001). Tahtamouni and his colleagues reported that, *in vitro* antifungal activity of some of the chitinolytic *Streptomyces* were isolated against *S. sclerotiorum.* Accordingly, they confirmed earlier findings of El-Tarabily et al. (2004) who had reported such activity against *Streptomyces minor* using of chitinolytic bacterium and Actinomycetes. Now, these findings confirm the importance of *Streptomyces* isolates as biocontrol agents and also, emphasize the importance of indigenous *Streptomyces* spp. as biocontrol agents against wide range of fungal phytopathogens (Aghighi et al., 2004). *In vitro* studies showed that, *Streptomyces* isolate No. 422 was able to control the growth of *S sclerotiorum*. In greenhouse study, *Streptomyces* isolate 422 suppressed fungal diseases in inoculated plant without any contamination in the soil and plant. These results confirm that, Actinomycetes (like *Streptomyces* isolate 422) are both biological control agents and some physiological results of *Streptomyces* isolate 422 showed that the active metabolite of this isolate had polar nature, long shelf life, low MIC, fungicidal activity and they are stable in high temperature. For this, the isolate is used to control phytopathogenes in nature.

In addition, this isolate had effective antagonistic characteristics against other phytopathogenes including *Rhizoctonia solani* (okra, sugar beet, melon, rice, cucumberaceae, pistachio and bean isolates), *Fusarium graminearum, Fusarium solani*, *Verticillim dahliae* and *Pythium aphanidermatum,* which foresights that *Streptomyces* isolate No. 422 and its purified effective components can be used as an important biocontrol agent in nature with the future perspective of replacement with chemical control measures.

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