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Possible Control of Potato Common Scab with Indigenous Nonpathogenic Species of *Streptomyces* in Egypt

Abd El-Hafez, O.A. and Abd El-Rahman, A.F.

Bacterial Dis. Res. Dept., Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

The potato common scab disease became a major threat for potato raised in light soil(s) in Egypt. In the present work, eight out of twenty-five isolates of *Streptomyces* recovered from four locations and rhizosphere soil of three different crops showed antagonistic potential against *Streptomyces scabies*. The partial 16S rRNA gene sequences of five of these eight isolates showed 99.13 to 99.67 % similarity with *Streptomyces rochei* strain NRRL B-1559.The other three isolates showed 99.73, 99.60, and100 % similarity with *Streptomyces geysiriensis* strain NRRL_B-12102, *Streptomyces tunisiensis* strain CN-207, and *Streptomyces djakartensis* strain NBRC 15409, respectively. The partial 16S rRNA gene sequences of the eight antagonistic isolates have been deposited in the GenBank of the National Center for Biotechnology Information (NCBI) under the accession numbers MT878417, MT878546, MT878545, MT878152, MT878547, MT878450, MT878478, and MT878497. The inhibition zones of the growth of *Streptomyces scabies* by the eight selected antagonistic isolates of *Streptomyces* were variably from 32.7 to 66.7 mm. Application of *Streptomyces geysiriensis* 7AS_GP8, *Streptomyces tunisiensis* 8AS_BNM2, *Streptomyces djakartensis* 4AS_MO2, and *Streptomyces rochei* 5AS_MO3 in pots experiment significantly decreased scab index from 68.6 % in control treatment to 39.1, 47.7, 25.3, and 31.7 %, respectively. *Streptomyces djakartensis* 4AS_MO2 gave the pronounced decrease (63.1%) in disease followed by *Streptomyces rochei* 5AS_MO3 (53.8 %), *Streptomyces geysiriensis* 7AS_GP8 (42.9 %), and *Streptomyces tunisiensis* 8AS_BNM2 (30.4%), respectively. *Streptomyces djakartensis* 4AS_MO2 and *Streptomyces rochei* 5AS_MO3 provided promising results as potential biocontrol agents against *Streptomyces scabies*. Further field studies may be advised.

Keywords: Potato, *Streptomyces scabies*, Biological control, *Streptomyces geysiriensis*, *Streptomyces tunisiensis*, *Streptomyces djakartensis*, *Streptomyces rochei*.

Streptomyces is a genus embracing (over 500 species) gram-positive microorganisms affiliated to the order Actinomycetales and the family Streptomycetaceae. *Streptomyces* are filamentous organisms and have the potential to produce bioactive secondary metabolites, especially antibiotics. The productions

of the bioactive secondary metabolites are important for *Streptomyces* in order to compete with other microorganisms even within the same genus (Procopio *et al*. 2012). *Streptomyces* spp. are viewed as free-living soil bacteria, and some species symbiosis with animals, plants, insects, and fungi and some strains live in marine soil. A few species of *Streptomyces* are plant pathogens, and the most economically important disease caused by *Streptomyces* species is the potato scab. Symptoms of potato scab are lesions (raised, pitted, or superficial) formed on the surface of tubers (Zhang and Loria, 2017).

Potato (*Solanum tuberosum* L.) is the most important food crop after cereals. Total world potato production is estimated at approximately four hundred million tons in 2017, of which about four and a half million tons were contributed by Egypt. Egypt ranks among the world's top potato exporters. Some diseases causing blemishes on the surface of the tubers reducing marketing ability (Arora and Khurama, 2004 and FAOSTAT, 2018). Many species of the genus *Streptomyces* as S. scabies, *S. turgidiscabies, S. acidiscabies, S. ipomoeae* (the causal agent of soft rot disease in sweet potatoes), *S. europaeiscabiei, S. stelliscabiei, S. luridiscabiei, S. puniciscabiei, S. niveiscabiei, S. reticuliscabiei,* and *S. caviscabies* cause scab or scab-like disease (s). The oldest and the most studied species of *Streptomyces* known to cause scab is *Streptomyces scabies* (Zhang and Loria, 2017). The Common scab of potatoes was placed among the most five serious diseases of potatoes (Sagova-Mareckova *et al*., 2015). This disease occurs worldwide and affects the quality of tubers due to lesions that are formed on the tuber surface. The potato scab disease has become a major problem for potato growers in Egypt, especially in the last few years (El-Sheikh *et al*., 2012 and Abd El-Rahman *et al.* 2018). El-Sheikh, 2010 found that the incidence and severity of common scab were ranged from 22.5 to 60.0 % and 11.4 to 25.8 %, respectively in three surveyed governorates El-Behera, El-Sharkia and El-Ismailia.

Control strategies of potato common scab are challenging due to limited understanding of the genetic diversity of *S. scabies* and genetic differences in various potato cultivars. Traditional control methods such as lowering soil pH, increasing irrigation intensity, and cultural practices such as crop rotation as well as chemical control methods are not usually effective. Several studies have used biocontrol agents such as *Pseudomonas* spp., *Bacillus* spp., and nonpathogenic *Streptomyces* spp. to control potato common scab. (Lin *et al*., 2018). Some nonpathogenic *Streptomyces* spp. were used to control pathogenic *Streptomyces* strains that cause potato common scab (Hiltunen *et al*., 2009 and Wanner *et al*., 2014).

The objective of this work was isolation and identification of nonpathogenic *Streptomyces* spp. from the rhizosphere of different crops at different locations in Egypt. Screening the antimicrobial activity of the nonpathogenic *Streptomyces* spp.

against *Streptomyces scabies*. Studying the biocontrol potential of nonpathogenic *Streptomyces* spp. on common scab severity.

Materials and Methods

Source of the pathogenic isolate of Streptomyces scabies:

The pathogenic isolate of *Streptomyces scabies* (T-47) was previously isolated from naturally infected potato tubers and identified by Mahdy *et al*. (2014). The pathogenicity test was re-performed on radish seedlings and leaf-bud cutting method (Lorang *et al*., 1995) to check virulence.

Isolation of nonpathogenic isolates of Streptomyces:

Nonpathogenic isolates of *Streptomyces* were isolated from the rhizosphere of different crops at different locations in Egypt using the serial dilution technique (Arifuzzaman *et al*. 2010 Rahman *et al*. 2011 and Janaki *et al*. 2014). The roots-adhering soil was collected and air-dried under room temperature for one week. One g soil in 10 ml of sterilized distilled water was heated in a water bath at 50ºC for 10 min and serially diluted up to 10^{-6} in sterile distilled water. A 100 µl of soil dilution was plated out on starch casein agar (SCA) medium (starch 10g, KNO_3 2g, casein 0.3 g, K_2HPO_4 2g, $MgSO_4.7H_2O$ 0.05 g, NaCl 2g, $CaCO_3$ 0.02 g, $FeSO_4.7H_2O$ 0.01 g, agar 20g, distilled water 1000 ml and pH 7.2) supplemented with cycloheximide (100 µg/ml) and incubated at 28°C. The medium was checked for the growth of typical *Streptomyces* colonies up to 14 days. Single colonies of *Streptomyces* were transferred to Petri dishes of glucose-yeast extract malt (GYM) agar medium (glucose 4g, yeast extract 4g, malt extract 10g, agar 20g, distilled water1000 ml and pH 7.2) and incubated at 28°C for 7 days. Subsequent transfer to fresh GYM agar medium was done using the streak-plate technique to obtain pure cultures.

Screening of antimicrobial activity of Streptomyces isolates:

Preliminary screening for antimicrobial activity of the *Streptomyces* isolates was carried out on the GYM agar medium. Isolates were grown separately in the GYM agar medium at 28°C for 7 days. Five milliliters suspension (10^8 CFU /ml) of *Streptomyces scabies* were used as inoculum for a flask containing 250 ml of melted GYM agar medium. Twenty milliliters of GYM were poured into each Petri dish. A loopful of growth of tested *Streptomyces* isolate was spot inoculated at the center of Petri dish containing GYM agar previously inoculated with *Streptomyces scabies*. The Petri dishes were incubated at 28°C for 3 days. The inhibition zone around the growth of the tested *Streptomyces* isolate was recorded as a positive (+) reaction (Abd El-Rahman and Shaheen 2016).

Identification of the Streptomyces isolates using 16S rRNA analysis:

Eight selected *Streptomyces* isolates were sent to Sigma Scientific Services Co., Giza, Egypt for 16S rRNA analysis as follows**:**

DNA extraction

DNA of each *Streptomyces* isolate was extracted using Quick-DNA™ Miniprep Plus Kit (Zymo Research Corporation, USA) according to the manufacturer's protocol. Water (95 µl), solid tissue buffer (blue, 95 µl) and proteinase K (10 µl) were added to 200 µl liquid media containing *Streptomyces* isolate in a microcentrifuge tube. Then the contents of the tube were mixed thoroughly and incubated at 55ºC for 2 hours. Then the contents of the tube were mixed thoroughly again and centrifuged at 12000 xg for 1 minute. The aqueous supernatant (300 µl) was transferred to a clean tube and 600 µl genomic binding buffer was added. Then the contents of the tube were mixed thoroughly, transferred to a Zymo-Spin™ IIC-XL Column in a collection tube and centrifuged at (≥ 12000 xg) for 1 minute (Lee *et al*., 2003). The collection tube was discarded, with the flowing through it. DNA Pre-Wash Buffer (400 µl) was added to the column in a new collection tube, and centrifuged at (12000 xg) for 1 minute. The collection tube was emptied, g-DNA Wash Buffer 700 µl was added and centrifuged at (12000 xg) for 1 minute. The collection tube was emptied, g-DNA Wash Buffer (200 µl) was added, centrifuged at (12000 xg) for 1 minute, the collection tube was discarded, with the flowing through it. The spin column was transferred to a clean microcentrifuge tube, elution buffer (30 µl) was added, incubated for 5 minutes at room temperature, and then centrifuged at (12.000 xg) for 1 minute to elute the DNA (Lee *et al*., 2003).

PCR amplification

Amplification of the 16S rRNA gene was carried out using a Thermo Scientific™ Arktik™ Thermal Cycler (Thermo Fisher Scientific Oy , Finland) with a 50 µl reaction mixture containing 25 µl My Taq Red Mix, 8 µL DNA template, 15 µl nuclease free water, 1 µl (20 Pico mol) forward primer F1 (5'-AGAGTTTGATCITGGCTCAG-3'; I=inosine) and 1 µl (20 Pico mol) reverse primer R5 (5'-ACGGITACCTTGTTACGACTT-3') (Cook and Meyers, 2003). The PCR program was set as follows: an initial denaturation step at 94 °C for 6 min; 35 cycles at 94 °C for 45 s, 56 °C for 45 s, 72°C for 1 min, followed by a final extension at 72°C for 5 min.

DNA purification and Sequencing

PCR products were purified using the DNA Clean & Concentrator-5 Kit procedure (Zymo Research Corporation, USA) according to the manufacturer's instructions. DNA was sequenced using ABI 3730xl DNA Sequencer (Applied Biosystems, Foster City, CA, USA) in GATC Company, Germany.

BLAST search and GenBank (NCBI) accession numbers:

The partial 16S rRNA gene sequences of the eight selected antagonistic isolates of *Streptomyces* were compared with the sequences in the GenBank of the National Center for Biotechnology Information (NCBI) by BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using the database of 16S ribosomal RNA sequences (Bacteria and Archaea) using Megablast (Optimize for highly similar

sequences) for alignments. DECIPHER version 2.17.1 was employed for chimeras check (Wright *et al*., 2012). The partial 16S rRNA gene sequences of the eight tested antagonistic isolates of *Streptomyces* were submitted to GenBank of the NCBI to receive the accession numbers.

The multiple alignment and preparing phylogenetic trees:

Multiple alignment and phylogenetic reconstructions were performed by the Multiple Clustal Alignment software from ClustalW (https://www.genome.jp/tools-bin/clustalw) using the function "build" of The Environment for Tree Exploration ETE3 v3.1.1 (Huerta-Cepas *et al*., 2016) as implemented on the Genome Net (https://www.genome.jp/tools/ete/). ML (Maximum likelihood) tree of the selected isolates of *Streptomyces* were inferred using Randomized Axelerated Maximum Likelihood RAxML v8.1.20 ran with model GTRGAMMA and default parameters (Stamatakis, 2014). Branch supports were computed out of 100 bootstrapped trees. ML (Maximum likelihood) tree of the selected isolates of *Streptomyces* and the closely related reference strains in GenBank of the NCBI was inferred using PhyML v20160115 ran with model and parameters: --alpha e -o tlr --nclasses 4 --bootstrap 100 --pinv e -f m (Guindon *et al*., 2010). Branch supports are computed out of 100 bootstrapped trees.

Measurement of the inhibition ability of Streptomyces isolates on the growth of Streptomyces scabies:

The same previous method used in the screening of antimicrobial activity of *Streptomyces* isolates was used to measure the inhibition ability of selected *Streptomyces* isolates on the growth of *Streptomyces scabies in vitro*. A loopful of growth of tested *Streptomyces* isolate was spot inoculated at the center of Petri dish containing GYM agar previously inoculated with *Streptomyces scabies*. The experiment was performed in three replicates. The Petri dishes were incubated at 28°C for 3 days. The inhibition zone around the growth of tested *Streptomyces* isolate was recorded in millimeters (mm).

Pots experiment:

Disease-free potato seed tubers (cv. Spunta) were obtained from potato brown rot project (PBRP), ARC, Egypt. The seed tubers were planted in Pots (20 cm in diam.) filled with infested soil mixture (sand: clay: compost at 2: 1: 1 ratio v/v/v) with *Streptomyces scabies*. The latter inoculum was prepared by inoculating 50 ml of GYM broth in a flask (250 ml) with 1 ml water suspension (10^6 CFU/ml) of *Streptomyces scabies* spores harvested from a 3-week old GYM agar plate. The flask was shake incubated for 5 days at 28°C. Cells were pelleted by centrifugation at 9000 xg and resuspended in 150 ml sterile distilled water. Five milliliters of this resuspended cells and 50 ml of sterile solution (sucrose 40 g, asparagine 2.4 g, K2HPO4 1.2 g, yeast extract 20 g in 1000 ml of sterile distilled water) were added to a bag containing 300 cm^3 of sterile vermiculite. Infested vermiculite bags were incubated for 14 days at 28°C, with regular shaking every other day during

incubation. After 14 days of incubation, the vermiculite bags were checked using the serial dilution technique to ensure even distribution of *Streptomyces* and to estimate inoculum density. Vermiculite inoculum containing 10^{10} CFU/g of *Streptomyces scabies* was added to the soil mixture (Vermiculite inoculum : soil mixture : at 1 : 9 ratio v/v) and mixed thoroughly (Wanner, 2007). Antagonistic isolates were propagated separately in GYM broth by shaking for 5 days at 28°C. Cells were pelleted by centrifuged at 9000 xg and were resuspended in sterile distilled water to adjust the inoculum (10^6CFU/ml) concentration (Sarwar *et al.,* 2019). The pots were drenched with 100ml/pot of antagonistic isolate suspension, with 5 replicates (3 pots each) after planting potato tubers. Control plants (without antagonistic applications) were considered in the experiment. Plants kept without *Streptomyces scabies* and antagonistic isolates were used as a negative control treatment .

Potato tubers were harvested after 100 days of seeding. The scab index was determined and the percent of disease decrease was estimated as described by Abd El-Rahman *et al.* (2018).

$$\text{Scab index \%} = (0A+1B+2C+3D+4E/4T) \times 100$$

Where:

0 = no scab; 1 = trace-10% tuber surface is scabbed; 2 = 11-25 % tuber surface is scabbed; 3 = 26-50 % tuber surface is scabbed; 4 = more than 50 % tuber surface is scabbed; A, B, C, D, and E are the number of tubers in grades 0, 1, 2, 3 and 4 respectively; T = is the total number of tubers.

Disease decrease % = [(scab index in control-scab index in treatment)/scab index in control) X 100.

Statistical analysis:

Completely randomized design was used in all experiments. The collected data were subjected to one-way analysis of variance (ANOVA) as illustrated by Snedecor and Cochran (1989). LSD values were used to compare the means at probability (P) value of ≤ 0.05.For performing the mentioned statistical analysis, SPSS version 13.0 (SPSS Inc. Chicago, IL, USA) statistical packages were used.

Results

Source and antimicrobial activity of the Streptomyces isolates:

Twenty-five isolates of *Streptomyces*, recovered from four different locations (Manshet Radwan, El Nubaria, Al-Wasta, and Mallawi) and rhizosphere soil of three different crops (Potato, maize, and onion) in Egypt, were tested for their ability to inhibit the growth of *Streptomyces scabies in vitro*. Eight isolates (GP8, BP1, BP6, BNM2, MO1, MO2, MO3, and MO4) out of the twenty-five showed potential to inhibit the growth of *Streptomyces scabies*. Three out of the eight isolates were isolated from the rhizosphere of potato plants, one (GP8) from Giza governorate (Manshet Radwan) and two (BP1, BP6) from Beheira governorate (El Nubaria).The other five isolates were selected, one (BNM2) from the rhizosphere of maize plants from Beni Suef governorate (Al-Wasta), and the other four (MO1, MO2, MO3, and

MO4) from the rhizosphere of onion plants from Minya governorate (Mallawi) (Table 1).

16S rRNA analysis and GenBank (NCBI) accession numbers:

The partial 16S rRNA gene sequences of the eight selected antagonistic solates of *Streptomyces* recovered from the rhizosphere of potato, maize, and onion from four governorates in Egypt compared to the sequences in the NCBI database are shown in (Table 2).The sequence of GP8 showed 99.73 % similarity with *Streptomyces geysiriensis* strain NRRL_B-12102. While the sequences of BP1, BP6, MO1, MO3, and MO4 showed 99.67, 99.67, 99.67, 99.67, and 99.13% similarity with *Streptomyces rochei* strain NRRL B-1559, respectively. The sequence of BNM2 showed 99.60 % similarity with *Streptomyces tunisiensi*s strain CN-207. Also, the sequence of MO2 showed 100 % similarity with *Streptomyces djakartensis* strain NBRC 15409.

Table (1): Source of *Streptomyces* isolates and their antibacterial potential against *Streptomyces scabies*

Isolate code	Location	Governorate	plant rhizosphere	Antibacterial ability against *Streptomyces scabies**
GP1	Manshet Radwan	Giza	Potato	-
GP2	Manshet Radwan	Giza	Potato	-
GP3	Manshet Radwan	Giza	Potato	-
GP4	Manshet Radwan	Giza	Potato	-
GP5	Manshet Radwan	Giza	Potato	-
GP6	Manshet Radwan	Giza	Potato	-
GP7	Manshet Radwan	Giza	Potato	-
GP8	Manshet Radwan	Giza	Potato	+
BP1	El Nubaria	Beheira	Potato	+
BP2	El Nubaria	Beheira	Potato	-
BP3	El Nubaria	Beheira	Potato	-
BP4	El Nubaria	Beheira	Potato	-
BP5	El Nubaria	Beheira	Potato	-
BP6	El Nubaria	Beheira	Potato	+
BP7	El Nubaria	Beheira	Potato	-
BNM1	Al-Wasta	Beni Suef	Maize	-
BNM2	Al-Wasta	Beni Suef	Maize	+
BNM3	Al-Wasta	Beni Suef	Maize	-
BNM4	Al-Wasta	Beni Suef	Maize	-
MO1	Mallawi	Minya	Onion	+
MO2	Mallawi	Minya	Onion	+
MO3	Mallawi	Minya	Onion	+
MO4	Mallawi	Minya	Onion	+
MO5	Mallawi	Minya	Onion	-
MO6	Mallawi	Minya	Onion	-

*Negative reaction (−), Positive reaction (+).

Table (2): Comparison of partial 16S rRNA gene sequences of the eight selected antagonistic isolates of *Streptomyces* recovered from four locations and rhizosphere soil of three different crops in Egypt with the sequences in GenBank (NCBI)

Isolate code	Identification by 16S rRNA sequence analysis	Similarity (%)
GP8	*Streptomyces geysiriensis* strain NRRL_B-12102	99.73%
BP1	*Streptomyces rochei* strain NRRL B-1559	99.67%
BP6	*Streptomyces rochei* strain NRRL B-1559	99.67%
BNM2	*Streptomyces tunisiensis* strain CN-207	99.60%
MO1	*Streptomyces rochei* strain NRRL B-1559	99.67%
MO2	*Streptomyces djakartensis* strain NBRC 15409	100%
MO3	*Streptomyces rochei* strain NRRL B-1559	99.67%
MO4	*Streptomyces rochei* strain NRRL B-1559	99.13%

The partial 16S rRNA gene sequences of the eight selected antagonistic isolates of *Streptomyces* GP8, BP1, BP6, BNM2, MO1, MO2, MO3, and MO4 have been deposited in GenBank (NCBI) under the accession numbers MT878417, MT878546, MT878545, MT878152, MT878547, MT878450, MT878478, and MT878497, respectively (Table 3).

Table (3): Accession numbers to the GenBank (NCBI) for the eight selected antagonistic isolates of *Streptomyces*

code	Isolate	Accession number
GP8	*Streptomyces geysiriensis* 7AS_GP8	MT878417
BP1	*Streptomyces rochei* 2AS_BP1	MT878546
BP6	*Streptomyces rochei* 1AS_BP6	MT878545
BNM2	*Streptomyces tunisiensis* 8AS_BNM2	MT878152
MO1	*Streptomyces rochei* 3AS_MO1	MT878547
MO2	*Streptomyces djakartensis* 4AS_MO2	MT878450
MO3	*Streptomyces rochei* 5AS_MO3	MT878478
MO4	*Streptomyces rochei* 6AS_MO4	MT878497

Multiple alignment and Phylogenetic analysis:

The alignment scores between eight selected antagonistic isolates of *Streptomyces* are shown in (Table 4). The alignments scores between *Streptomyces geysiriensis* 7AS_GP8 and the five isolates of *Streptomyces rochei* i.e., 2AS_BP1, 1AS_BP6, 3AS_MO1, 5AS_MO3, and 6AS_MO4 were ranged from 98 to 99. The alignments scores between *Streptomyces geysiriensis* 7AS_GP8 and the two isolates *Streptomyces tunisiensis* 8AS_BNM2 and *Streptomyces djakartensis* 4AS_MO2 were 93 and 36, respectively. While the alignments scores between the five isolates

of *Streptomyces rochei*, 2AS_BP1, 1AS_BP6, 3AS_MO1, 5AS_MO3, and 6AS_MO4 were 99. The alignments scores between the five previous isolates and the two isolates *Streptomyces tunisiensis* 8AS_BNM2 and *Streptomyces djakartensis* 4AS_MO2 ranged from 96 to 97 and 53 to 54, respectively. Also, the alignments score between *Streptomyces tunisiensis* 8AS_BNM2 and *Streptomyces djakartensis* 4AS_MO2 was 42.

Phylogenetic analysis of the sequence data of the eight selected antagonistic isolates of *Streptomyces* revealed that all isolates were located in two distinct clusters (Fig. 1). Cluster 1 consisted of *Streptomyces djakartensis* 4AS_MO2. Whereas Cluster 2 comprised the isolates of *Streptomyces rochei* (3AS_MO1, 1AS_BP6, 5AS_MO3, 2AS_BP1, and 6AS_MO4), *Streptomyces tunisiensis* 8AS_BNM2, and *Streptomyces geysiriensis* 7AS_GP8. *Streptomyces tunisiensis* 8AS_BNM2 and *Streptomyces geysiriensis* 7AS_GP8 formed a separate cluster within the main Cluster 2.

The phylogenetic analysis of the sequences data of the eight selected antagonistic isolates of *Streptomyces* and the closely related reference strains in GenBank of the NCBI revealed that all eight isolates and their closely related reference strains were located in two distinct clusters (Fig. 2). Cluster 1 consisted of the isolate *Streptomyces tunisiensis* 8AS_BNM2 and the closely related reference strain *Streptomyces tunisiensis* CN-207. Whereas Cluster 2 comprised the isolates *Streptomyces djakartensis* 4AS_MO2, *Streptomyces geysiriensis* 7AS_GP8, and the isolates of *Streptomyces rochei* (3AS_MO1, 1AS_BP6, 5AS_MO3, 6AS_MO4, and 2AS_BP1) as well the closely related reference strains, *Streptomyces djakartensis* NBRC 15409, *Streptomyces geysiriensis* NRRL_B-12102, and *Streptomyces rochei* NRRL B-1559. *Streptomyces djakartensis* 4AS_MO2 and *Streptomyces djakartensis* NBRC 15409 formed a separate cluster within the main Cluster 2.

Table (4): The alignment score in between the eight selected antagonistic isolates of *Streptomyces*

Sequence	6AS_ MO4 **	5AS_ MO3 **	4AS_ MO2 ****	3AS_ MO1 **	8AS_ BNM2 ***	1AS_ BP6 **	2AS_ BP1 **	7AS_ GP8 *
7AS_GP8*	98	99	36	99	93	99	99	100
2AS_BP1 **	99	99	54	99	97	99	100	-
1AS_BP6 **	99	99	54	99	97	100	-	-
8AS_BNM2 ***	96	97	42	97	100	-	-	-
3AS_MO1 **	99	99	54	100	-	-	-	-
4AS_MO2 ****	53	54	100	-	-	-	-	-
5AS_MO3 **	99	100	-	-	-	-	-	-
6AS_MO4 **	100	-	-	-	-	-	-	-

***** *Streptomyces geysiriensis* ** *Streptomyces rochei*
*** *Streptomyces tunisiensis* **** *Streptomyces djakartensis*

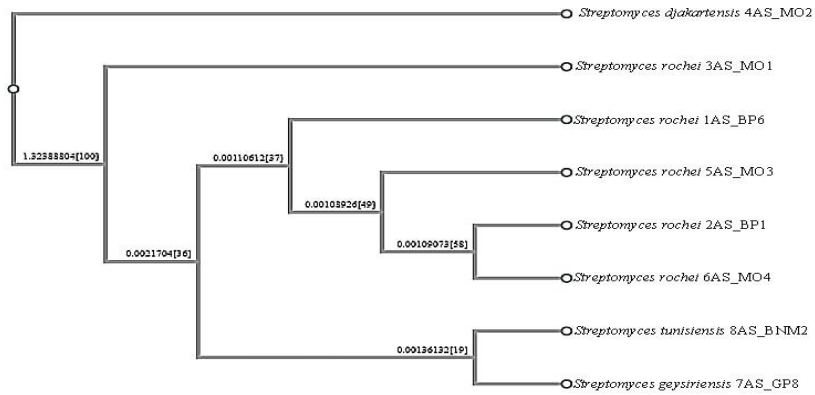

Fig. (1): Maximum likelihood phylogenetic tree based on 16S rRNA multiple sequence alignment of the eight selected antagonistic isolates of *Streptomyces*

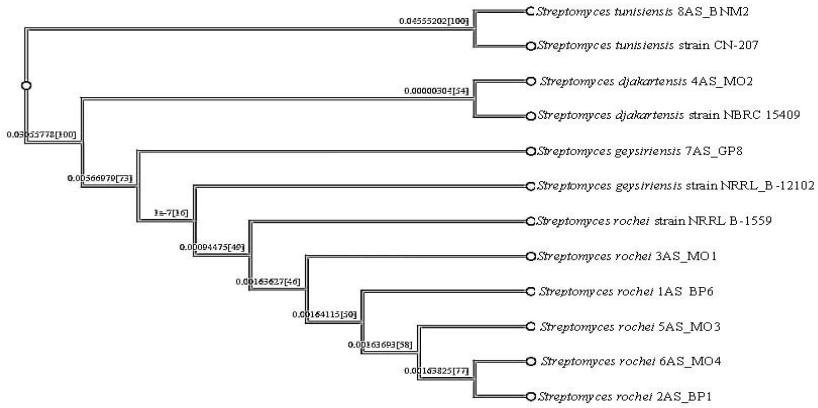

Fig. (2): Maximum likelihood phylogenetic tree based on 16S rRNA multiple sequence alignment of the eight selected antagonistic isolates of *Streptomyces* and the closely related reference strains in the GenBank of the NCBI

Inhibition potential of Streptomyces isolates for growth of Streptomyces scabies:

The results in Table (5) showed that the eight selected nonpathogenic isolates of *Streptomyces* can inhibit the growth of *Streptomyces scabies* on GYM agar medium with different inhibition zone diameters. The diameters of inhibition zones ranged from 32.7 to 66.7 mm. The maximum inhibition zone diameter (66.7mm) was

recorded by the *Streptomyces djakartensis* 4AS_MO2. The *Streptomyces rochei* isolates recorded inhibition zones diameter ranged from 54.3 to 63.3 mm. The *Streptomyces geysiriensis* 7AS_GP8 recorded an inhibition zone diameter of 51.0 mm. The minimum inhibition zone diameter (32.7 mm) was recorded by the *Streptomyces tunisiensis* 8AS_BNM2 (Fig. 3).

Table (5): Antagonism of eight isolates of *Streptomyces* to the growth of *Streptomyces scabies in vitro*

Isolate	Inhibition zone (mm)
Streptomyces geysiriensis 7AS_GP8	51.0
Streptomyces rochei 2AS_BP1	54.3
Streptomyces rochei 1AS_BP6	54.3
Streptomyces tunisiensis 8AS_BNM2	32.7
Streptomyces rochei 3AS_MO1	57.7
Streptomyces djakartensis 4AS_MO2	66.7
Streptomyces rochei 5AS_MO3	63.3
Streptomyces rochei 6AS_MO4	62.0
L.S.D. 0.05	4.70

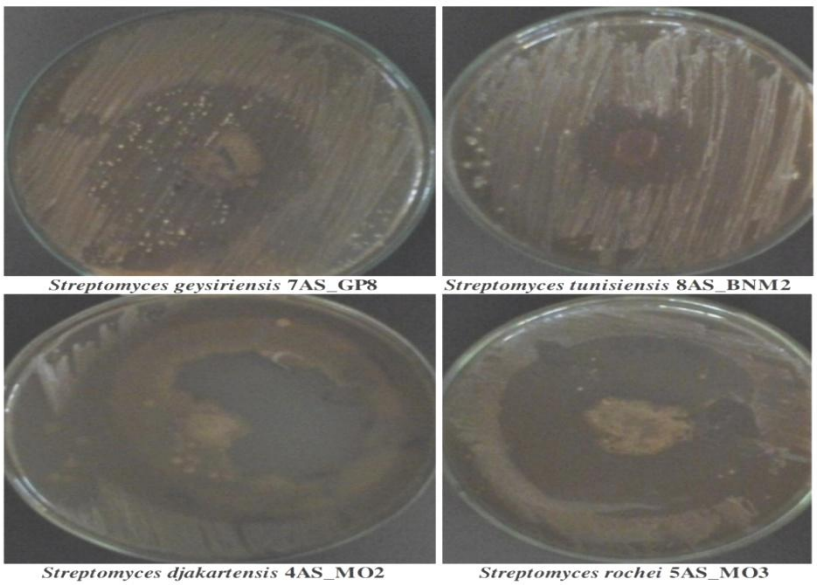

Fig. (3): Inhibition of *Streptomyces scabies* by isolates of non-pathogenic species of *Streptomyces* on GYM agar medium

Influence of different isolates of non-pathogenic species of Streptomyces on common scab severity:

Application of *Streptomyces geysiriensis* 7AS_GP8, *Streptomyces tunisiensis* 8AS_BNM2, *Streptomyces djakartensis* 4AS_MO2, and *Streptomyces rochei* 5AS_MO3 in pots significantly decreased scab index from 68.6 % in control treatment to 39.1, 47.7, 25.3, and 31.7 %, respectively. *Streptomyces djakartensis* 4AS_MO2 gave the best decrease (63.1%) in common scab disease followed by *Streptomyces rochei* 5AS_MO3 (53.8 %), *Streptomyces geysiriensis* 7AS_GP8 (42.9 %), and *Streptomyces tunisiensis* 8AS_BNM2 (30.4%), respectively (Table 6).

Table (6): Influence of different isolates of non-pathogenic species of *Streptomyces* on common scab severity

isolate	scab index %	Disease decrease %
Streptomyces geysiriensis 7AS_GP8	39.1	42.9
Streptomyces tunisiensis 8AS_BNM2	47.7	30.4
Streptomyces djakartensis 4AS_MO2	25.3	63.1
Streptomyces rochei 5AS_MO3	31.7	53.8
Control (+)	68.6	00.0
Control (-)	00.0	-
L.S.D. 0.05	3.4	2.9

Discussion

The common scab of potatoes was placed among the most five serious diseases of potatoes (Sagova-Mareckova *et al*., 2015), and became a marketing problem for potato growers in Egypt (El-Sheikh *et al*., 2012 and Abd El-Rahman *et al.* 2018). It has been described that some nonpathogenic *Streptomyces* spp. are used to control pathogenic *Streptomyces* strains that cause potato common scab (Hiltunen *et al*., 2009 and Wanner *et al*., 2014).

In the present study, eight out of twenty-five isolates of *Streptomyces*, recovered from four different locations and three different crops in Egypt, showed the potential to inhibit the growth of *Streptomyces scabies*. The partial 16S rRNA gene sequences of five of these eight isolates showed 99.13 to 99.67 % similarity with *Streptomyces rochei* strain NRRL B-1559. *Streptomyces rochei* produces antimicrobial compounds such as streptothricin and there are experiments to use it for biological control of soil-borne fungal plant pathogens (Anukool *et al*., 2004 and Kanini *et al*., 2013). The other three isolates of *Streptomyces* showed 99.73, 99.60, and100 % similarity with *Streptomyces geysiriensis* strain NRRL_B-12102, *Streptomyces*

tunisiensis strain CN-207, and *Streptomyces djakartensis* strain NBRC 15409, respectively. *Streptomyces geysiriensis* has been isolated from the soil and produces antibacterial antibiotic, moenomycin (Komatsu *et al*., 1980). *Streptomyces tunisiensis* has been isolated from forest soil in Tunisia and has antibacterial activity against *Staphylococcus* species and several other Gram-positive and Gram-negative bacteria (Slama *et al*., 2014). *Streptomyces djakartensis* has been isolated from soil and produces N-acetyltryptamine (Zhang *et al*., 2013). The maximum inhibition zone diameter (66.7 mm) against *Streptomyces scabies* was recorded by the *Streptomyces djakartensis* 4AS_MO2. Two compounds were isolated from *Streptomyces djakartensis* (*N*-acetyltryptamine and (*E*)-2- methoxy-1, 4 naphthoquinone-1-oxime) and showed significant antibacterial activities against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseuomonas syringaepv. Actinidiae* and *Erwinia* carotovora (Zhang *et al*., 2013). *Streptomyces djakartensis* 4AS_MO2 gave the best decrease (63.1%) in common scab disease, followed by *Streptomyces rochei* 5AS_MO3 (53.8%), *Streptomyces geysiriensis* 7AS_GP8 (42.9%) and *Streptomyces tunisiensis* 8AS_BNM2 (30.4%) in pots experiment, respectively. Application of *Streptomyces rochei* as a biocontrol agent against *Fusarium oxysporum* protect tomato seeds infection in vivo (Kanini *et al*., 2013). Therefore, this experiment showed that *Streptomyces djakartensis* 4AS_MO2 and *Streptomyces rochei* 5AS_MO3 provided promising results as potential biocontrol agents against *Streptomyces scabies*. Less promising results were obtained by *Streptomyces geysiriensis* 7AS_GP8 and *Streptomyces tunisiensis* 8AS_BNM2. More experiments should be conducted in this regard. Further detailed field studies may be advised.

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Corresponding author: Abd El-Rahman, A.F.
E-mail: aabdelrahman2012@gmail.com

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امكانية مكافحة مرض الجرب العادي في البطاطس بأنواع محليه من الإستربتوميستات الغير ممرضة في مصر

أمنية أحمد عبدالحافظ ،وأشرف فتحي عبدالرحمن

قسم بحوث الأمراض البكتيرية ، معهد بحوث أمراض النباتات ، مركز البحوث الزراعية ، الجيزة ، مصر

أصبح مرض الجرب العادي في البطاطس تهديدًا كبيرًا للبطاطس التي تزرع في التربة الخفيفة في مصر. في العمل الحالي ، أظهرت ثمانية من أصل خمسة وعشرين عزلة من الاستربتوميستات المعزولة من أربعة مواقع ومن تربة الجذور لثلاثة محاصيل مختلفة امكانية على ان تضاد *Streptomyces scabies*. أظهرت تسلسلات 16S rRNA الجزئية لخمس من هذه العزلات الثمانية تشابهًا بنسبة ٩٩,١٣ إلى ٩٩,٦٧ ٪ مع *Streptomyces rochei* السلالة NRRL B-1559 . أظهرت العزلات الثلاثة الأخرى تشابهًا بنسبة ٩٩,٧٣ و ٩٩,٦٠ و ١٠٠٪ مع *Streptomyces geysiriensis* السلالة NRRL_B-12102 و *Streptomyces tunisiensis* السلالة CN-207 و *Streptomyces djakartensis* السلالة NBRC 15409 على التوالي. تم إيداع تسلسلات 16S rRNA الجزئية للثماني عزلات المضادة في بنك الجينات (NCBI) تحت أرقام الانضمام MT878417 و MT878546 و MT878545 و MT878152 و MT878547 و MT878450 و MT878478 و MT878497. تراوحت منطقة تثبيط نمو *Streptomyces scabies* بواسطة العزلات الثمانية المضادة المختارة من الاستربتوميستات بين ٣٢,٧ و ٦٦,٧ مليمتر. يؤدي تطبيق *Streptomyces geysiriensis* 7AS_GB8 و *Streptomyces tunisiensis* 8AS_BNM2 و *Streptomyces djakartensis* 4AS_MO2 و *Streptomyces rochei* 5AS_MO3 في تجربة الاصص إلى انخفاض كبير في مؤشر الجرب من ٦٨,٦٪ في معاملة المقارنة إلى ٣٩,١ و ٤٧,٧ و ٢٥,٣ و ٣١,٧٪ ، على التوالي. أعطت *Streptomyces djakartensis* 4AS_MO2 أفضل انخفاض (٦٣,١٪) في المرض يليها *Streptomyces rochei* 5AS_MO3 (٥٣,٨٪) و *Streptomyces geysiriensis* 7AS_GB8 (٤٢,٩٪) و *Streptomyces tunisiensis* 8AS_BNM2 (٣٠,٤٪) ، على التوالي. *Streptomyces djakartensis* 4AS_MO2 و*Streptomyces rochei* 5AS_MO3 قدمتا نتائج واعدة كعوامل مقاومة حيوية محتملة ضد *Streptomyces scabies*. قد نحتاج الى إجراء المزيد من الدراسات في الحقل.