Gebily et al. Egyptian Journal of Biological Pest Control (2021) 31:33
https://doi.org/10.1186/s41938-021-00373-x

Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Characterization and potential antifungal activities of three *Streptomyces* spp. as biocontrol agents against *Sclerotinia sclerotiorum* (Lib.) de Bary infecting green bean

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Abstract

Background: White mold disease, caused by *Sclerotinia sclerotiorum* the devastating pathogen, attacks green beans (*Phaseolus vulgaris* L.) and several crops worldwide. The present investigation was carried out to introduce some antagonistic microorganisms as novel antifungal substances to be an alternative and secure method to effectively control the disease.

Results: Three *Streptomyces* species, i.e., *S. griseus* (MT210913 "DG5"), *S. rochei* (MN700192 "DG4"), and *S. sampsonii* (MN700191 "DG1") were isolated, biologically, molecularly characterized, and evaluated in vitro and in vivo. Molecularly, polymerase chain reaction (PCR) amplification and nucleotide sequencing were used to characterize the pathogen and bio-agents. PCR amplification of the pathogen and *Streptomyces* species (bioagents) exhibited amplicons of around 535 bp and 1300 bp, respectively. The nucleotide sequence analysis of the three *Streptomyces* spp. indicated that *S. rochei* was closely related to *S. griseus*, and both had a distance relationship with *S. sampsonii*. The evaluation of bioagents was carried out against *S. sclerotiorum*. Reduction percentages in the mycelial growth of the pathogen ranged between 60.17 and 52.30%, indicating that *S. rochie* gave the highest inhibition percent. Incorporations of *Streptomyces* spp. culture filtrate components into culture media proved that *S. sampsonii* was more efficient as a bioagent in reducing mycelial growth pathogen by 84.50%. The effectiveness of the bioagent volatile compounds inhibited the pathogen growth at a rate of 54.50-72.54%, respectively, revealing that *S. rochei* was the highest inhibitor followed by *S. griseus*. The parasitic activity of *Streptomyces* spp. upon *S. sclerotiorum* showed deformation, contraction, and collapse when observed by light and scanning electron microscopy. Molecular characterization of the 3 *Streptomyces* spp. revealed that *S. griseus* was closely related to *S. sampsonii* (96%), secondly ranked by *S. rochei* (93.1%). Viability and germination of pathogen sclerotia were reduced when they dipped into the *Streptomyces* spore suspensions for 10, 20, and 30 days. Application of the 3 *Streptomyces* spp. in the field proved a great potential to control the disease.

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Conclusions: The results suggested that the 3 *Streptomyces* strains and their secondary metabolites can be potential biocontrol agents and biofertilizers for controlling *S. sclerotiorum*, the causative agent of bean white mold disease.

Keywords: *Sclerotinia sclerotiorum*, *Streptomyces* spp., Antifungal activity, Green bean

Background

Beans are a staple food source of protein for billions of people worldwide and are suspected of infection by various diseases. The white rot (mold) caused by the destructive soil-borne pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary, affects over 600 plant species including almost all dicotyledonous and some monocotyledonous plants (Shitou et al. 2020). It is considered one of the most important limiting factors in producing green beans worldwide (Zheng et al. 2019). In Egypt, bean (*Phaseolus vulgaris* L.) plants are grown usually in high moist and cool conditions. In both dry and green beans, the pathogen causes white mold disease leading to losses ranged from 30 to 100% depending on the favorable conditions (Mohamed and Atallah 2020). Such conditions (cold weather) seemed to be subsidizing factors for white rot infection, thus the growers have been battling large yield losses due to a disease caused by the fungus (Alsum et al. 2017). Globally, many challenges confront the management strategies of *S. sclerotiorum* due to the long-term persistence and tolerance of reproductive structures (sclerotia), a wide host range, and unpredictability of infection (Arfaoui et al. 2018). Due to its unique life cycle, the pathogen infects host plants either through aeciospores that can be discharged reluctantly upwards from apothecia into the air or by mycelium emerging from infected tissue and germinated sclerotia (Zheng et al. 2019). Synthetic pesticides are capable to effectively control plant diseases, but some fungicides result in severe environmental and health problems.

Out of all rhizosphere microbes, actinomycetes are considered as potential biocontrol agents because they exhibit many useful features. In this context, actinomycetes have been utilized to defend various plants against a wide range of phytopathogenic fungi and bacteria as well as human and insects (Zhao et al. 2019). The genus *Streptomyces*, a unique subgroup of actinomycetes bacteria, is best-known for their ability to produce a variety of weapons as bioactive secondary metabolites, i.e., antibiotics, antifungal, antibacterial, antioxidants, antiparasitic, anticancer, plant growth-promoting substances, insecticidal (Dongli et al. 2019; Zhao et al. 2019).

Recently, microbial antagonists such as *Streptomyces* spp. have been widely applied for the biocontrol of plant diseases (Katarzyna et al. 2018). The interaction of *Streptomyces* with the pathogenic fungi is usually due to the production of cell wall-degrading enzymes such as cellulases, chitinases, amylases, and glucanases. Also, the parasitic activity of *Streptomyces* species upon phytopathogenic fungi was demonstrated by light and scanning electron micrographs (Zamoum Miyada et al. 2017 and Kong et al. 2019). Furthermore, *Streptomyces* spp. are recognized for the production of an exceptionally large number of the natural bioactive secondary metabolites (70-80%) and volatile organic compounds "VOCs" well-known for their pharmaceutical or agrochemical applications (Salwan and Sharma 2020; Sharma et al. 2020). Nowadays, the bioformulation of VOCs derived from *Streptomyces* spp. available are used as biofungicide and inhibited hyphal growth against different soilborne pathogenic fungi (Sharma et al. 2020).

The highlights of the present investigation were conducted to determine the capabilities of parasitic activities, the potency of secondary metabolites, and volatile compounds of different *Streptomyces* isolates in vitro and in vivo to introduce alternative fungicides such as biological agents to manage *S. sclerotiorum*. For this reason, the utilization of antagonistic microorganisms and/or their novel antibacterial, antifungal, and antiviral substances becomes necessary as an alternative and secure method to effectively control the fungal and bacterial diseases of crops.

Methods

Collection, isolation, and purification of the pathogen (*S. sclerotiorum*)

Pure culture of *S. sclerotiorum* (green bean isolate) was obtained from infected green bean plants grown in the fields of Fayoum governorate, Egypt. The fungus was isolated from the sclerotia, formed on the pods of the diseased plants. The sclerotia were surface sterilized by sodium hypochlorite (0.5%) for 2-3 min, washed several times with sterilized distilled water, then dried between sterilized filter papers, and transferred directly into plate 9 cm contained Cook's medium. The purified *S. sclerotiorum* isolate was identified according to Kora et al. (2003) and grown on Cook (1970), which contained 15 g glucose; 1 g K_2HPO_4; 0.5 g K_2Cl; 0.5 g $MgSO_4.7H_2O$; 0.01 g $FeSO_4.7H_2O$; 2 g asparagine; 20 g agar/l liter distilled water). The fungus was kept onto the slant tubes medium at 5 °C for further experiments.

Molecular identification of the pathogen

Total genomic DNA extraction was carried out as described by Toda et al. (1999). Polymerase chain reaction

(PCR) was conducted by the universal primers, i.e., forward primer ITS-1(5′-TCC GTA GGT GAACCT GCG G-3′) and reverse primer ITS-4 (5′-TCC TCC GCT TAT TGATAT GC-3′) as described by White et al. (1990). Amplification targeted is the sequence divergence within the ribosomal DNA internal transcribed spacer (rDNA-ITS) regions of the fungal. PCR was conducted according to the method described by Hayakawa et al. (2006) in GATC Biotech German Company (Under License of Sigma). PCR products were separated on 2 %agarose gel electrophoresis. The DNA ladder (100 bp) was also loaded in the gels to estimate the proper band size of amplified products and photographed. PCR cleanup was carried out to the PCR product, using GeneJET™ PCR Purification Kit (Thermo K0701). Finally, the sequencing of the PCR product was made, using ABI 3730xl DNA sequencer plus forward and reverse primers. The sequenced PCR product was confirmed, using NCBI (The National Center for Biotechnology Information) mega blast for the species identity. According to the percentage of homogencity between our isolate and identified isolates, the name of species was detected. After identification, the isolate has been deposited at the National Center for Biotechnology Information (NCBI) under the GenBank accession number (MT645495). The nucleotide sequences of the corresponding 16S rRNA gene were compared with 15 sequences of other fungus isolates from different geographical regions that were kept in the GenBank. Alignment program and phylogenetic tree were conducted to compute evolutionary distances for both sequences of nucleotides of the isolated fungus using DNAMan Ver.7.

Isolation and purification of Actinobacteria isolates

Twenty-one random rhizosphere soil samples were collected from different plant sources (Table 1). Also, samples were collected from phyllosphere plants and compost as well as saltwater from different Egyptian governorates. Isolation of Actinobacteria from soil samples was accomplished according to Lawrence (1956) and Hamada Eman (2006). One gram or milliliter of each collected sample was suspended in a flask containing 19-ml sterilized water to be a dilution of 1/20 and shaken in a rotary shaker (150 rpm). Afterward, two drops of each previously mentioned suspension were transferred separately from each flask to cylinder 25 ml in capacity containing 10 ml of phenol diluted by water (1:140) just to reduce the bacterial and fungal contamination. Subsequently, 0.1 ml of diluted soil suspension was streaked separately onto Petri dishes each containing 15 ml of solidified starch nitrate agar (StNA) medium contained (g/l); 20 g soluble starch; 2 g KNO_3; 0.5 g NaCl; 1 g K_2HPO_4; 0.5 g $MgSO_4.7H_2O$; 3 g $CaCO_3$; 0.001 g $FeSO_4.7H_2O$; 20 g agar; pH 7.2. Five plates were used for each sample, incubated at 28 °C, and examined periodically. After 3-5 days, colonies of actinomycetes on the plates were picked based on their morphological characteristics and purified on the same medium, then incubated for 7 days. Actinobacteria colonies were transferred to Petri dishes containing starch nitrate agar (ST.N.A.) medium supplemented with sodium propionate to suppress any bacterial or fungal contamination, then incubated at 25 °C (Crook et al. 1950; Waksman 1959).

Table 1 Sources and locations of the isolated Actinobacteria

No.	Source	Location	Code
1	Sugarcane	Rhizosphere	1
2	Sugarcane	Rhizosphere	2
3	Sugar cane	Rhizosphere	4
4	Hibiscus	Rhizosphere	6
5	Onion	Rhizosphere	7
6	Chamomile	Rhizosphere	13
7	Olive	Rhizosphere	15
8	Pepper	Rhizosphere	25
9	Mint	Rhizosphere	26
10	Bean	Rhizosphere	27
11	Orange	Rhizosphere	33
12	Quinoa	Rhizosphere	35
13	Tomato	Rhizosphere	36
14	Clover	Rhizosphere	42
15	Sugar beet	Rhizosphere	43
16	Papyrus	Rhizosphere	30
17	Fennel A1	Rhizosphere	51
18	Fennel A6	Rhizosphere	52
19	Fennel A7	Rhizosphere	53
20	Egg plant	Phyllosphere	10
21	Pepper	Phyllosphere	31
22	Compost	Plant compost heap	16
23	Water	Salt water (Red Sea)	54
24	Water	Salt water (Mediterranean Sea)	46

Molecular characterization of *Streptomyces* isolates

Three *Streptomyces* species were characterized according to a molecular biological protocol by DNA extraction, amplification (PCR), and sequencing of amplification of 16S rDNA gene. DNA extraction of antagonist bacteria was implemented using Maxima Hot Start PCR Master Mix (Thermo K1051) according to the manufacturing instructions of GATC Biotech German Company. The target base pair DNA fragment generated from genomic DNA was amplified using forward primer “63f” (5′-CAGGCCTAACACATGCAAGTC-3′) and reverse primer “1387r” (5′-GGGCGGWGTGTACAAGGC-3′) as

designated by Marchesi et al. (1998). PCR reaction was performed by a first initial denaturation at 95 °C for 10 min/one cycle, followed by denaturation 95 °C/30 s/35-cycles, annealing 57 °C/1 min/35-cycles, and extension 72 °C/1.5 min/35-cycles and final extension step at 72 °C/10 min/1-cycle. PCR amplicons were analyzed using 2.0% agarose gel electrophoresis in 1x TAE buffer staining with ethidium bromide. The amplified bands were visualized by UV illumination using a gel documentation system. The DNA Marker (100 bp) was also loaded in the gels to estimate the proper band size of amplified products and photographed. Then, PCR cleanup was carried out to the PCR product, using GeneJET™ PCR Purification Kit (Thermo K0701). Finally, the sequencing of the PCR products was achieved utilizing the ABI 3730xl DNA sequencer. The sequenced PCR product was confirmed, using NCBI (The National Center for Biotechnology Information) mega blast for the species identity. According to the percentage of homogencity between our *Streptomyces* isolates and identified isolates, names of the genus and species were determined as *Streptomyces griseus*, *S. rochei*, and *S. sampsonii*. After identification, species were deposited at the National Center for Biotechnology Information (NCBI) under the GenBank accession numbers, i.e., *S. griseus* (MT210913 "DG5"), *S. rochei* (MN700192 "DG4"), and *S. sampsonii* (MN700191 "DG1"). The nucleotide sequences of the corresponding 16S rRNA gene were compared with various sequences of other *Streptomyces* genera from different geographical regions that were kept in the GenBank. The nucleotide sequence of *Bacillus subtilis* (AJ276351.1) was used as out-group. For the alignment program, sequences retrieved were aligned by the most similar type strains obtained from the GenBank, using CLUSTAL W method by DNAMan Ver.7. While the phylogenetic tree was statistically tested, using the Maximum Like Hood method (Bootstrap phylogeny analysis with 1000 replications).

Determine the efficacy of *Streptomyces* spp. in vivo

This experiment was carried out at the greenhouse. Five seeds (Green bean "*Phaseolus vulgaris* L." cv. Polista) were sown in pots (25 cm in diameter) containing 2.5 kg Peatmoss/sand. Inocula of *S. sclerotiorum* were prepared as the following: *S. sclerotiorum* was subcultured on Cook's medium and kept in an incubator at 22 °C/15 days for the development of sclerotia. Five sclerotia were placed to infest each pot and 3 replicates (each replicate contained 4 pots)/treatment. Each treatment was applied to 60 plants. Concerning the application of *Streptomyces* species as bio-agents, the six species, i.e., *Streptomyces griseus* strain DG5 (MT210913), *S. rochei* strain DG4 (MN700192), *S. sampsonii* strain DG1 (MN700191), and *Streptomyces* spp. (no. 2, 27, and 30) were grown on a liquid starch casein medium for 7 days. All *Streptomyces* spp. were applied at the rate of 1/100-liter water (1 ml contain 15×10^6 cfu). Applications of *Streptomyces* were sprayed on 15-day-old plants 2 times (15 days interval). Data were recorded after 45 days of planting. All pots were irrigated periodically using the same amount of irrigation and fertilization.

Antifungal activity assays of *Streptomyces* spp.

Various techniques were carried out to determine the bioactivities of the 3 isolated *Streptomyces* spp. against *S. sclerotiorum* utilized dual culture techniques, mechanism of parasitism, culture filtrate, and volatile organic compounds (VOCs).

Detection of antagonistic activity by dual culture techniques

A dual culture technique was carried out to determine the antagonistic activity of *Streptomyces* spp. against the pathogen. *Streptomyces* spp. (*S. griseus* strain DG5 (MT210913), *S. rochei* strain DG4 (MN700192), and *S. sampsonii* strain DG1 (MN700191) were tested separately as antagonistic against *S. sclerotiorum* in vitro. Petri dishes (9 cm in diameter) each contained 15 ml of starch nitrate agar medium were inoculated on one side using a loopful of Streptomyces isolate and incubated at 28 °C for 4 days. The Petri dishes on opposite sides were inoculated by 5 mm mycelial disk from a 7-day-old culture of *S. sclerotiorum*. Plates inoculated only with *S. sclerotiorum* served as controls. Three replicate plates were used for each species. All inoculated plates were incubated at 22 °C for 7 days then examined. The percentages of reduction in mycelium growth of the pathogen were calculated and compared with control treatment as elaborated by Abd El-Moity (1985) as follows:

$$X = 100 - (G2/G1 \times 100)$$

where X is the % of reduction in the growth of pathogenic fungus

G1 is the growth of the pathogenic fungus in the control plate

G2 is the growth of the pathogenic fungus in treated plates with antagonist

Evaluation of the parasitic mechanism of *Streptomyces rochei*

The parasitic activity of *Streptomyces rochei* upon *S. sclerotiorum* was confirmed by light and scanning electron micrographs (SEM). The slide technique provides a clear view of the interaction between *Streptomyces* spp. and *S. sclerotiorum* malformation, lyses, or parasitism. In this technique, a sterilized microscope glass slide was covered by a thin film sterilized diluted starch nitrate

(0.1%). A loopful of *S. rochei* was stretched at one side, whereas the mycelial disk of *S. sclerotiorum* was inoculated at the other side of the microscope glass slide. Inoculated slides were placed in sterilized Petri dishes contained filter paper saturated with 10 ml of sterilized water just to maintain humidity, then incubated at (22 ± 2 °C). Slides were observed periodically using a light microscope with a fixed camera to examine the interaction between the pathogen and antagonist. The mycoparasitism of *S. rochei* on the mycelium of *S. sclerotiorum* was studied using SEM. The preparation of the samples was undertaken according to Loliam et al. (2013). Examinations were carried out, using the light microscopy (CHS, Olympus optical Co. Ltd.) and SEM (QUANTA FEG 250, Japan) in the National Research Center.

Impact of *Streptomyces* culture filtrates on mycelial growth suppression of *S. sclerotiorum*

Streptomyces species (*S. griseus*, *S. rochei*, *and S. sampsonii*) were grown separately in flasks containing 100 cm starch casein liquid (StC) medium consisted of 10 g soluble starch; 0.3 g casein; 2 g KNO_3; 2 g NaCl; 2 g K_2HPO_4; 0.05 g $MgSO_4.7H_2O$; 0.02 g $CaCO_3$; 0.01 g $FeSO_4$ $7H_2O$; distilled water 1000 ml, then adjusted to pH 7.2 and incubated at 28 °C for 7 days. Flasks were shaken on a rotary shaker (at 130 rpm). The culture broth was centrifuged (at 6000 rpm for 10 min), then filtered through a 0.2 μm. Each of the culture filtrates was mixed separately at a rate of 30% to sterilized Cook's medium when reached 45 °C. Then the disks of *S. sclerotiorum* mycelium were placed onto the center of the plates containing Streptomyces culture filtrate. The diameter of mycelial growth was measured after 7 days of incubation at 22 °C. Three replicates were used for each genus of Streptomyces. The inhibition percentages of mycelial growth were determined as described by the equation of Gamliel et al. (1989) as the following:

$$\%\text{Hyphal growth inhibition} = 100 - \left[\left(r^2/R^2\right)\right] \times 100$$

where r is the colony radius of the pathogen on Cook's medium incorporated with the culture filtrate of the Streptomyces

R is the colony radius of the fungal pathogen on Cook's medium without *Streptomyces* culture filtrate.

Antifungal activity of volatile compounds produced by Streptomyces

The volatile organic compounds (VOCs) produced by *Streptomyces* spp. (*S. griseus*, *S. rochei*, *S. sampsonii*) was tested by the double-sealed plate method according to Fiddaman et al. (1993). Petri dishes (9 cm in diameter), each containing 15 ml of soluble starch casein agar medium was inoculated by *Streptomyces* spp., separately using loopful. After incubation at 28 °C for 3 days, a second Petri plate containing Cook's medium was inoculated by a 5-mm desk of *S. sclerotiorum* and placed over the plate inoculated with the Streptomyces. The two dishes were sealed together by a parafilm and incubated at 22 °C. A Petri dish containing agar medium without bioagent was placed over the plate containing Cook's medium, inoculated with the fungal pathogen served as control. % inhibition in radial growth of the tested fungus was measured after 7 days as previously mentioned (Gamliel et al. 1989).

Impact of *Streptomyces* spp. broth on the fungus sclerotia germination

Inhibition of sclerotia germination was conducted using *Streptomyces* spp. broth as the following: Mycelium of *S. sclerotiorum* was inoculated on Cook's medium plates and then incubated at 22 °C. After 2 weeks, the developed sclerotia of *S. sclerotiorum* were harvested in the same sizes. *Streptomyces* spp. (*S. griseus*, *S. rochei*, and *S. sampsonii*) were grown in flasks containing 100 cm starch casein liquid media at 28 °C for 7 days. The sclerotia were soaked into individual streptomyces suspension (10^7 cfu/ml) for 10, 20, and 30 days. The soaked sclerotia were collected and cultured onto the center of the plates contained Cook's medium. The plates were incubated at 22 °C for 7 days to test the germination of sclerotia and percentage of reduction in sclerotinia mycelial growth as described by Gamliel et al. (1989) as previously mentioned.

Impact of single or combined treatments of *Streptomyces* spp. under field (in vivo) conditions

A field experiment was carried out during the 2 successive seasons 2017/18 and 2018/19 at the farming plots located at Dahshur District, Giza Governorate, Egypt, where the soil was light sandy soil naturally infested with the pathogen. *S. griseus* strain DG5, *S. roche*i strain DG4, and *S. sampsonii* strain DG1 were applied individually or in combinations as a broth (containing cell-free extract, mycelia + spores). Three replicates were used for each treatment containing 100 bean seeds cv. Paulista. The plants were irrigated and fertilized. Corporal Max was used as a positive control (commercial fungicide produced by the Central Laboratory for Organic Agriculture). All *Streptomyces* spp. were applied at the rate of 1/100-liter water (1 ml contain 15×10^6 cfu). The application was carried out 4 times (15 days interval) on the plants after 30 days post sowing. Control treatment plants were sprayed by water similarly. The percentages of disease incidence were calculated and the percentages of reduction in white rot disease incidence were calculated after the development of natural disease symptoms

on the control plants (60 days post sowing). Also, the total chlorophyll was measured according to Askar and Treptow (1993). The total yield was recorded for all pickings during the harvesting period.

Statistical analysis

Statistical analyses were performed by SPSS (2008), version 17.0, using the General Linear Model's procedure. Significant differences among treatments were evaluated using Duncan's multiple range test ($P \leq 0.05$).

Results

Identification, purification, and molecular characterization of the pathogen

A pure culture of *S. sclerotiorum* was started from one sclerotia obtained from infected pods of green bean plants grown in the fields of Fayoum governorate, Egypt, and kept for further experiments. PCR amplification with the primers (ITS-1/ITS-4) resulted in an approximately 553-bp product within the internal transcribed spacer (rDNA-ITS) region of the fungal isolate tested (Fig. 1a). The sequence of the isolate was submitted to NCBI GenBank and gave an accession number MT645495. Phylogenetic tree of the Egyptian isolate *S. sclerotiorum* and other 15 isolates spread worldwide has been constructed based on nuclear ribosomal internal transcribed spacer the ITS rDNA (amplicons from primers ITS-1 and ITS-4) from a subset of isolates (Egyptian isolate and other 15 worldwide isolates) using DNAMan Ver.7. The result revealed that the nucleotide sequence identity of the ITS rDNA gene between the Egyptian isolate of *S. sclerotiorum* is closely related to some worldwide isolates and ranged from 98 to 100%. Meanwhile, the lowest homology percentage with other corresponding nucleotide sequences from different geographic origins was recorded at 93% as shown in Fig. 1b. The result indicated that PCR amplification of the ITS gene, using a specifically designed primer pair (ITS-1/ITS-4), followed by sequencing and phylogenetic analysis identified the isolate as *S. sclerotiorum*.

Molecular characterization of the *Streptomyces* spp.

The 16S rRNA gene was successfully amplified by both primer pairs (63f and 1387r) from the 3 tested Streptomycetes, i.e., *S. griseus* strain (MT210913 "DG1"), *S. rochei* strain (MN700192 "DG1") and *S. sampsonii* strain (MN700191 "DG1"). PCR amplification of its 16S rDNA gene sequence exhibited amplicons of around 1300 bp (Fig. 2a). The result indicated that the PCR technique, as a molecular method, has been extensively used for the identification of *Streptomycetes* species. It is favored over the traditional methods due to its high efficiency, accuracy rapidity specificity in amplification of the target gene. The alignment program and phylogenetic tree using the Maximum Like Hood method based on 16S rRNA gene sequences showed the close and distance relationships between the nucleotide sequences of *Streptomycetes* strains in the present study with the nucleotide sequences of available strains in the NCBI GenBank database as shown in (Table 2 and Fig. 2b). Concerning the 3 Egyptian *Streptomycetes* species, *S. griseus* strain had the highest sequence similarity (96%) with *S. sampsonii* strain, while the lowest similarity (93.1%) with *S. rochei* strain. However, the result indicated the

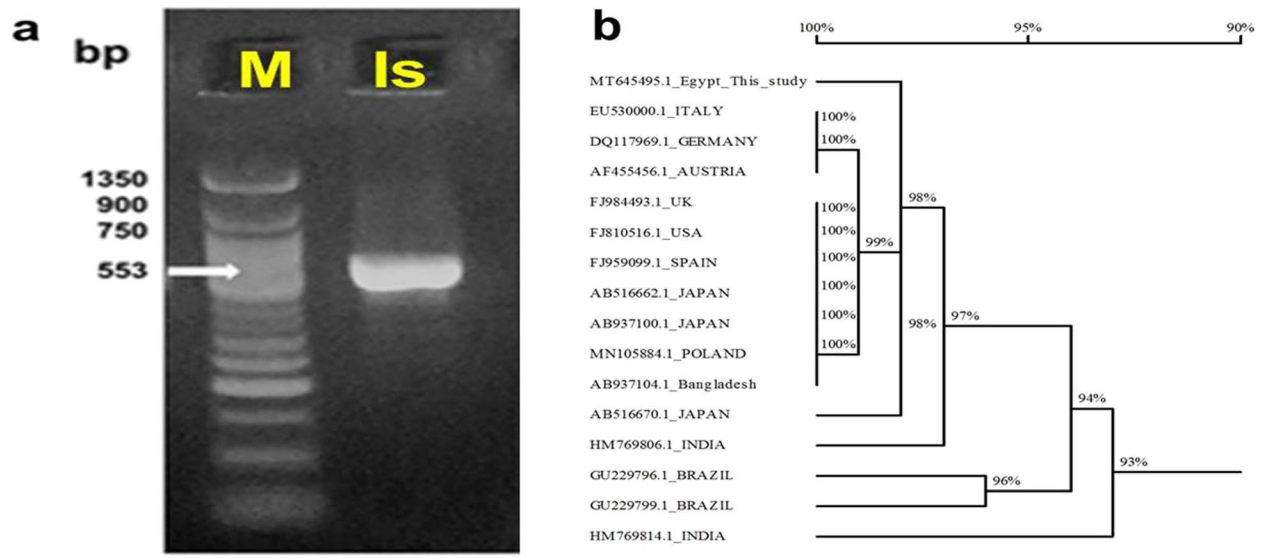

Fig. 1 **a** Products of polymerase chain reaction amplification isolate using the universal primers ITS-1 and ITS-1. A product (amplicon) of 553 bp length was amplified in Egyptian isolate (Is). M = Leader and Is = isolated fungus. **b** Phylogenetic tree obtained from the alignment of nucleotide sequences of the Egyptian isolate and fifteen sequences from GenBank. All 15 sequences show their GenBank codes and countries of origin

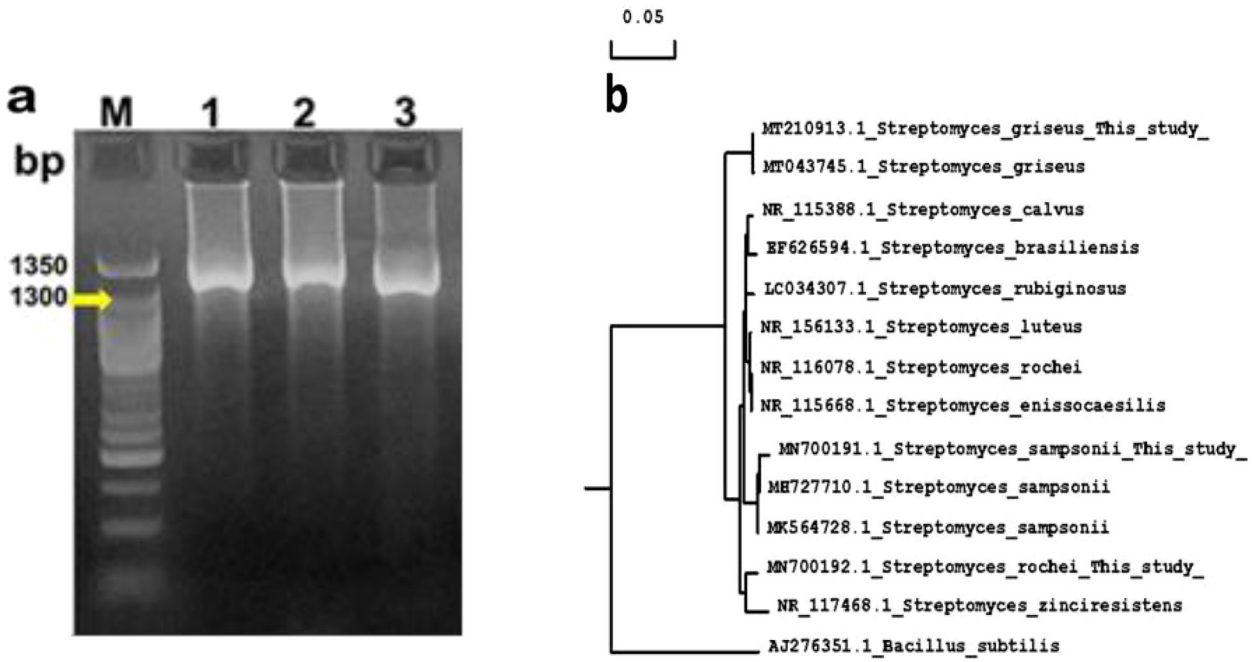

Fig. 2 **a** Products of polymerase chain reaction amplification isolates using the universal primers 63-forward and 1387-reverse. Products (amplicons) of 1300 bp length were amplified from the three isolates of Streptomyces (Is). M = Leader, 1,2 and 3 = isolated *S. sampsonii*, *S. rochei*, and *S. griseus*. **b** Phylogenetic tree based on 16S rRNA gene sequence of *S. rochei*, *S. griseus*, and *S. sampsonii* with the reference Streptomycetes 16S rRNAgene available in the GenBank

divergence in nucleotide sequence between *Streptomyces* isolates with *Bacillus subtilis* out-group exceeded 82.9 %. Further, *S. griseus* MT210913 shared 100% sequence identity with *S .griseus* MT043745 in the GenBank. While *S. griseus* homology was 97% with *S. luteus*, *S. zinciresistens* (96.5%), and *S. rochei* (96.8%), which present in the NCBI GenBank. Homology of *S. rochei* MN700192 was (95.5%) with *S. luteus*, *S. zinciresistens* (95.4%), and *S. rochei* (95.7%), which present in the NCBI GenBank. Whereas *S. sampsonii* MN700191 had 99% homology with each of *S. sampsonii* MK564728 and *S. sampsonii* MH727710 in the GenBank. The aforementioned result indicated that *S. griseus* MT210913 was closely related to *S. sampsonii* MN700191 (96%), secondly ranked by *S. rochei* MN700192 (93.1%).

Screening of *Streptomyces* spp. antifungal activity against *S. sclerotiorum* under greenhouse conditions

Out of 24 *Streptomyces* spp. isolated from the plant rhizosphere, only 6 isolates proved to be effective as antagonistic agents against the devastating pathogen (*Sclerotinia sclerotiorum* "Lib." de Bary) infecting green bean. Among the 6 tested isolates, 3 turned to be more

Table 2 Sharing (%) of nucleotide sequences between the three Streptomyces isolates and others isolates from different geographical regions available in the GenBank

Homology matrix of 14 sequences
MT210913.1_Streptomyces griseusThis _study 100%
MN700192.1_Streptomyces_rocheiThis_study 93.1% 100%
MN700191.1_S._sampsonii _- This- study 96.0% 94.6% 100%
NR_156133.1_Streptomyces_luteus 97.0% 95.5% 98.7% 100%
NR_117468.1_Streptomyces_zinciresistens 96.5% 95.4% 98.2% 99.2% 100%
NR_116078.1_Streptomyces_rochei 96.8% 95.7% 98.6% 99.8% 99.4% 100%
LC034307.1_Streptomyces_rubiginosus 97.1% 95.0% 98.2% 99.2% 99.0% 99.0% 100%
NR_115668.1_Streptomyces_enissocaesilis 96.8% 95.7% 98.6% 99.8% 99.4% 100.0% 99.0% 100%
NR_115388.1_Streptomyces_calvus 96.6% 95.5% 98.4% 99.4% 99.5% 99.5% 99.5% 99.5% 100%
EF626594.1_Streptomyces_brasiliensis 96.5% 95.4% 98.2% 99.2% 99.4% 99.4% 99.0% 99.4% 99.5% 100%
MK564728.1_Streptomyces_sampsonii 97.0% 95.5% 99.0% 99.7% 99.2% 99.5% 99.2% 99.5% 99.4% 99.2% 100%
MT043745.1_Streptomyces_griseus 100.0% 93.1% 96.0% 97.0% 96.5% 96.8% 97.1% 96.8% 96.6% 96.5% 97.0% 100%
MH727710.1_Streptomyces_sampsonii 97.0% 95.5% 99.0% 99.7% 99.2% 99.5% 99.2% 99.5% 99.4% 99.2% 100.0% 97.0% 100%
AJ276351.1_Bacillus_subtilis 82.9% 79.7% 81.8% 82.6% 82.2% 82.7% 82.4% 82.7% 82.2% 82.2% 82.2% 82.9% 82.2% 100%

Table 3 Screening of *Streptomyces* spp. on controlling *S. sclerotiorum* under greenhouse conditions

Isolate and code	% Disease incidence	% Disease reduction
S. sampsonii (1)	0.0[g]	100[a]
Streptomyces sp. (2)	20.00[d]	39.90[d]
S. rochei (4)	16.67[e]	49.90[c]
Streptomyces sp. (30)	26.67[c]	19.90[e]
S. griseus (51)	10.00[f]	69.90[b]
Streptomyces sp. (27)	30.00[b]	9.90[f]
Control	**33.33[a]**	**0.00[g]**
SE	**1.09**	**2.67**

*Values with the same letter are not significantly different

efficient. Data presented in Table 3 revealed that the percentages of disease incidence were reduced to 16.67, 10, and 0.0%, when green bean plants were treated by *S. rochei*, *S. griseus*, and *S. sampsonii*, respectively. Among the 3 *Streptomyces* spp., *S. sampsonii* was the most effective bio-agent, followed by *S. griseus* and *S. rochei* reached 100, 69.6, and 49.9%, respectively, in the reduction of the disease. The results indicated that the 3 *Streptomyces* spp. might have many useful and vital bioactive compounds, which act as a wide variety of modes of antifungal against *S. sclerotiorum* the causal agent of white disease of bean.

In vitro evaluation of the antifungal activity of *Streptomyces* spp.

In this context, 4 techniques, i.e., dual culture techniques, culture filtrate, mechanism of parasitism, and volatile organic compounds (VOCs) were applied as the following:

In vitro inhibitory potential of Streptomyces spp.

Results of dual culture technique in Table 4 and Figs. 3, 4, and 5 indicated that all *Streptomyces* spp. significantly inhibited linear growth of pathogenic fungus, *S. sclerotiorum* compared with control treatment. Percentages of growth reduction of *S. sclerotiorum* ranged between

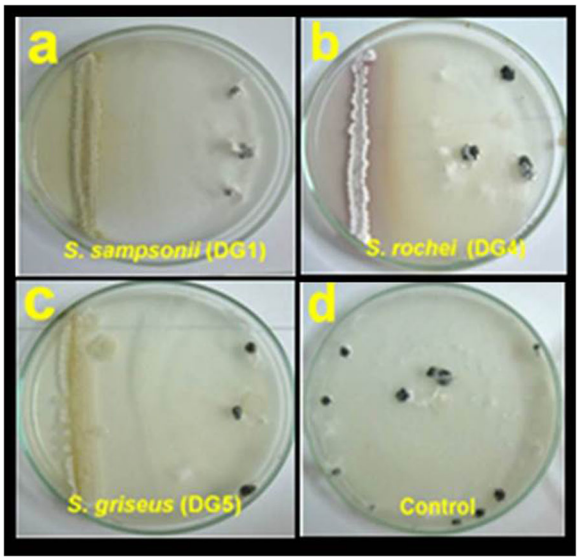

Fig. 3 In vitro antagonistic effect of *S. sampsonii*, *S. rochei*, and *S. griseus* against *S. sclerotiorum*

60.17 and 52.30% (Fig. 3). *S. rochie* showed the highest percentage of reduction in mycelial growth. Obtained results displayed that the plate assays are an indication of whether strains can act as biocontrol agents.

On the other hand, the antagonistic activity of the *Streptomyces* spp. upon *S. sclerotiorum* was demonstrated by light and scanning electron micrographs (SEM). Light and scanning electron micrographs showed that *S. rochei* was colonized on *S. sclerotiorum* mycelium as well as penetrated and grown inside the hyphae. The growth of *S. sclerotiorum* could be suppressed by *S. rochei* appeared to alter the structure of *S. sclerotiorum* mycelia by lysing hyphal tips that caused swelling, pores, irregular membrane border and shrinkage of the hyphal tip as well as grown inside hyphae of the pathogen (Figs. 4 and 5). This result supports the potency and efficacy of *S. rochei* as a bioagent, able to produce secondary metabolites, and penetrated as well as destroy and lysis

Table 4 Impact of antifungal activity of *Streptomyces* spp. on the growth linear of *S. sclerotiorum*

Isolate	% Reduction of linear growth after applying		% Growth inhibition due to volatile compounds (VOCs)
	Dual culture	Culture filtrate	
S. sampsonii	55.47[b]	84.0[a]	54.50[c]
S. rochei	60.17[a]	79.41[c]	72.54[a]
S. griseus	52.30[c]	80.17[b]	64.70[b]
Control	0.00[d]	0.00[d]	0.00[d]
SE	0.15	0.16	1.39

Values with the same letter are not significantly different

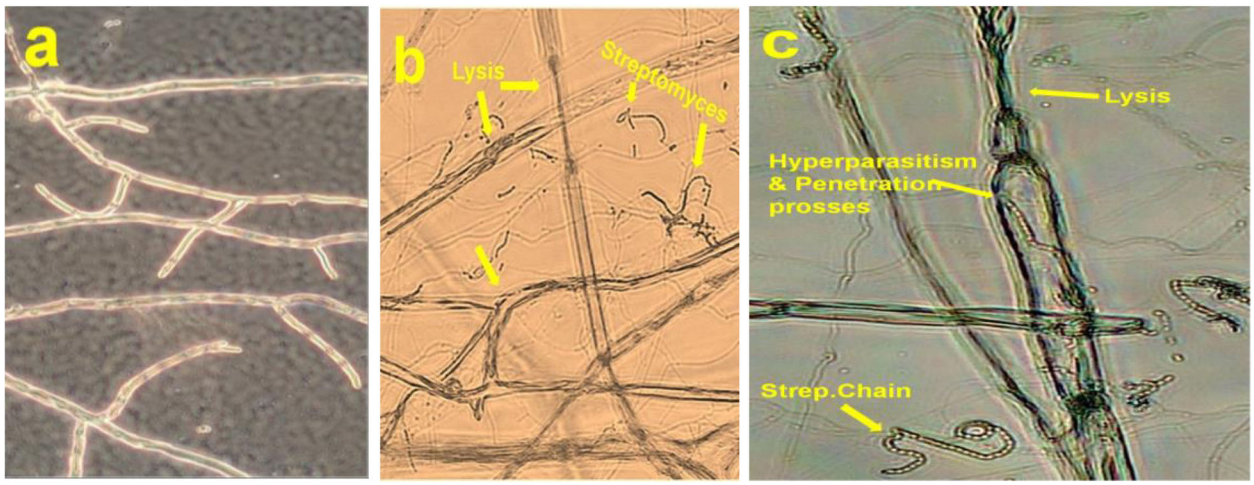

Fig. 4 Light micrograph shows the colonization of *S. rochei* on S. *sclerotiorum* mycelium: (**a**) Untreated mycelium of *S. sclerotiorum*; (**b**) smaller and thinner *S. rochei* mycelium, broken, and distorted of *S. sclerotiorum* mycelium; and (**c**) *S. rochei* growing a long *S. sclerotiorum* mycelium

the hyphae of *S. sclerotiorum*, the devastating soil-borne fungal pathogen.

Antifungal activity of the culture filtrate

The secondary metabolites produced by these 3 *Streptomyces* spp. were extracted and partially purified. Suppression of mycelial growth of *S. sclerotiorum* by culture filtrate of *Streptomyces* spp. proved that they secreted high antifungal activity bioactive secondary metabolites expressed in vitro significant reduction in the growth of pathogenic fungus than in the control treatment. Data in Table 4 showed that the highest percentage of reduction in mycelial growth (84.50%) was obtained when the culture filtrate of *S. sampsonii* was used (Fig. 6).

Inhibitory effects of VOCs produced by *Streptomyces* spp. on mycelium growth of *S. sclerotiorum*

Growth inhibition of *S. sclerotiorum* was tested by the 3 *Streptomyces* spp. The growth inhibition of the pathogen was ranged between 54.50 and 72.54%. *S. rochei* showed the highest inhibition percentage in mycelial growth, followed by *S. griseus* (64.70%) as shown in Table 4. This result indicated that *S. sclerotiorum* was suppressed but not completely killed after exposure to volatile organic compounds (VOCs) of *Streptomyces* spp. Test of growth inhibition in the sealed plates method exhibited that all the *Streptomyces* spp. were able to produce secondary metabolites such as antifungal or VOCs inhibited and deformed the growth of *S. sclerotiorum* mycelium (Figs. 7 and 8).

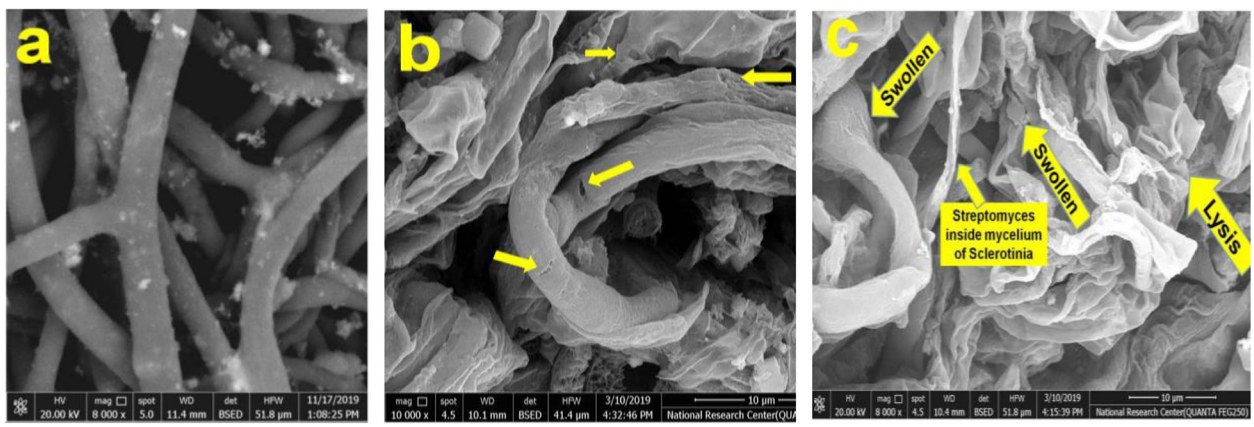

Fig. 5 Scanning electron micrographs of the deformation of the fungal mycelium. (**a**) Untreated mycelium of *S. sclerotiorum*, (**b**) Bioagent *S. rochei* induced pores in the mycelium of *S. sclerotiorum*, and (**c**) *S. rochei* induced swollen, deformation, distortion, and lysis of the pathogen mycelium as well as it is grown inside the hyphae

Fig. 6 Suppression of mycelial growth of S. sclerotiorum by culture filtrate *of S. sampsonii*, *S. rochei*, and *S. griseus*

Impact of *Streptomyces* spp. broth on the fungus sclerotia germination

The 3 antagonistic *Streptomyces* spp. inhibited significantly the viability and sclerotia germination of *S. sclerotiorum* compared with the control. Also, the radial mycelial growth of the germinated sclerotia was reduced after soaking into the spore suspensions of *Streptomyces* spp. separately for 10, 20, and 30 days (Fig. 9). The highest inhibition in mycelial growth of the germinated sclerotia was obtained with *S. sampsonii* by 90.32, 100, and 100%, when soaked for 10, 20, and 30 days, respectively. In this trial, *S. sampsonii*, followed by *S. griseus* proved to be a more inhibitory effect than *S. rochei*, which reduced mycelial growth to 35.32, 42.38, and 90.32%, respectively after same exposure periods (Table 5).

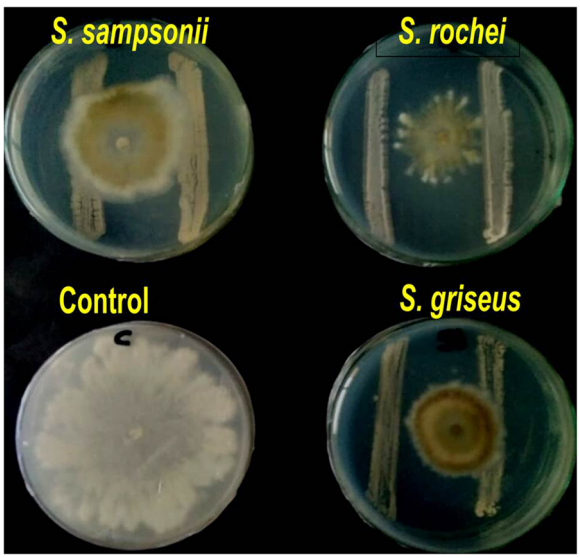

Fig. 7 Effect of volatile organic compounds produced by *Streptomyces* spp. on mycelium growth of *S. sclerotiorum*

Application of the three *Streptomyces* spp. under the field conditions (in vivo)

To check the ability of selected 3 *Streptomyces* spp. to control the white rot disease caused by *S. sclerotiorum*, bioagents were applied on bean plants grown in the field as inocula in the form of suspension (containing cell free extract, mycelia+ spores). The tested *Streptomyces* spp. exhibited a significant reduction of *S. sclerotiorum* in the field. These results indicated a good correlation between in vitro inhibitory action and in vivo activity. Data in Table 4 showed that all *Streptomyces* spp. either in a single form or mixture resulted in a significant reduction in disease incidence compared with check treatment. The lowest intensity of disease incidence was achieved by *S. rochei* DG4 (12%), followed by *S. griseus* DG5 (13%) and (25%) in check treatment (Table 6). Non-significant differences in percentages of disease incidence and percentages of disease reduction were noticed when *S. griseus* DG5 and *S. rochei* DG4 were used separately. A clear significant effect was recorded when the mixtures of *Streptomyces* spp. were used in comparison with using any single isolate. The highest percentages of disease reduction were noticed when the mixtures of DG4 + DG5 or DG1 + DG5 were used, recording 76.00 and 77.33%, respectively. Clear significant deference was noticed when the mixture of the three *Streptomyces* spp. (DG1, DG4, and DG5) compared with any other treatment. Using *S. rochei* (DG4) alone, it proved to be a good protective bioagent and increased the chlorophyll content and pods yield. Also, both of *S. rochei* and *S. griseus* played an important role in controlling *S. sclerotiorum* due to their various modes of action, i.e., hyperparasitism, bioactive metabolites, and volatiles.

Total chlorophyll and yield in the treated plants were measured and compared with the control treatment. Obtained data in Table 5 indicated that all treatments resulted in a reduction of disease incidence and increase in chlorophyll content as well as pods yield than the control. The increase of chlorophyll means increasing in photosynthesis which is reflected as a rise in enzyme level and sugars consequently as well as flourishing in the plant growth and total yield. The relation between reduction in disease incidence and increase of total chlorophyll and yield was very clear when using the most effective treatment (DG1 + DG4 + DG5) in contrast with the control. Further, mixing different isolates increases the scope of the mode of action consequently increases the efficacy of the treatment. Thus, the use of

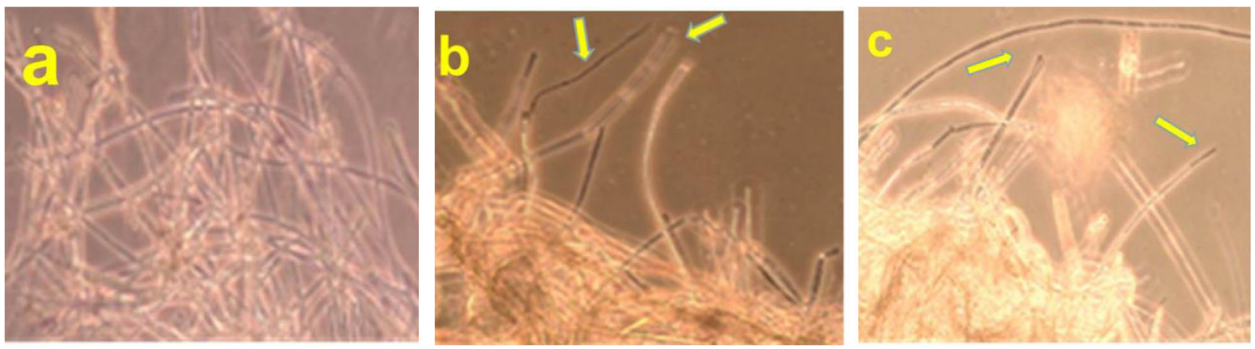

Fig. 8 Light micrograph shows that volatiles compounds of *S. rochei* induced mycelial growth abnormalities in *S. sclerotiorum* by (**a**) mycelium of *S. sclerotiorum* (**b** and **c**) hyphal deformation, swollen, and dark staining indicating hyphae death

biological control agents (*Streptomyces* spp.) is considered as an alternative and sustainable strategy to control *S. sclerotiorum*.

Discussion

White mold disease incited by *S. sclerotiorum* is notably challenging. Thus, the present research aimed to isolate, characterize, and introduce new useful biological agents to manage the disease. Based on the present investigation findings, *Streptomyces* spp. revealed antifungal activity against *S. sclerotiorum* under in vitro conditions as well as successful biocontrol in the greenhouse experiment.

Regarding characterization of the pathogen *S. sclerotiorum*, the results of PCR amplification (553-bp product), sequence, and phylogenetic tree of the Egyptian isolate *S. sclerotiorum* shared with other 15 isolates are in agreement with Prova et al. (2018) as they demonstrated that the PCR amplified 564 bp product in the rDNA-ITS region of the fungal *S. sclerotiorum* isolated from Hyacinth Bean (*Lablab purpureus*). Also, a consistent trend indicated by several investigators highlights the effectiveness of the nuclear ribosomal internal transcribed spacer (ITS) region sequences located between the nuclear small- and large-subunit rRNA genes in species identification (Prova et al. 2018).

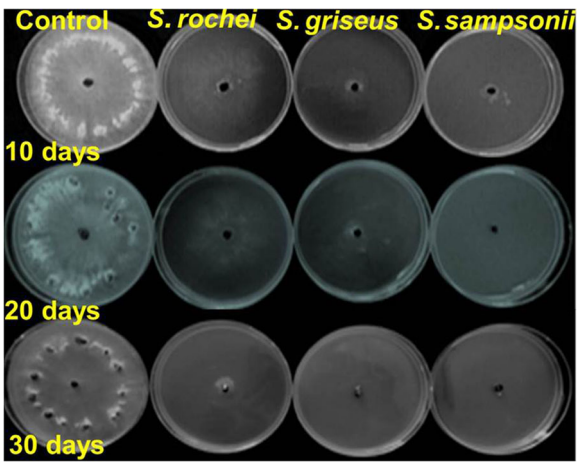

Fig. 9 Suppression of sclerotia germination and mycelial growth after soaking into broth of the three *Streptomyces* spp. for 10, 20, and 30 days

Concerning molecular characterization of the *Streptomyces* spp., the 16S rRNA gene sequences (1300 bp) were checked and aligned, then statistically tested using the Maximum Like Hood method. The aforementioned results indicated that the three isolated *Streptomyces* spp. were closely related together; they had close and/or distant relationships with the nucleotide sequences of available strains in the NCBI GenBank database. These results are in harmony with those observed by Marchesi et al. (1998); Anderson and Wellington (2001); and Rintala et al. (2001), who demonstrated that PCR detection had many features in *Streptomycetes* species identification compared with the morphological, biochemicals, and physiological methods. Further, in consensus with the obtained results, various workers elaborated that the phylogeny tree in the *Streptomycetes* based on 16S rRNA gene sequences proved to be a useful characterization method for determining the relationships among *Streptomycetes* species (Sharma et al. 2020; Yu et al. 2020).

In a preliminary experiment to evaluate antifungal activity against *S. sclerotiorum* under greenhouse conditions, the results proved that the 3 *Streptomyces* spp. might have antifungal activity against *S. sclerotiorum* the causal agent of white disease of the bean. A similar trend was observed by those obtained by Zamoum Miyada et al. (2017) who stated that *Streptomyces* spp. are regarded as significant biological resources, due to their secondary metabolites; these antimicrobial compounds play roles in protecting plants against different pathogens. Furthermore, Srivastava et al. (2015) revealed that *S. rochei* strain SM3 suppressed mycelial growth and disease development of *S. sclerotiorum* at an approximate rate of 74%. In vitro antagonistic activity trials of

Table 5 Effect of soaking *S. sclerotiorum* sclerotia into *Streptomyces* spp. broth on sclerotia germination

Streptomyces spp.	Percentage of reduction in mycelial growth of *S. sclerotiorum* after sclerotia soaking for		
	10 days	20 days	30 days
S. sampsonii DG1	90.32[b]	100[a]	100[a]
S. rochei DG4	35.32[g]	42.38[d]	90.32[b]
S. griseus DG5	67.48[c]	90.32[b]	100[a]
Control	0.00[i]	0.00[i]	0.00[i]
SE	**0.10**		

Values with the same letter are not significantly different

Streptomyces spp. were implemented to ensure the greenhouse results and prove antifungal bioactive compounds to be applied in the field as follows:

In vitro inhibitory potential of *Streptomyces* spp.

Obtained results, when using the dual culture technique, showed that *Streptomyces* spp. could act as biocontrol agents. Such results are consistent with those by Dongli et al. (2019) who declared that *Streptomyces* spp. NEAU-S7GS2 induced a significant inhibitory activity against the mycelial growth of the phytopathogenic fungi *S. sclerotiorum* and *Rhizoctonia solani* affecting soybean (*Glycine max*) plants at a rate of 99.1 and 65.3%, respectively, compared with the control. Moreover, Evangelista-Martínez et al. (2020) showed that the antagonistic activity of the *Streptomyces* isolate CACIS-1.5CA was similar to the commercial strain *Streptomyces lydicus* WYEC 108 against the pathogenic fungi; *Colletotrichum* sp., *Alternaria* sp., *A.* sp., *Botrytis* sp., *Rhizoctonia* sp., and *Rhizopus* sp. with percentages ranging from 30 to 63%. Furthermore, Yu et al. (2020) showed that *S. triticiradicis* caused significant inhibitory effects against 4 phytopathogenic fungi, i.e., *Colletotrichum orbiculare*, *Corynespora cassiicola*, *S. sclerotiorum*, and *Exserohilum turcicum* with an inhibition rate ranging from 48.4 to 69.0%. Further, many investigators stated that *Streptomyces* spp. caused a reduction in mycelial growth of *S. sclerotiorum* due to antifungal substances produced by *Streptomyces* isolates as bioactive compounds and secondary metabolites such as enzymes and antibiotics (Baharlouei et al. 2013).

Examination of the antagonistic activity of the *Streptomyces* spp. upon *S. sclerotiorum* using light and scanning electron micrographs (SEM) supported the potency and efficacy of *S. rochei* as bioagent, which was able to produce secondary metabolites, and penetrated as well as destroyed and lysis the hyphae of the fungal pathogen. Similary, other investigators (Yang et al. 2019) working with *Aspergillus flavus* examined severe destruction of cell membrane integrity, irregular morphological shapes after exposure to VOCs produced by *Streptomyces philanthi* RM-1-138 and *S. alboflavus* TD-1. Moreover,

Table 6 Application of single and/or mixture *Streptomyces* spp. on white rot incidence, total leaf chlorophyll and pods yield in bean plants (under field conditions)

Streptomyces isolate	% White rot disease		Chlorophyll mg/g	Pods yield Ton/ feddan
	Incidence	Reduction		
S. sampsonii DG1	15.00[b]	40.00[e]	23.54	3.00[d]
S. rochei DG4	12.00[c]	52.00[d]	24.68	3.50[c]
S. griseus DG5	13.00[c]	48.00[d]	23.93	2.50[e]
S. sampsonii DG1 plus *S. rochei* DG4	9.00[d]	64.00[c]	25.20	3.90[b]
S. sampsonii DG1 plus *S. griseus* DG5	5.67[e]	77.33[b]	24.22	4.00[b]
S. rochei DG4 plus *S. griseus* DG5	6.00[e]	76.00[b]	26.49	4.00[b]
S. sampsonii DG1 plus *S. rochei* DG4 plus *S. griseus* DG5	3.33[f]	86.67[a]	30.00	5.00[a]
Corporal Max (Fungicide)	6.33[e]	74.67[b]	22.29	2.40[e]
Control	**25.00[a]**	**0.00[f]**	**18.79**	**1.53[f]**

Values with the same letter are not significantly different

Dongli et al. (2019) mentioned that the SEM and light microscopy examination revealed that *Streptomyces* NEAU-S7GS2 led to cell wall degradation in *S. sclerotiorum*, indicating its production of extracellular lysis enzymes. Similarly, Evangelista-Martínez et al. (2020) examined the morphological changes as irregular membrane border, deformation, shrinkage, and collapsed conidia of postharvest fungal pathogens such as *Alternaria* sp., *Botrytis* sp., *Rhizoctonia* sp., and *Colletotrichum musae* after exposure to bioactive extract of *Streptomyces* sp. CACIS-1.5CA.

Antifungal activity of the culture filtrate

The applications of *Streptomyces* spp. culture filtrate in the present experiments demonstrated their ability to reduce mycelial growth of *S. sclerotiorum* due to their secretion of secondary metabolites. It could be attributed to the fact that *Streptomyces* spp. produce a variety of extracellular hydrolytic enzymes (degrading cell wall enzymes such as extracellular hydrolytic enzymes) (degrading cell wall enzymes such as cellulases, hemicellulases, chitinases, amylases, and glucanases) and antibiotics as secondary metabolites (Elleuch et al. 2010). Further, Kanini et al. (2013) tested various *Streptomyces* isolates from diverse Greek habitats for their antifungal activity against the common phytopathogenic fungus *Fusarium oxysporum* f. sp. *lycopersici* affecting tomato. They mentioned that the isolate was characterized molecularly as *S. rochei* ACTA1551 strongly suppressed the fungal growth when examined in antagonistic bioassays in vitro. Also, *S. rochei* secreted antimicrobial compounds able to inhibit the growth of all common microbial indicators (*Escherichia coli*, *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Saccharomyces cerevisiae*). Additionally, Li et al. (2018) stated that the antifungal activity of *S. sampsonii* crude extract inhibited the hyphal growth and destroyed fungal cell walls of 4 different pathogenic fungi, i.e., Cylindrocladium *scoparium*, *Cryphonectria parasitica*, *Neofusicoccum parvum*, and *Fusarium oxysporum*). Nevertheless, many researchers recorded that the Actinobacteria proved to be great importance in the field of biotechnology, as producers of benefits and bioactive secondary metabolites, i.e., antifungal, insecticidal, antibacterial, enzymes such as chitinase, antibiotics, and antihelminthic activities with extensive industrial, medical use, and as a tool for eco-friendly management in agricultural applications of plant pathogens (Katarzyna et al. 2018; Salwan and Sharma 2020; and Sharma et al. 2020).

Inhibitory effects of VOCs produced by *Streptomyces* spp. on mycelium growth of *S. sclerotiorum*

All of the tested *Streptomyces* spp. showed more or less antifungal activity against the pathogen both on Petri plate and pot assays. In the same respect, Wu et al. (2015) revealed that volatile organic compounds produced by *Streptomyces* spp. were reported to induce growth abnormalities in *S. sclerotiorum*. The produced VOCs by *S. albulus* NJZJSA2 not only inhibited *S. sclerotiorum* mycelial growth by 100% but also significantly inhibited the sclerotia germination. Similarly, Cordovez et al. (2015), in their studies on *Streptomyces* spp. over a disease-suppressive soil, identified Actinobacteria as the most dynamic phylum in a soil suppressive to the fungal root pathogen *Rhizoctonia solani*. All *Streptomyces* strains were able to significantly retard the growth of *R. solani*, an economically important soil-borne fungal pathogen of many crops. VOCs produced by *S. canus* strains (W47 and W214) were the most inhibitory of radial hyphal growth at a rate of 57 and 41%, respectively, and significantly enhanced plant shoot and root biomass. On the other hand, some investigators discussed that this phenomenon depends on the diversity of the pathogens that have different levels of resistance or susceptibility to VOCs (Gotorvila et al. 2017).

Impact of *Streptomyces* spp. broth on the fungus sclerotia germination

Application of the 3 antagonistic *Streptomyces* spp. inhibited significantly the viability and sclerotia germination, which reflected on the growth of the mycelium. The result in consensus with Errakhi et al. (2007), who stated that the highest inhibition in sclerotial germination of *Sclerotium rolfsii* was obtained by streptomyces isolate J-2 by 93 and 88%, when applied as biomass and filtrate culture, respectively. While the lowest inhibition in sclerotial germination was obtained with the isolate B-5 which was 47, 44, and 17% with biomass inocula, culture filtrate, and spore suspension, respectively. These results are consistent with those recorded by Dongli et al. (2019) who revealed that *Streptomyces* sp. NEAU-S7GS2 completely inhibited sclerotia germination *S. sclerotiorum*. Furthermore, Smoliñska and Kowalska (2018) indicated that the antibiosis is likely to be a major mechanism employed by *Streptomyces* spp. on control *S. sclerotiorum* sclerotia. Certain bacterial genera can be used as a biological control against *S. sclerotiorum* by inhibiting the germination of ascospores by the production of antimicrobial substances or direct growth on the ascospores (Huang and Erickson 2008).

Application of the three *Streptomyces* spp. under the field conditions (in vivo)

Field experiments clearly showed that *Streptomyces* spp. had the potential to be used as highly effective biocontrol agents due to their various beneficial properties against White mold disease and contribution in enhancement of plant growth as well as they are inexpensive, long lasting, and safe toward the environment and

living organisms. Relevant to this work, the results obtained by several investigators, highlighted the contribution of *Streptomyces* genera to plant growth and protection. Another role of *Streptomyces* has been revealed by Jogaiah et al. (2016) who coated the seeds of the pearl millet with *S. griseus* SJ_UOM-07-09 and *Streptosporangium roseum* SJ_UOM-18-09 to protect the plants against downy mildew disease caused by *Sclerospora graminicola*. The two strains possessed a direct anti-mildew activity in inhibiting the sporangial formation, zoospore release from sporangia, and enhanced the induced resistance to protect plants from disease. Application of (DG1 + DG4 + DG5) treatment led to a reduction in disease incidence and noticeable total chlorophyll and yield increase compared to control. Furthermore, mixing different isolates improved treatment efficacy. Thus, *Streptomyces* spp. proved to be as an alternative and sustainable strategy to control *S. sclerotiorum*. This result supports the earlier explanations that the mixtures of bio-agents with different mechanisms in disease control will have an additive efficient impact in improving disease control compared to their separate application (Huang and Erickson 2008).

Conclusions

Obtained findings demonstrated that *Streptomyces* spp. (*S. griseus*, *S. rochei*, and *S. sampsonii*) had a great potential to be used as biocontrol agents against *S. sclerotiorum* in both in vitro and in vivo. The 3 isolates seemed to be a promising contribution to the development of new methods for the management of plant diseases and reducing the deleterious impacts of using chemical pesticides in the future.

Abbreviations

PCR: Polymerase chain reaction; rDNA-ITS: Ribosomal DNA internal transcribed spacer; SEM: Scanning electron microscope; *S.*: *Sclerotinia*; Spp.: Species; StNA: Starch nitrate agar; S: *Streptomyces*; VOCs: Volatile organic compounds

Acknowledgements

We would like to acknowledge Dr. Ahmed Abd-El Aziz Kheder, who provided a profound contribution in the part of molecular biology.

Authors' contributions

GG suggested the plan of study and followed up on the carried-out work in addition to scripting the manuscript. DG executed the plan of work, analyzed the output data, and contributed in writing the manuscript. AA assisted in writing the manuscript. MR reviewed the manuscript. NS and TA helped in the design of the work plan until they both, sadly, passed away. All authors except NS and TA (passed away) read and approved the final manuscript.

Funding

The authors declare that the current study is self-funded.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 19 October 2020 Accepted: 28 January 2021
Published online: 15 February 2021

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