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ANTIFUNGAL ACTIVITY OF TWO *STREPTOMYCES* SPECIES ISOLATED FROM EGYPTIAN SOIL AGAINST SOME PHYTOPATHOGENIC FUNGI

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ABSTRACT

The objective of this study was to isolate and identify *Streptomyces* species with high antifungal activities against some phytopathogenic fungal strains. Fourteen different *Streptomyces* isolates were isolated from the soils of the Damietta Governorate, Egypt. The results indicated that two isolates (O5 and M7) showed potent antifungal actions against the tested fungal strains including *Alternaria alternata*, *Macrophomina phaseolina*, *Fusarium oxysporum*, *Penicillium italicum*, *Rhizoctonia solani* and *Trichoderma harzianum*. The strongest inhibition zones of the isolates O5 and M7 were obtained against *A. alternata* (3.5±0.04 cm and 3.63±0.02 cm, respectively) and *M. phaseolina* (3.23±0.03 cm and 3.43±0.01 cm, respectively). The two isolates were identified classically and using 16S rDNA sequencing as *Streptomyces flavovirens* (accession number: EGDA-O5 OM301640) and *S. gougerotii* (accession number: EGDA-M7 OM301639). The obtained results confirmed that *S. flavovirens* and *S. gougerotii* might be used as promising antifungal agents against various fungal strains.

Keywords: *Streptomyces; Actinomycetes; Characterization; Molecular identification; Antifungal activity*

INTRODUCTION

According to **Strange and Scott (2005)**, plant infections are a significant issue for plant cultivation and are to blame for 10% of the world's agricultural production loss. Typically, chemical fungicides are used to eradicate fungi, which are among the most destructive plant infections. An alternate, ecologically benign method of managing phytopathogens is provided by biological control **(Kishore et al., 2005; Prapagdee et al., 2008; Sharma et al., 2011)**. Both host-specific infections and microbial antagonists are used to reduce plant diseases and manage weed populations. The biological control agent (BCA) is the organism that inhibits the pathogen **(Vurukonda et al., 2018)**. In a broader sense, the use of naturally occurring compounds that have been isolated or fermented from diverse sources has also been referred to as biological control. Numerous microbial antagonists have been studied as possible plant disease biocontrol agents that combat fungal infections. Actinomycetes are widely recognized as biocontrol agents that suppress or lyse a variety of soil-borne and airborne plant pathogenic fungus. This is notably true of species from the genus *Streptomyces* **(El-Tarabily, 2006; Lee et al., 2008; Sousa et al., 2008)**. The only member of the family Streptomycetaceae (Phylum: Actinobacteria, Order: Actinomycetales) is the genus *Streptomyces*, which contains more than 500 species of Gram-positive, filamentous bacteria **(Anderson and Wellington, 2001)**. They are extensively found in the rhizosphere and soil, accounting for around 40% of soil bacteria **(Vetsigian et al., 2011; Viaene et al., 2016)**. Streptomycetes are extensively found in soil, water, and other natural settings **(Seong et al., 2001; Singh et al., 2006)**. A variety of physical, chemical, and biological factors influence the population of the spores within an ecosystem **(Kharat et al., 2009)**. According to **Wang et al. (1999)**, the identification of novel ecological systems is consequently essential for the discovery of novel Streptomycetes. *Streptomyces* spp. are the many microbial populations in dry, alkaline soil that, due to their filamentous shape, give the soil strength and shield it from erosion by wind and rain **(Vetsigian et al., 2011)**.
The present study reported on the isolation, characterisation, and inhibitory activities of local isolates of *Streptomyces* that were tested against several phytopathogenic fungi.

MATERIALS AND METHODS

Materials

Phytopathogenic fungal strains

Tested phytopathogenic fungi, *Alternaria alternata* Fr. Keissler, *Penicillium italicum* CECT 2294, *Fusarium oxysporum* f. sp. *lycopersici* Fol4287, *F. solani* DSM 62413, *Rhizoctonia solani* (JG Kühn) AG4, *Sclerotinia sclerotiorum* (Lib.) de Bary, *Aspergillus niger van Tiegh niger*, *A. fumigatus Fresenius*, *A. flavus Link ex Fries group*, *A. nidulans* NK002, *A. terreus* ATCC 20542, *Trichoderma harzianum* T-203 and *Rhizopus stolonifer* NBRC 30816 were obtained from Microbiology laboratory, Department of Botany and Microbiology, Faculty of Science, Damietta University, Egypt. *Macrophomina phaseolina* (Tassi) Goid. was obtained from Plant Diseases Laboratory, Faculty of Agriculture, Mansoura University, Egypt. The phytopathogenic fungal strains were grown on potato-dextrose agar medium at 30°C for five days for further work.

Methods

Isolation and purification of *Streptomyces*

Random soil samples were taken from a few locations inside the Egyptian governorate of Damietta. Soil samples were taken using a shovel and put in fresh polythene bags. The methodology used for sampling followed **Johnson et al. (1959)** and **Muratova et al. (2003)** methods. To isolate *Streptomyces*, the dilution agar plating method as reported by **Johnson et al.** in **1959** was employed. Eighteen milliliters of sterile distilled water were mixed with two grams of soil samples under aseptic conditions. After vortexing the suspension, sterile distilled water was added to dilute it. 150µl aliquots of each dilution were distributed on the surface of every starch-nitrate medium in turn. For seven days, the plates were incubated at 28°C. The formed single colonies of *Streptomyces*, which varied in size and color, were removed from the incubation time and streaked on brand-new starch-nitrate agar plates to purify them. For future usage, the refined *Streptomyces* isolates were routinely subcultured and kept on starch-nitrate agar slants at 4°C.

Detection of antifungal activities from *Streptomyces species*

Agar well diffusion method

The obtained *Streptomyces* isolates were coded as O5 and M7. The O5 isolate was grown in conical flasks containing starch-nitrate broth medium **(Waksman, 1959)**. The pH of the media was adjusted to 7.2 ± 0.2 using NaOH and HCl (1M). While M7 was grown in conical flasks containing glycerol-yeast extract medium and pH was 7.0 **(Waksman, 1961)**. Flasks were inoculated using spore suspension (1×10^6 cell/ml) and incubated at 30°C with shaking at 150 rpm for 5 to 7 days. Subsequently, the cultures were gathered and centrifuged for 20 minutes at 4000 rpm. The supernatants were then promptly transferred and filtered through a Millipore filter (0.45µm) to get a supernatant free of cells. To investigate antifungal activity, 100–300 µl of the supernatant were evaluated in an agar diffusion assay against all identified phytopathogenic fungi **(Rabah et al., 2007; Atta et al., 2009)**. All the antifungal activities were assayed in triplicate.

Classical identification and Characterization of the most active actinomycetes

In order to examine the cultural characteristics of the experimental isolates, the isolates were cultured on a variety of media, including CM-I agar, Oatmeal agar, Glycerol-Yeast Extract Agar, Glycerol-Yeast Extract Agar, Starch-Casein Agar, Czapek-dox Agar, and starch-nitrate Agar. Based on Waksman (1961) and Bergey's Manual of Systematic Bacteriology **(Goodfellow et al., 2012)**, the most active isolates, O5 and M7, were identified. Additionally, their morphological, physiological, biochemical, and cultural characteristics were considered.

Morphological characteristics

The morphology of spore chains

For microscopical observation, the colonies of isolates were cultivated on starch-nitrate **(Waksman, 1959)** and glycerol-yeast extract agar media **(Waksman, 1961)** by simple "Inclined cover slip culture technique" **(Gibbs and Shapton, 1968)**. The coverslip was carefully withdrawn and placed directly onto light microscopy using a magnification of range of 200-400X. Microphotographs range were produced using a Karl-Zeiss-Jena microscope.

Electron-microscopy of spores

Samples for electron microscopy were prepared using the spore print technique. Without further treatment, sporulating starch nitrate and glycerol-yeast extract cultures were detected using transmission electron microscopy (TEM) with colloidal film grids gently placed over the surfaces **(El-Zahed et al., 2021)**. First, the grids were vacuum-shadowed with chromium for scanning electron microscopy (SEM) at 100 kV (JEOL-100 CX electron microscope). Actinomycetes mycelia and spores were examined for size and shape using a scanning electron microscope (SEM) operating at 15 kV (JEM-1400 Plus unit, Alexandria University).

16S rDNA sequencing and phylogenetic analysis

Genomic DNA extraction

The highly active isolates O5 and M7 had their bacterial genomic DNA extracted using a modified version of the phenol/chloroform procedure **(Ausubel et al., 2003)**. A 1.5 ml overnight culture was centrifuged for 5 minutes at 6,500 rpm. After breaking down the pellet and denaturing the cells with liquid nitrogen to extract DNA, the samples were combined and added to 0.8 ml to 1 ml of SET buffer (20% sucrose, 50 mM Tris HCl, pH 7.6, 50 mM ethylene diamine tetra acetic acid (EDTA). After adding 50µL of 10% Sodium dodecyl sulphate (SDS), the mixture was incubated for 60 minutes at 70°C with 15-minute interval vortexing. Introduced 50µL–100µL NaCl (5M) after incubation. 500 µL of a phenol/chloroform/isoamyl alcohol solution (25:24:1) was used to extract DNA, which was then precipitated with isopropanol and cleaned with 75% cold ethanol. The DNA was then suspended in 1/10 TE buffer and kept at -20°C until it was needed for DNA sequencing.

Agarose gel electrophoresis and DNA detection

Using 1% agarose gel electrophoresis dissolved in TAE buffer pH 8.0 (0.04 M Tris-acetate and 0.001 M EDTA), the genomic DNA was analyzed. To the DNA staining, 2µL of ethidium bromide (10 mg/mL) was added. Typically, the sample was mixed with 2 µL of loading buffer (0.25% bromophenol blue, 70% glycerol, and 10 mM Tris-HCl pH 7.0). A UV transilluminator was used to visualize the DNA on the gel.

DNA sequencing

Prokaryotic universal primer pairs (27F: 5'AGAGTTTGATCMTGGCTCAG3' and 1492R: 5'TACGGYTACCTTGTTACGACTT3') were utilized to amplify the 16S rRNA gene **(Ausubel et al., 2003)**. Using the same earlier primers, Macrogen Co., Korea sequenced the amplified PCR result.

Alignment and phylogenetic analyses

The generated 16S rDNA sequence was sent to BLAST **(Altschul et al., 1990 and 1997)** to find the best similarities with other related sequences in the database. The NCBI GenBank provided the strongest DNA sequence similarities with the 16S rDNA region, which were then aligned using CLUSTAL Omega **(Sievers et al., 2011)**. Sequences belonging to the same species and unidentifiable organisms were removed, and unalienable areas were manually excluded. Lastly, MEGA version 4 was used to visualize and interpret phylogenetic tree analyses **(Tamura et al., 2007)**. We used maximum composite likelihood approaches for neighbor-joining. According to **Tamura et al. (2007)**, only values 20 or above were taken into account and displayed next to the branches of the phylogenetic tree with confidence levels determined using 1000 bootstrap replicates.

Statistical analysis

The Statistical analysis was performed using the SPSS version 22. One-way analysis of variance ANOVA was done followed by the Tuckey' test in order to determine the significant difference between means. All the data were presented as mean ± SE and differences were regarded as statistically significant when $p < 0.05$ **(Aldrich, 2018)**.

RESULTS

Isolation, cultivation, and primary characterization of the actinomycetes

Fourteen actinomycetes species were isolated from the soils of the Damietta Governorate, Egypt. Nine isolates had white color and five isolates were grey to light grey color. All the isolates had a powdery appearance on starch-nitrate agar media except M7 had a leathery appearance (Table 1). All actinomycetes isolates were grown on starch-nitrate agar medium at 28°C±2 except one isolate M7 could grow only on glycerol-yeast agar medium at 35°C±2. Identification of actinomycetes was made on the basis of cultural, morphological, physiological, biochemical, and DNA properties.

Table 1 Collection sites of soil samples and color grouping of the actinomycetes isolates.

Sample No.	Code of isolate	Color of aerial mycelia	Site of sample	Cover plant
1	O5	Grey	Alruwda	*Citrus sinensis*
2	La5	white	Alruwda	*Citrus aurantium*
3	Gnl1	grey	Alsnania	*Citrus limon*
4	Gnk3	grey	Alsnania	*Pyrus communis*
5	O6	white	Alruwda	*Citrus sinensis*
6	8F	Light grey	Kafr saad	*Citrus limon*
7	9B	white	Kafr saad	*Citrus limon*
8	Ma11	white	Alruwda	*Mangifera indica*
9	7F	white	Kafr saad	*Mangifera indica*
10	E2	white	New Damietta	*Citrus reticulata*
11	M7	white	Alsnania	*Mangifera indica*
12	E4	white	New Damietta	*Mangifera indica*
13	E6	white	New Damietta	*Citrus reticulata*
14	6F	Light grey	Kafr saad	*Mangifera indica*

Antifungal activity of selected actinomycetes against tested phytopathogenic fungi

Screening for the ability of the isolated actinomycetes to produce antifungal metabolites was carried out using a plate detection system. These fourteen actinomycetes were tested against some phytopathogenic fungi (*P. italicum*, *M. phaseolina*, *F. oxysporum*, *F. solani*, *A. alternata*, *R. solani*., *S. sclerotiorum*, *A. niger*, *A. fumigatus*, *A. flavus*, *A. nidulans*, *A. terreus*, *T. harzianum* and *R. stolonifera*) and showed antifungal activity as indicated by inhibition zones (Table 2 & Figure 1). The effectiveness of different isolates of actinomycetes against tested phytopathogenic fungi varied widely as shown in Table 2. Some of these isolates showed antifungal activity against most of the tested fungi. In primary screening, there were some of the tested actinomycetes could inhibit the growth of some phytopathogenic fungi. Isolates coded O5, Gnl1, M7 and 8f have the highest antifungal activity against most tested pathogenic fungi in different degrees. The isolates O5 could inhibit the growth of some tested fungi, the strongest inhibition zones were obtained against *A. alternata* and *M. phaseolina* (3.5±0.04 cm and 3.23±0.03 cm, respectively) and could moderately inhibit the growth of *P. italicum*, *F. oxysporum*, *F. solani*, *T. harzianum* and *R. solani* (1.96±0.02 cm, 2.0±0.05 cm, 1.8±0.03 cm, 2±0.04 cm and 1.56±0.03 cm, respectively). Isolate O5 had no antifungal activity against other tested phytopathogenic fungi. The isolate La5 had a moderate antifungal activity against *F. oxysporum* and *F. solani* (2±0.05 cm and 1.8±0.03 cm, respectively) and weak antifungal activity against *A. alternata* (1.5±0.03 cm). The isolate Gnl1 had moderate antifungal activity against

P. italicum (1.4±0.05 cm), *S. sclerotiorum* (1.23±0.01 cm), *A. alternata* (1.36±0.05 cm) and *M. phaseolina* (1.66±0.03 cm) while isolate Gnk3 showed little antifungal activity against only *P. italicum* (1.16±0.06 cm). The isolate M7 had the highest antifungal activity against *M. phaseolina* and *A. alternata* (3.43±0.01 cm and 3.63±0.02 cm) respectively. It also could inhibit the growth of *P. italicum*, *F. oxysporum*, *F. solani* (2±0.03 cm, 1.73±0.03 cm and 1.86±0.06 cm, respectively). Isolate 8F, had moderate antifungal activity against *M. phaseolina* (2.43±0.01 cm) and *A. alternata* (2.7±0.05 cm) and weak antifungal activity against *P. italicum* and *R. solani*. The isolate O6 showed little antifungal activity against only *A. alternata* and the isolate Ma11 showed moderate antifungal activity against *M. phaseolina* only. Other tested actinomycetes isolates had no antifungal activity against tested phytopathogenic fungi. Finally, from these results isolates O5, Gnl1, 8F and M7 have shown good antifungal activity as indicated by inhibition zones against phytopathogenic fungi (*P. italicum*., *M. phaseolina*, *F. oxysporum*, *F. solani*, *A. alternata*, *S. sclerotiorum* and *R. solani*. The most affected phytopathogenic fungi by actinomycetes isolates are *M. phaseolina* and *A. alternata*. So, we selected the actinomycetes isolates O5 and M7 for further work against *M. phaseolina* and *A. alternata*.

Table 2 Effect of actinomycete isolates on growth of tested phytopathogenic fungi. Values are the diameter of the inhibition zone (cm). Each value is the mean of three replicates±SE.

Tested Fungi	Isolated actinomycetes													
	O5	La5	Gnl1	Gnk3	O6	M7	8F	9B	Ma11	7F	E2	6F	N4	N6
A. alternata	3.5±0.04	1.5±0.04	1.36±0.05	-	1.33±0.01	3.63±0.02	2.7±0.05	-	-	-	-	-	-	-
A. flavus	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. fumigatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. nidulans	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. terreus	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F. oxysporum	2±0.05	1.86±0.03	-	-	-	1.73±.03	1.46±.06	-	-	-	-	-	-	-
F. solani	1.8±0.03	1.93±0.02	-	-	-	1.86±0.06	1.66±0.07	-	-	-	-	-	-	-
M. phaseolina	3.23±0.03	-	1.66±0.03	-	-	3.43±0.01	3.43±0.05	-	1.86±0.03	-	-	-	-	-
P. italicum	1.96±0.02	-	1.4±0.05	1.16±0.06	-	2±0.03	1.26±0.02	-	-	-	-	-	-	-
R. solani	1.56±0.03	-	-	-	-	-	1.6±0.05	-	-	-	-	-	-	-
R. stolonifera	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. sclerotiorum	-	-	1.23±0.01	-	-	-	-	-	-	-	-	-	-	-
T. harzianum	2 ±0.04	-	-	-	-	-	-	-	-	-	-	-	-	-

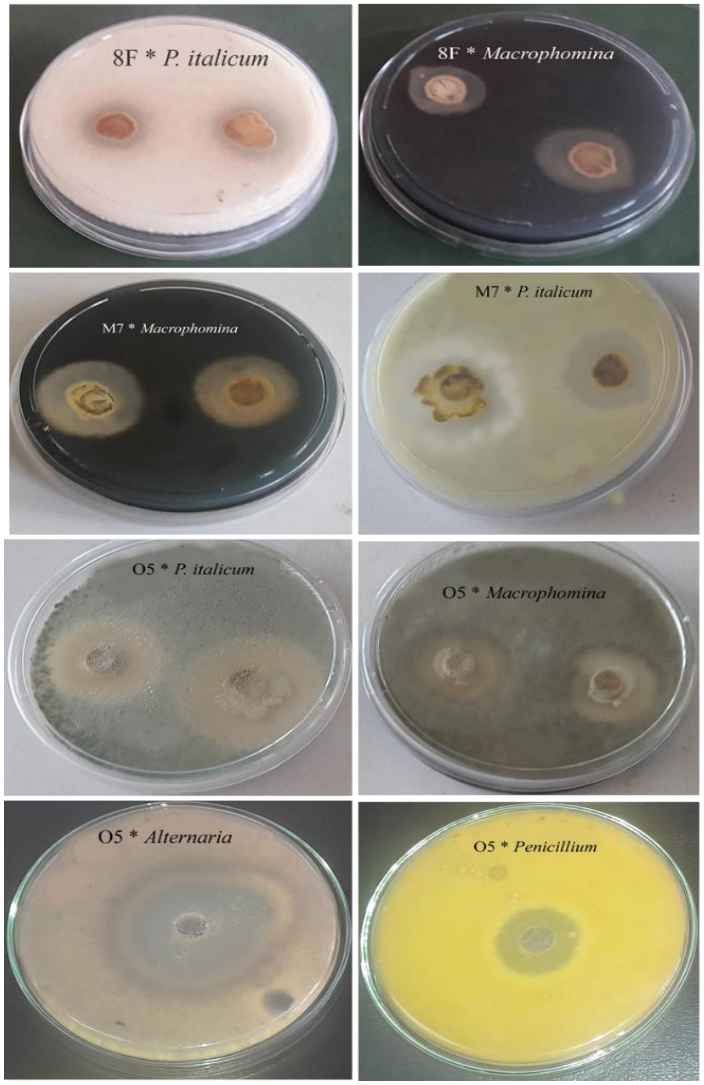

Figure 1 Effect of some actinomycete isolates on growth of some tested phytopathogenic fungi.

Biological characteristics and identification of the isolates O5 and M7

The spore chain morphology of isolates O5 and M7 were straight to flexuous sporophore, as indicated by electron microscope and light microscope (Figure 2, A & B). Spore surface of isolate O5 was smooth with a diameter of 466.6 X 838.63 nm while the isolate M7 spore surface was smooth with a diameter of 688.38 X 488.50 nm (Figure 2, C & D). Tables 3-6 displayed the morphological, cultural, and some physiological characteristics of the isolates O5 and M7, as well as the cultural attributes of 7-day-old cultures on various media and the use of various carbon and nitrogen sources.

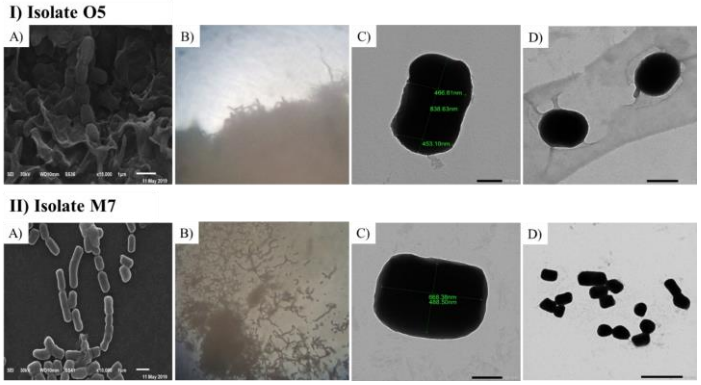

Figure 2 Characterization of the isolates O5 and M7. (A) SEM of sporophore. (B) light microscope of sporophore. (C) & (D) TEM of spore surface.

Table 3 The cultural characteristics of isolate O5 cultures that are 7 days old on various mediums

Media	Type of growth	Color			Growth intensity	pH sensitivity of pigment	
		Aerial mycelium	Substrate mycelium	Medium pigmentation		NaOH	HCl
Starch nitrate agar	Powdery	Grey	Brownish grey	Brown	+++	-	+ve
Starch casein agar	Powdery	Faint Grey	Faint grey	-	+	+ve	-
Starch amm.sulphate agar	Powdery	Grey	Brownish	-	+++	-	-
Czapex- Dox agar	Powdery	White	White	-	Weak growth	-	-
Glycerol yeast extract	leathery	Buff	buff	-	Weak growth	-	-
Glycerol asparagine agar	Powdery	Yellowish White	White	-	+	-	-
Oatmeal agar	Powdery	Grey	Brownish	-	++	-	-

Tabel 4 The cultural properties of 7 days old cultures of isolate M7 on different media

Media	Type of growth	Color			Growth intensity	pH sensitivity of pigment	
		Aerial mycelium	Substrate mycelium	Medium pigmentation		NaOH	HCl
Starch nitrate agar	Powdery	White	White	-	++	-	-
Starch casein agar	Powdery	White	White	-	Low growth (Traces)	-	-
Starch amm.sulphate agar	Powdery	White	Yellowish	-	++	-	-
Czapex- Dox agar	Powdery	White	White	-	Low growth	-	-
Glycerol yeast extract	Powdery	White	Yellowish brown	Faint yellow	+++	-	-
Glycerol asparagine agar	Powdery	White	Yellowish brown	-	++	-	-
Oatmeal agar	Leathery	Faint white	Yellowish brown	Faint yellow	++	+ve	-

Table 5 Utilization of different carbon sources during the growth of tested isolates

Carbon Sources	Isolate O5	Isolate M7	Nitrogen Sources	Isolate O5	Isolate M7
D-glucose	+ve	+ve	Potassium nitrate	+ve	+ve
D-fructose	+ve	+ve	L-phenylalanine	+ve	+ve
D-mannose	+ve	+ve	L-methionine	+ve	++ve
L-arabinose	+ve	+ve	L-histidine	+ve	+++ve
D-xylose	+ve	+ve	Peptone	+ve	+++ve
L-rhamnose	+ve	+ve	Tryptone	+ve	++ve
Sucrose	-ve	+ve	Casein	+ve	-ve
Raffinose	-ve	+ve	Acmonium sulphate	+ve	-ve
D-mannitol	+ve	+ve	L-ydroxyproline	+ve	-ve
Meso-nositol	+ve	+ve			
Cellulose	-ve	-ve			

Table 6 Cultural, morphological and some physiological properties of the isolates

Test	Isolate O5	Isolate M7
Aerial mass color	Grey	White
Melanoid pigment	None	None
Reverse side pigment	Brownish grey	Yellowish brown
Soluble pigment	Brown	Faint yellow
Spore chain	Straight to flexuous	Straight
Spore surface	Smooth	Smooth
Liquification of gelatin	+ ve	+ve
Coagulation of milk	+ ve	+ve
Hydrolysis of starch	-ve	-ve
Decomposition of cellulose	-ve	-ve

Phylogenetic analysis

The morphological, molecular, and physiological studies of isolate O5 and M7 indicated that these strains should be classified in the genus *Streptomyces*, after following the information provided in Bergey's Manual of Systematic Bacteriology **(Goodfellow et al., 2012)**. The DNA of the isolates was prepared, and purified and then the region of 16S rRNA was amplified using either universal primers or specific-specific primers. The nucleotide sequence was analyzed with the previously recorded sequences using BLASTN analysis and compared for homology to known sequences currently available in the NCBI database and the most significant alignment is illustrated. BLASTN analysis of isolate O5, indicates that the experimental isolate 16S rRNA sequences showed from 99.9 % to 100% sequence homology to different *Streptomyces* strains and clustered in a way with *Streptomyces flavovirens* with dissimilarity reached 1.5%. The M7 experimental isolate 16S rRNA sequences exhibited close identity and reached 99.7 % with different *Streptomyces* strains and clustered a way with *Streptomyces gougeroti* with dissimilarity reached to 1.0%. The main morphological, physiological and molecular properties of isolate O5 and M7 indicate that they are more related to *Streptomyces flavovirens* and *S. gougerotii* respectively. The strains code, accession numbers, and homology percentages are represented in Tables 7 & 8. The sequences were also subjected to phylogenetic analysis. Phylogenetic trees were constructed to illustrate the relation between strains in Figures 3 & 4.

Table 7 Alignment identity % of *Streptomyces* sp. isolate O5 with the other related strains found on the NCBI database.

Strain	Query Cover %	Identity %	Accession No.
S. rochei strain NRRL B-1559	100%	100.00%	NR_116078
S. enissocaesilis strain NRRL B-16365	100%	100.00%	NR_115668
S. vinaceusdrappus strain NBRC 13099	100%	100.00%	NR_112368
S. plicatus strain NBRC 13071	100%	100.00%	NR_112357
S. geysiriensis strain NRRL B-12102	100%	99.93%	NR_043818
S. viridosporus strain CSSP718	100%	99.93%	NR_043366
S. mutabilis strain NRRL ISP-5169	100%	99.72%	NR_044139
S. vinaceusdrappus strain NRRL 2363 e	100%	99.65%	NR_043383
S. violaceorubidus strain LMG 20319	100%	99.08%	NR_042309
S. flavoviridis strain NBRC 12772	100%	99.08%	NR_041218
S. virens strain NBRC 15901	100%	98.87%	NR_112480
S. viridochromogenes strain NBRC 13347	100%	98.73%	NR_112526
S. violaceoruber strain NBRC 12826	100%	98.66%	NR_112292
S. griseoviridis strain NBRC 12874	100%	98.66%	NR_112313
S. viridiviolaceus strain NBRC 13359	100%	98.59%	NR_112378
S. flavovirens strain NBRC 12771	100%	98.50%	NR_112269
S. flavovirens strain NRRL B-2685	100%	98.50%	DQ_026635

Table 8 Alignment identity % of *Streptomyces* sp. isolate M7 with the other related strains found on the NCBI database.

Strain	Query Cover %	Identity %	Accession No.
S. sampsonii Strain NBRC 13083	99%	99.79%	NR_11236
S. coelicolor Strain NBRC 12854	99%	99.79%	NR_112305
S. limosus Strain NBRC 12790	99%	99.79%	NR_112279
S. felleus Strain NBRC 12766	99%	99.79%	NR_112266
S. globisporus subsp. Caucasicus Strain NBRC 100770	99%	99.72%	NR_112593
S. champavatii Strain NBRC 15392	99%	99.65%	NR_112451
S. daghestanicus Strain NRRL B-5418	99%	99.58%	NR_043816
S. violascens Strain CSSP183	98%	99.64%	NR_043338
S. intermedius Strain NBRC 13049	99%	98.73%	NR_041103
S. gougerotii Strain DSM 40324	99%	98.59%	NR_119348
S. gougerotii Strain NBRC 13043	99%	98.52%	NR_112610
S. rutgersensis Strain NBRC 3727	99%	98.52%	NR_112495.1
S. violaceus Strain NBRC 13103	99%	98.52%	NR_041115.1
S. roseoviolaceus Strain NBRC 13081	99%	98.52%	NR_112361.1
S. levis Strain NRRL B-16370	99%	98.38%	NR_115778.1
S. arenae Strain NBRC 13016	99%	98.24%	NR_112609.1
S. griseoviridis Strain NBRC 12874	99%	98.24%	NR_112313.1
S. mutabilis Strain NRRL ISP-5169	99%	98.17%	NR_044139.1
S. griseostramineus Strain NBRC 12781	99%	98.10%	NR_041067.1
S. griseomycini Strain NBRC 12778	99%	98.10%	NR_041065.1
S. graminearus Strain LMG 19904	99%	98.10%	NR_042296.1

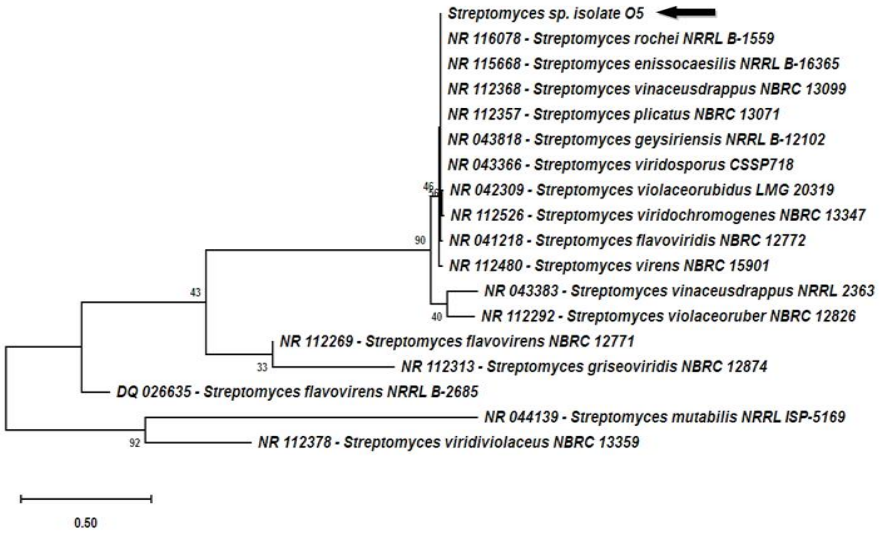

Figure 3 Phylogenetic tree of *Streptomyces* O5 with some other related based on *Streptomyces* species based on 16S rRNA sequence alignment.

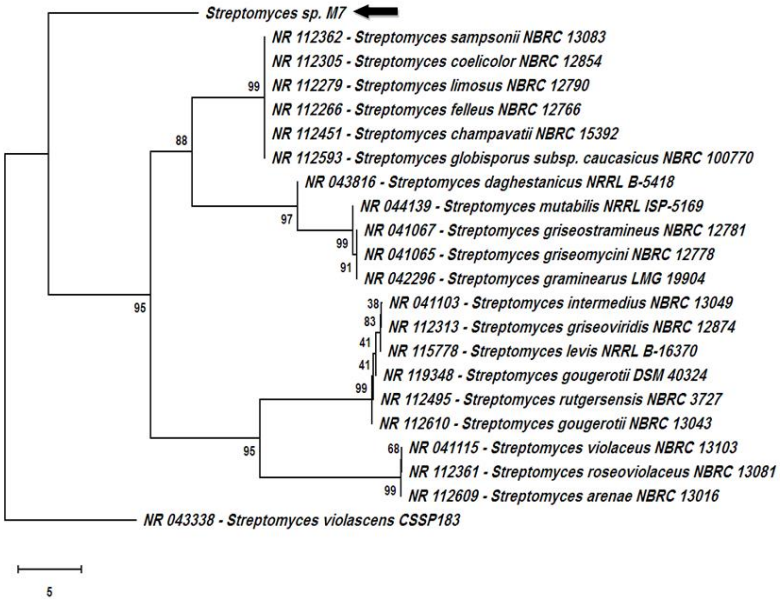

Figure 4 Phylogenetic tree of *Streptomyces* M7 with some other related based on *Streptomyces* species based on 16S rRNA sequence alignment.

DISCUSSION

An appealing and environmentally friendly substitute for chemical pesticides is the employment of antagonist microorganisms against fungal plant diseases **(Evangelista-Martínez, 2014)**. The inhibition of a harmful organism's activity by one or more other species is known as biological control. Biological control offers numerous environmental benefits, such as reduced pesticide residues in food, safety for humans and other non-target organisms, increased activity of most other natural enemies, and increased biodiversity in managed ecosystems **(Ouda, 2014)**. Because of their propensity to synthesize a variety of secondary metabolites with unique structures and exceptional biological activity, actinomycetes, especially *Streptomyces* spp., become an invaluable biological control resource **(Pérez Rojas et al., 2015; Yang et al., 2019)**. The antifungal activity of *Streptomyces* species against a variety of pathogenic fungi was assessed. During vegetative growth, these particular infections reduce crop yields in a number of economically significant crops. Bio-fungicides that employ *Streptomyces* sp. to suppress infections have frequently replaced synthetic fungicides **(Bressan, 2003; Errakhi et al., 2007; Hassan et al., 2011; Kim et al., 2014)**. Beneficial soil bacteria called *Streptomyces* spp. have the potential to be used as biocontrol agents **(Soares et al., 2006)**. Species of *Streptomyces* are commonly employed as BCAs to manage soil-borne illnesses due to their antifungal properties **(Lee et al., 2012)**.

Around the world, *Streptomyces* spp. is biologically screened to determine their antifungal activity **(Cao et al., 2005; Bonaldi et al., 2014)**. Microorganisms found in soil offer a valuable resource for the separation and recognition of compounds with significant medicinal applications. Actinomycetales is a significant group among them **(Berdy, 2005)**. Fourteen actinomycetes isolates were obtained from various Damietta soil sites for this study. *Streptomyces* sp. was shown to be the majority of actinomycetes strains recovered from soil **(Baltz, 2008; Sapkota et al., 2020)**. **Šantrić et al. (2018)** reported that *S. flavovirens* exhibited strong antagonistic activity against *T. harzianum* and *T. aggressivum*. This suggests that it could be employed to improve mushroom yield and aid in disease management by inhibiting the growth of the aggressive compost green mold agent, *T. aggressivum*. According to **Ogundare et al. (2015)**, *S. flavovirens* exhibited strong antifungal activity against *A. flavus*. *S. flavovirens* and *S. gougeroti* isolates showed strong antifungal activity against the majority of tested phytopathogenic fungi in our investigation.

Volatile organic compounds (VOCs), or volatile organic compounds, were produced by *Streptomyces* sp. and were found in metabolites **(Li et al., 2024)**. Compared to alternative bio-control tactics that necessitate direct physical contact with pathogens, **Gong et al. (2015)** discovered that bacterium-emitted VOCs had advantages for the control of air-borne diseases, such as *Aspergillus* pathogens. Since food and feed products are frequently stored in settings with regulated atmospheres, the benefits of using VOCs over direct contact approaches are especially noticeable in this context. According to **Naqvi et al. (2019)**, bis(2-ethylhexyl) phthalate exhibited antifungal efficacy against *M. phaseolina and F. oxysporum.*

Streptomyces sp. was shown to be the majority of actinomycetes strains recovered from soil **(Baltz, 2008 and Sapkota et al., 2020)**. *Streptomyces* sp. was isolated from soil samples using starch nitrate agar medium, which is consistent with findings by **Atta et al. (2009)**, **El-Naggar et al. (2009)**, **El-Naggar (2015)**, and **Korayem et al. (2015)**. Furthermore, actinomycetes were isolated using a starch-casein agar medium **(Hasani et al., 2014; Duddu and Guntuku, 2016; El Karkouri et al., 2019; Sapkota et al., 2020)**. Glycerol yeast-extract agar medium allowed the isolate M7 to thrive, as reported by **Duddu and Guntuku (2016)** and **Bawazir et al. (2018)**. According to **Yadav et al. (2014)**, *Streptomyces* spp. have antifungal efficacy against *R. solani*, *A. alternata*, *F. oxysporum*, and *M. phaseolina* in this investigation. *Phytophthora capsici*, *Colletotrichum* spp., *Rhizoctonia* spp., *Penicillium* spp., *Cercospora canescens*, *Diaporthe citri*, *Curvularia* spp., *A. niger*, *Helminthosporium* spp., *Fusarium* spp., *Alternaria* spp., and *Sclerotinia soleroforum* are among the pathogens that *Streptomyces* spp. have antifungal effects against in soil and crops **(Welscher et al., 2008; Liu et al., 2009; Oskay, 2009; Nguyen et al., 2017; Law et al., 2017; Zhang et al., 2020)**. Only two isolates exhibited the strongest antagonistic activity against all of the examined fungus, out of the five that demonstrated antagonistic ability against all of the fungi. These isolates were chosen to be used in the ensuing trials. No antagonistic action was demonstrated by other tested *Streptomyces* against any of the tested fungi. The sensitivity of the pathogen under test to the bioactive metabolites generated by *Streptomyces* isolates may indicate, according to **Ndonde and Semu (2000)**, that the pathogens under test had not previously been exposed to bioactive metabolites that were comparable. They were still so vulnerable to these metabolites. The limited antibacterial activity may also have been caused by the antimicrobial compounds' sensitivity to light and temperature, their inherent instability after extended storage, or the low concentration of bioactive chemicals in the crude extracts.

The mature aerial mycelium's white or grey color, the spore chain morphology type with smooth spore wall ornamentation, and the absence of melanoid pigment production all clearly indicated that the isolates belonged to the genus *Streptomyces*, according to Bergey's Manual of Systematic Bacteriology **(Yashchuk et al., 2014)**. According to **Willemse et al. (2011)**, the spores are typically 1 µm in diameter or less, and the colonies have smooth surfaces **(Ambarwati et al., 2012; Abdel-Rahman et al., 2012; Taddei et al., 2006)**. According to **Al-Garni et al. (2014)**, the active *Streptomyces* isolates belong to the grey and white groups. *Streptomyces* sp. exhibit the same lack of melanoid pigment production in this investigation as **Taddei et al. (2006)**.

Bacterial polyphasic taxonomy may include all genotypic, phenotypic, and phylogenetic data. The unique phenotypic characteristics of the *Streptomyces* genus, which are derived from chemotaxonomic markers and a variety of other stable expressed features like micro- and macro-morphology, physiology, and biochemical properties, allow it to be easily distinguished from all other bacterial groups **(Yashchuk et al., 2014)**. In actuality, the primary method for classifying the genus *Streptomyces* according to conventional taxonomy is the use of the phenotypic feature. The combined obtained morphological, physiological, chemotaxonomic, and biochemical features unambiguously confirmed that the isolates under investigation are members of the *Streptomyces* genus. A contemporary method of identifying *Streptomyces* relies on the 16S rRNA gene sequence, which has yielded significant insights into the systematics of Streptomycetes and has been applied to the identification of multiple recently isolated *Streptomyces* **(Begani et al., 2019; Yun et al., 2021)**. For certain applications, the conventional approaches are still useful **(Anderson and Wellington, 2001)**. 16S rRNA gene sequencing analysis of a few chosen strains confirms this early identification. Utilizing 16S rRNA sequences in conjunction with the traditional actinomycete identification system proved to be quite beneficial in evaluating the strains' potential novelty and biodiversity. In this study, in spite of the 16S rRNA sequence alignment for *Streptomyces* O5 isolate showed

high similarity to *Streptomyces rochei* NRRL B-1559, *S. enissocaesilis* NRRL B-16365, *S. vinaceusdrappus* NBRC 13099, *S. plicatus* NBRC 13071, *S. geysiriensis* NRRL B-12102 and *S. viridosporus* CSSP718, but its morphological and cultural properties are different with them. For example, *S. rochei* had the ability to produce melanin pigment in tyrosine agar medium but *Streptomyces* O5 could not produce melanin pigment. Furthermore, the spores of *S. rochei* have wrinkled surface and sporophore was spiral while *Streptomyces* O5 isolate possessed smooth spore and straight to flexuous sporophore. Also, spores of *S. geysiriensis* were hairy and had different color of mycelia from *Streptomyces* O5. Although *Streptomyces* O5 isolate was cluster a way with *S. flavovirens*, they possessed considerable similarity of morphological and cultural characters. Also, they share most of the physiological and biochemical properties. The combination of these results indicated that *Streptomyces* O5 is identified as *S. flavovirens*.

The 16S rRNA nucleotide sequence of *Streptomyces* M7 isolate exhibited high similarity with *S. gougerotii* (98.59%) and *S. coelicolor* (99.79%). The morphological, physiological and cultural characters of *Streptomyces* M7 isolate are more similar to *S. gougerotii* than *S. coelicolor*. Neither *S. gougerotii* DSM-40324 and *Streptomyces* M7 had the ability to produce melanin pigment compared to *S. coelicolor* NBRC12854 which can produce melanin pigment. Also, *S. gougerotii* DSM-40324 and *Streptomyces* M7 have smooth spore surface in rectiflexibiles (RF) chain while *S. coelicolor* NBRC12854 spores appeared as slightly granular without periodic substructure. *S. coelicolor* NBRC12854 had different colour of mycelium (grayish yellow) from *S. gougerotii* DSM-40324 and *Streptomyces* M7 (white). In addition, *S. gougerotii* DSM-40324 and *Streptomyces* M7 shared most of the physiological and biochemical characteristics. So, the combination of molecular and traditional identification encouraged us to name isolate M7 as *S. gougerotii*.

CONCLUSIONS

A total of 14 different *Streptomyces* spp. isolates were recovered from Egyptian soil samples. Antifungal activity of the isolates was tested against different phytopathogenic fungal strains. According to some morphological and cultural characteristics, the high antifungal strains belonged to *Streptomyces* spp (*Streptomyces flavovirens* (accession number: OM301640) and *S. gougerotii* (accession number: OM301639).

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