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Phenotypic and Molecular Characterization of *Streptomyces enissocaesilis* and *Streptomyces caviscabies* Induced Potato Common Scab in Egypt

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ABSTRACT

Potato common scab incited by pathogenic *Streptomyces* spp. is a significant soil-borne disease leading to serious economic losses in potato tubers worldwide. However, there is limited information available in Egypt regarding the pathogenicity, prevalence, and variety of *Streptomyces* spp. inciting common scab. Therefore, this study aims to clarify the aspects of identifying and characterizing *Streptomyces* spp. obtained from scabby tubers as well as to evaluate their pathogenicity. In the present investigation, nine isolates of *Streptomyces* spp. were obtained from various scab lesion symptoms. Of these, the Ag2 and Man strains exhibited pathogenic traits. The pathogenicity assays demonstrated that the strains induced necrotic lesions on tuber slices and abnormal growth of radish seedlings. In potato pot trail, The Ag2 isolates caused deep-pitted lesions with a disease index of 73.30%. Additionally, tubers inoculated with the Man isolate exhibited visible brown raised lesions, resulting in a disease index of 63.97%. Subsequently, the strains were characterized based on morphological, physiological, biochemical and phylogenetic levels. Phylogenetic tree derived from 16S rRNA gene sequences revealed that Ag2 and Man strains share 100% sequence similarity with *Streptomyces caviscabies* ATCC 51928 and *Streptomyces enissocaesilis* NRRL B-16365, respectively. The results of this study demonstrate that *S. caviscabies* and *S. enissocaesilis* are capable of causing CS disease in potatoes and may pose a potential threat to potato cultivation in Egypt.

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Introduction

The potato (*Solanum tuberosum*) is the world's third-largest crop and a critical component of global food security. Potato tubers provide essential carbohydrates, protein, antioxidants, minerals, and vitamins. they also have various industrial processes and serve as animal feed (Dongyu, 2022). In Egypt, potatoes are a major export commodity, with production reaching approximately 6.9 million metric tons (FAO, 2020).

Among the numerous diseases affecting potato crops, potato common scab (CS) is a significant disease affecting most potato-growing regions worldwide (Braun et al., 2017). Symptoms of CS disease appear as scab-like surface, shallow-pitted, netted, raised, or russet lesions on the tuber (Gong et al., 2017; Li et al., 2019; Loria et al., 1997; Natsume et al., 2005). Recently, deep longitudinal cracks with scabby lesions (fissure scab) have also been produced (Cruywagen et al., 2021). These symptoms cause economic losses for farmers, decrease the quality of the potatoes, and impact potato marketability (Lankau et al., 2020; Loria et al., 1995). The CS disease of potatoes is induced by gram-positive bacteria in the genus *Streptomyces* which comprises over 900 species (Parte, 2018). Although *Streptomyces* exhibits a wide range of morphological, ecological, and molecular genetic variations, only a specific closely related group is responsible for the widespread occurrence of this disease worldwide (Braun et al., 2017). To date, many studies have reported that potato scab is caused by more than 27 species of pathogenic *Streptomyces*, including *S. scabies, S. acidiscabies, S. europaeiscabiei, S. luridiscabiei, S. reticuliscabiei, S. stelliscabiei, S. turgidiscabies, S. puniciscabiei,* and *S. niveiscabiei* (Aallam et al., 2021; Braun et al., 2017; Cruywagen et al., 2021; Vincent & Bignell, 2024). Other species that are also associated with CS include *S. caviscabies*, (found in Canada, China, Iran, and Brazil) and *S. enissocaesilis*, (found in China) (Corrêa et al., 2015; Gong et al., 2017; Goyer et al., 1996; Khodakaramian & Khodakaramian, 2013; Yang et al., 2018).

In Egypt, the prevalence of potato scab disease has recently become a significant concern for potato growers, and the incidence of CS across three governorates ranges from 22.5% to 60% (El-Sheikh, 2010; El-Sheikh et al., 2012). Several reports indicate that *S. scabies* is the most frequent pathogen associated with CS in potato cultivation (Abd El-Hafez & El Shishtawy, 2021; Eid et al., 2022; El-Sayed, 2000; Hosny et al., 2016; Hussein et al., 2019). Potato CS is also associated with *S. acidiscabies* (El-Sayed, 2000) and *S. alkaliscabies,* (Abdel-Rahman et al., 2012).

The current investigation aims to identify and characterize the *Streptomyces* species that have the ability to cause scab disease in potatoes under Egyptian conditions.

Materials and Methods

Streptomyces Isolation from Potato Tubers

Scabby potato tubers were collected from major potato-producing areas in Egypt from 2021 and 2022. Each scabby tuber was rinsed with water to get rid of any soil particles and dried with a paper towel. Then a small piece of infected potato tissue was excised with a sterile scalpel, ensuring it included tissue beneath the necrotic zone. The tissues were disinfected on the surface using 1.5% NaOCl for 1.5 minutes, followed by four rinses with sterile distilled water (SDW). Thereafter, the tissue was ground in 1 ml of SDW and incubated for 60 min. at room temperature. 100 µL of the ground solution was plated onto oatmeal agar (OMA, oatmeal 20g, agar 18g, sterile water to 1000 mL, pH 7.2). After 10 -15 days of incubation at 28°C, Streptomyces-like colonies were selected and purified on OMA medium. These colonies were subsequently maintained in 15% glycerol at -80°C.

Pathogenicity Tests

Tuber slice assay, radish seedling bioassay, and potato pot trial were performed to assess the pathogenicity of *Streptomyces* isolates according (Bignell et al., 2010; Loria et al., 1995) with some modifications.

For tuber slices pathogenicity assay, healthy tubers were surface sterilized with NaOCl, 1.5% for 1 min. and washed several times with SDW. Subsequently, the cores of the tubers were aseptically extracted, sliced into disks with a thickness of 0.5cm and placed on moistened filter paper (Whatman No.1, 9cm) in petri dishes. The isolates of *Streptomyces* were cultured in OMA medium for 10 days at 28°C. Agar mycelial plugs from the actively growing colonies were then inoculated upside-down onto potato disks. An agar plug of OMA was placed onto potato disks as a control. There were five replicates for each isolate. All petri dishes were incubated for seven days at 28°C. Following the incubation period, potato disks were observed for necrosis and photographed.

The pathogenicity assessment of the isolates was further investigated using a radish seedling bioassay. Radish seeds were surface disinfected with NaOCl for 2 min, afterwards, the seeds were washed 5 times with SDW. After that, they were allowed to germinate for a day at room temperature (23–25°C) in a petri dish containing moist filter paper. Germinated seedlings were placed into tubes (100ml) with water agar (1.5% w/v) and inoculated with 200 µl cultures of *Streptomyces* cell suspension from 7-days-old Oatmeal Broth (OMB). Control seedlings were treated with non-inoculated OMB. There were three replicates of one isolate (for a total of 15 seedlings per isolate). Seedlings were grown at 25°C for 10 days and examined for the presence of necrosis as well as abnormal growth in the root or shoot system.

In addition, the pathogenicity of the *Streptomyces* isolates was tested using a potato pot trial. To prepare spore suspension for soil inoculation, 50 ml of Yeast-malt extract (YME) broth (0.4% Yeast Extract; 1% Malt Extract; 0.4% Glucose; PH 7.2) was inoculated with 1 ml of spore suspension collected from 15-day-old plate of *Streptomyces* isolates grown in YME agar. Cultures were shaken at 200 rpm at 28°C. After incubating for 7 days, the cultures were centrifuged to obtain spores which were then resuspended in sterile H_2O and adjusted to a concentration of 10^7 CFU/ml. Potato tubers, cv. Spunta were procured from the Potato Brown Rot Project, Agricultural Research Center, Egypt, and were maintained at room temperature for several days. The tubers with sprouting eyes were planted into pots (35 cm in diameter) filled with a sterile mixture of sand-clay-soil (1:1; v/v). Inoculations (200ml, 10^7 CFU/ml) were added to the soil of the pots at the time of sowing. Non-inoculated pots were used as a control. Each treatment was repeated in three pots. After 90 days of planting, the disease incidence and disease index of potato scab were computed on harvested tubers (Hao et al., 2009; Liang et al., 2019). The disease severity was grouped as the following scale; 0 = no symptoms, 1 = 1 to 10% surface area with superficial or raised lesions, 2 = 11 to 25% surface area with superficial or raised lesions, 3 = 26 to 50% surface area with superficial or raised lesions, 4 = more than 50% surface area with superficial or raised lesions or 6 to 25% pitted lesion area, and 5 = >50% surface area with superficial or raised lesions or >25% pitted area. The disease index (percentage) was calculated as follows; [Σ (number of diseased potatoes at each scale × representative value at each scale)] / (number of total potatoes investigated × highest representative scale) × 100. The percentage of tubers in each treatment exhibiting CS symptoms was used to express the disease incidence. Data was subjected to a one-way analysis of variance using SPSS software, version 24 (SPSS Inc. USA).

Morphological, Physiological, and Biochemical Traits of Pathogens

Morphological, physiological, and biochemical characterization were performed on the *Streptomyces* isolates identified as pathogens according to the International *Streptomyces* Project (ISP) (Shirling & Gottlieb, 1966). For the morphological observations, the isolates were cultured on YME agar and incubated at 28°C for two weeks. The substrate mycelia, aerial mycelia, spore chains, and soluble pigments from the medium were examined. Melanin production assay was detected on peptone yeast iron (PYI) and tyrosine agar (TYR) plates. Cultures used for light microscope and transmission electron microscope (TEM) examination were obtained after incubation of tested isolates at 28°C for 15 days in OMA media. For TEM analysis, the carbon coated copper grids were gently pressed to the aerial surface of a culture with mature spores. Spore chains that adhere to the coated

surface of the grids were observed using a JEOL GEM-1010 transmission electron microscope at 80 kV at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Egypt.

Physiological and biochemical characteristics were assessed following the methods described in (Williams et al., 1983). Briefly, the utilization of the sole carbon (1% w/v) and nitrogen sources (1g/l) was conducted, and growth results were recorded after 7 days of incubation (Goyer et al., 1996). The growth of strains was assessed at pH values (4-10), and NaCl concentrations (0-10) as described by Pridham et al. (1957). The potential effectiveness of penicillin G (10 IU/ml), oleandomycin (100 pg/ml), and streptomycin sulfate (20 µml) on bacterial cultures was evaluated (Lambert & Loria, 1989). The strains were tested for their capacity to hydrolyze xylan, Polygalacturonic acid, and arbutin (Williams et al., 1983). Additionally, The inhibitory effects of crystal violet (0.5 µg/ml), and phenol (0.1%) were assessed (Lambert & Loria, 1989).

Molecular Identification

Isolates were cultured for two weeks on YME agar medium at 28°C (Shirling & Gottlieb, 1966). Then, mycelium and spores were collected from the plate and ground in liquid nitrogen. Genomic DNA was extracted using a commercial kit (Thermo Fisher Scientific, U.S.A) following the preparation instructions given by the manufacturers. The extracted DNA was quantified using gel electrophoresis and UV spectrophotometry (A260/A280) (Thermo/Scientific NanoDrop 200 Spectrometer). The 16S rRNA gene sequence of the isolates was obtained from fragments generated by PCR with the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Lane, 1991). The PCR reaction mixture contained 2 µl of template DNA (25 ng/µL), 1µl of each of forward and reverse primer (10µM), 12.5 µl of 1x Master Mix (Takara Bio Inc.), and 8.5 µL of PCR grade water. The cycling conditions were as follows: 94 ◦C for 5 min; 35 cycles of 94 ◦C for 30 s, 55 ◦C for 30 s and 72 ◦C for 1 min; and a final extension at 72 ◦C for 10 min. The amplified DNA was electrophoresed on 1.5 % agarose gel stained with ethidium bromide. Sanger sequencing of the resultant products was carried out at Macrogen (Seoul, South Korea). The resulting sequences were examined for sequence homology using BLASTn at the NCBI nucleotide sequence database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The 16S rRNA gene sequences from the highest homology with isolates Ag2 and Man along with sequences of *S. caviscabies* and *S. enissocaesilis* strains induced potato CS, were included in subsequent phylogenetic analyses. All sequences were aligned using the Clustal W algorithm in MEGA X software (Kumar et al., 2018). Phylogenetic trees were reconstructed with the neighbour-joining (Saitou & Nei, 1987). The topology stability of the resultant tree was evaluated using the bootstrap method with 1000 repetitions (Felsenstein, 1985).

Result

Samples of scabby tubers were collected from various regions of Egypt between 2021 and 2022. The disease symptoms of potatoes exhibited netted, superficial, raised, or pitted lesions (Table 1). Nine isolates of *Streptomyces* spp. were recovered from diverse symptoms of CS lesions.

To validate the pathogenicity of these isolates, tuber slice and radish seedling assays were performed. Among nine isolated *Streptomyces* spp. Ag2 and Man isolates showed pathogenicity traits (Table 1, and Figure 1). Ag2 and Man isolates produced brown, necrotize tissues associated with hyphal growth on potato slices (Figure 1g, h) and caused dwarfing abnormal growth of the radish seedlings (Figure 1d, e).

Positive isolates from the potato slices and radish seedlings were tested for pathogenicity using a potato pot trail. Additionally, the types of scab lesions and disease severity on harvested tubers were scored. Both Ag2 and Man isolates induced CS symptoms on potato tubers. The Ag2 isolate produced deep- pitted lesions with an incidence 98.33% and a disease index 73.30% (Figure 1j, and Table 2). Furthermore, tubers that were inoculated with the Man isolate showed apparent brown-raise lesions with an incidence 95.00% and a disease index 63.97% (Figure 1k, and Table 1). The scab symptoms did not observed on the control tubers (Figure 1, i). Bacteria that inoculated onto potato pot trail (tubers) were reisolated from scabbed lesions to confirm Koch's postulates by using morphological characteristics identity. So, Ag2 and Man isolates become known as potato scab pathogens and will be investigated further.

Table 1. Preliminary pathogenicity screening of *Streptomyces* spp. isolated from different potato growing areas and scab symptoms.

Isolate	Geographic origin	Lesion appearance	Pathogenicity	
			Potato	Radish
Beh-1	El-Beheira	Netted	-	-
Beh-9	El-Beheira	Raised	-	-
Nub-1	Nubaria	Netted	-	-
Nub-2	Nubaria	superficial	-	-
Nub-3	Nubaria	Netted	-	-
Qal-1	Qalyubia	Netted	-	-
IS-1	Ismailia	Netted	-	-
Ag2	Cairo	Pitted	+	+
Man	Mansoura	Raised	+	+

Note: (+) = indicates that the isolate induces necrosis on potato tuber slices and abnormal growth of radish seedlings. (-) indicates that the isolate did not induce necrosis on potato tuber slices and normal growth of radish seedlings.

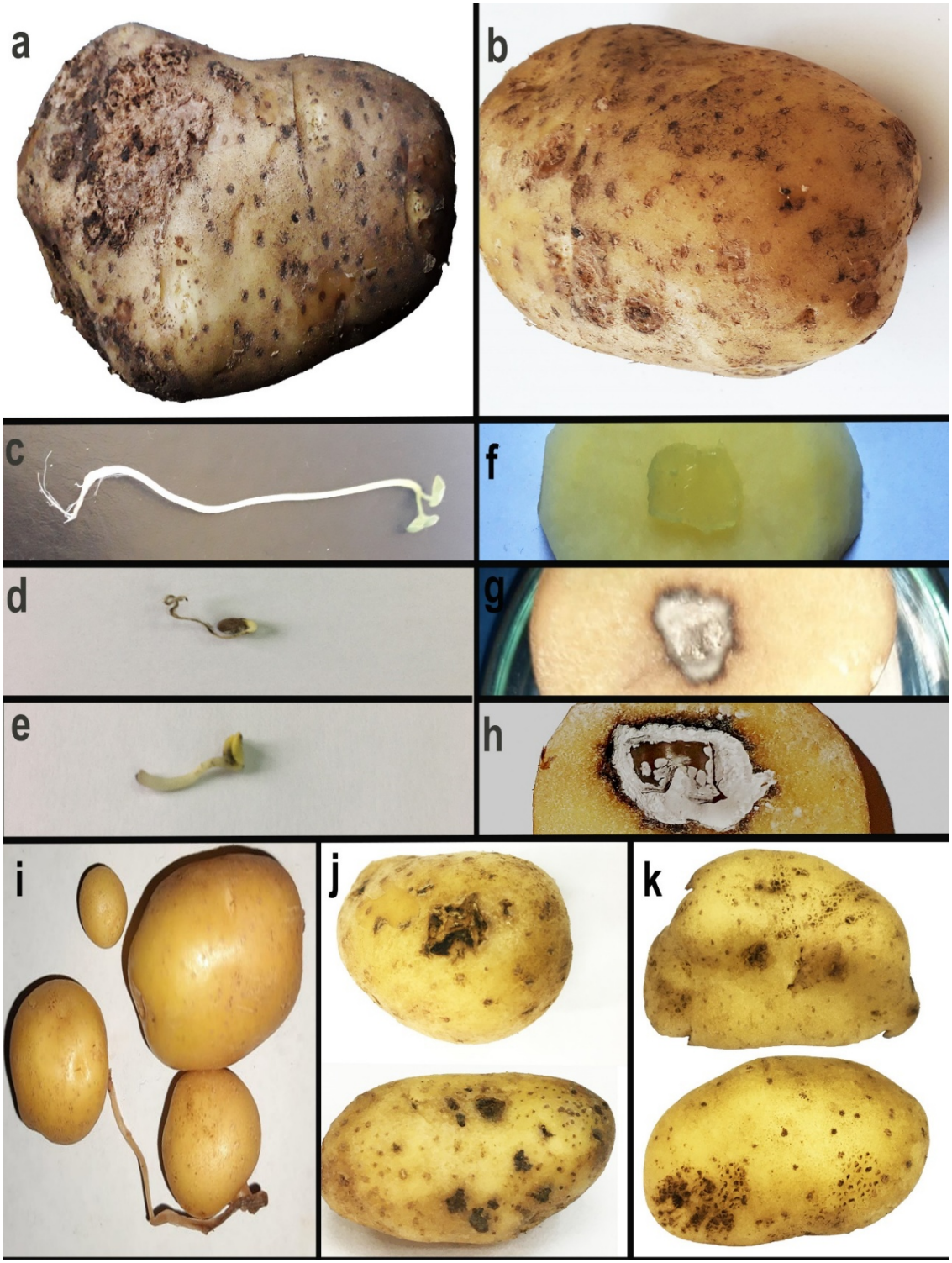

Figure 1. The field symptoms (Naturally infected potato showing symptoms of scab) of potato scab and Pathogenicity verification of Streptomyces isolates Ag2 and Man. Pitted and raised natural symptoms that are source of *Streptomyces* sp. Ag2 (a) and Man (b). Symptoms developed on radish seedlings treated with oatmeal culture of *Streptomyces* sp. Ag2 (d) and Man (e). Necrosis on potato tuber slices produced by agar plug from sporulating colonies of *Streptomyces* sp. Ag-2 (g) and Mans (h). Deep-pitted and raised lesions on potato cv. Spunta induced by *Streptomyces* sp. Ag2 (j) and Man (k). Blank control without pathogen inoculation (c,f, and i).

Table 2. Pathogenic properties of potato scab-causing *Streptomyces* isolates.

Isolate	Disease incidence	Disease index
Ag2	98.33[a*]	73.30[a]
Man	95.00[a]	63.97[a]
control	0.0[b]	0.00[b]
$LSD_{0.05}$	6.66	13.18

* Means with the same letters are not significantly different at 0.05 probability level.

Table 3. Comparison of Phenotypic, physiological and biochemical characteristics of strains Ag2 and Man with references strains.

Characteristics	Ag2	*S. caviscabies**	Man	*S. enissocaesilis***
Colony color on YME medium[@]	Gold	Gold to light brown	gray	N
Spore color	White	White	gray	gray
Spore ornamentation	Smooth	Smooth	Smooth	Smooth
Spore chain morphology	Flexuous	Flexuous	Spiral	Spiral
Melanin production on PYI[#]	–	–	–	–
Production of diffusible pigment	–	v	+	N
Utilization of carbon sources				
L-Arabinose	–	–	+	+
D-Fructose	–	–	+	+
D-Mannitol	–	–	+	+
Raffinose	+	+	+	+
Rhamnose	–	–	–	–
Sucrose	–	–	–	–
D-Xylose	–	–	+	+
D-Glucose	+	N	+	+
Meso-insiotol	+	N	+	+
Utilization of nitrogen sources				
L - P r o l in e	+	+	+	+
L-Methionine	+	+	–	+
L - Tyrosine	+	N	+	N
L - Histidine	+	N	+	+
Asparagine	+	N	+	N
L - Alanine	+	N		N
Hydrolysis of				
Arbutin	-	-	+	N
Polygalacturonic acid	-	-	–	N
Xylan	+	+	–	N
Minimum growth at pH 4.5	–	–	+	+
Growth in the presence of				
NaCl (4%)	+	+	+	+
NaCl (7%)	–	v	–	–
NaCl (10%)	–	–	–	–
Crystal violet (0.5 µg/ml)	+	v	+	+
Phenol (0.1%)	+	+	+	+
Penicillin (10 IU/ml)	+	+	+	+
Oleandomycin (100 µg/ml)	+	v	+	N
Streptomycin (20 µg/ml)	+	+	–	–

The phenotypic, physiological and biochemical characteristics data of *S. caviscabies* and *S. enissocaesilis* from references Goyer et al., 1996; Faucher et al 1995; + : Positive reaction ; ー : Negative reaction ; V: variable; N : Not available; @: YME medium, yeast malt extract medium; #: PYI, Peptone–yeast extract–iron medium.

The morphological, biochemical, and physiological properties of the pathogenic strains are given in Table 3 and Figure. 2. Strain Ag2 exhibited a golden-colored mycelium on YME medium (Figure 2a) and lacked melanin pigment production on PYI. No soluble pigments were detected in the media used. Additionally, it produced white, cylindrical, smooth spores in flexuous chains (Figure 2c). The Ag-2 utilized raffinose, D-glucose, and meso-insitol as carbon sources as well as grew on all tested nitrogen sources. Notably, it could degrade xylan but not hydrolyze polygalacturonic acid or arbutin. Furthermore, this strain cannot grow at a minimum pH of 4.5 but can grow with 4% NaCl or less. It was tolerant to penicillin (10 IU/ml), oleandonycin (100 µg/ml), and streptomycin (20 µg/ml). Also, this strain exhibited growth in the presence of crystal violet (0.5 µg/ml) and phenol (0.1%).

In contrast, strain Man developed gray aerial mycelium, which developed smooth and gray spores arranged in spiral chains (Figure. 2b, d). It produced a brown diffusible pigment but no melanin pigment was detected. The strain used almost all sugars as a carbon source except rhamnose and sucrose. Also, proline, tyrosine, histidine, asparagine, and alanine were utilized as nitrogen sources, but no growth was observed on methionine. The strain had a minimum growth pH of 4.5 and growth in the presence of NaCl 4%. This strain was resistant to oleandonycin (100 µg/ml), penicillin (10 IU/ml), crystal violet (0.5 µg/ml), phenol (0.1%), and arbutin, but its growth was inhibited by polygalacturonic acid, xylan, and streptomycin (20 µg/ml).

A comparison of the phenotypic, biochemical, and physiological traits of pathogenic strains Ag2 and Man with reference *Streptomyces* strains is given in Table 3 and Figure. 2. Therefore, Ag2 and Man strain are nearly identical to *S. caviscabies* and *S. enissocaesilis* respectively.

PCR and sequencing of the 16S rDNA gene were performed to confirm the identity of strains Ag2 and Man (Figure 3).

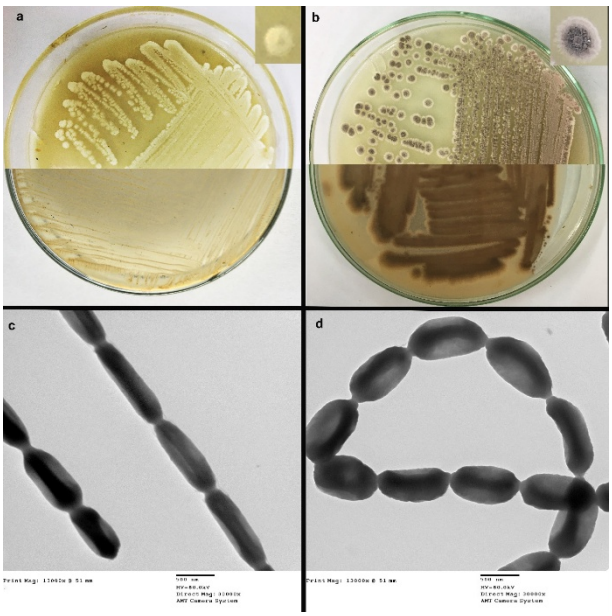

Figure 2. Morphological observation of potato scab-inducing Streptomyces isolates Ag2 and Man. spore color and substrate mycelium of Ag2 (a) and Man (b) culture on yeast malt extract medium incubated at 28°C for 14 days. Spores chain morphology and spores ornamentation under transmission electron microscope of Ag2 (c) and Man (d) grown on oatmeal agar at 28°C for 14 days.

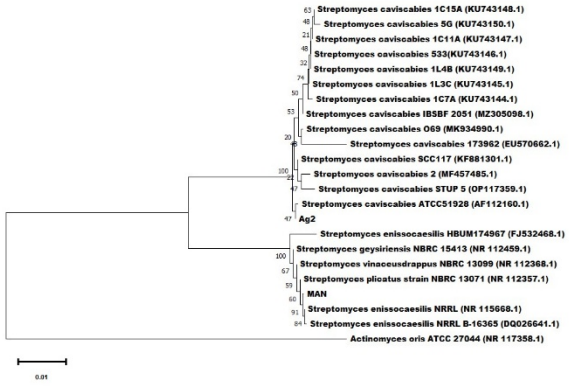

Figure 3. Phylogenetic tree derived from 16S rDNA gene sequences, showing the relationships of strains Ag2 and Man with related *Streptomyces* spp. The tree was rooted with *Actinomyces oris* and constructed using the neighbor-joining method with bootstrap values calculated from 1,000 repetitions. The bar indicates 1% estimated sequence divergence

The sequencing data of the Ag2 and Man strains have been deposited in the GenBank (NCBI) under accession numbers OR447472 and OR447473, respectively. A nucleotide BLAST search revealed that the Ag2 and Man strains share 100% similarity with *S. caviscabies* ATCC 51928 and *S. enissocaesilis* NRRL B-16365, respectively. The 16S rDNA phylogenetic tree (Figure 3) demonstrated that the pathogenic strains (Ag2 and Man) were separated into two distinct groups supported by high bootstrap values (100%). All *S. caviscabies* strains and the Ag2 strain formed a cluster in the first group, with Ag2 closely related to the *S. caviscabies* (ATCC 51928) type strain. The second group comprised *Streptomyces* species with nearly identical 16s DNA sequences, and the Man strain clustered closely with type strain of S. *enissocaesillis* NRRL B-16365 with a bootstrap value of 91%.

Discussion

Potato CS is a widespread disease incited by different *Streptomyces* species that significantly decreases the marketable yield for potato growers (Braun et al., 2017). While *S. scabies* is widely recognized as the main cause of scab disease, there are actually over 27 scab-causing *Streptomyces* species that have been reported worldwide(Vincent & Bignell, 2024).

Despite the frequent occurrence of scab disease in Egypt, which poses a significant threat to potato crops and has been reported as early as 1966 (EL.Kashier, 1966; Mehiar & El-Samra, 1978), there is a noticeable absence of comprehensive studies that specifically aim to characterize the pathogenic *Streptomyces* species in the country.

In this investigation, nine isolates of *Streptomyces* spp. were recovered from potato tubers naturally infected and collected from various locations in Egypt. Out of the nine isolates, only the Ag2 and Man strains showed positive results in both the tuber slice and radish seedling tests. In the potato pot trail, these strains induced symptoms of CS on potato tubers that were identical to those observed on the original infected potatoes. A significant number of *Streptomyces* spp. isolated from scabby potatoes have been previously reported to be non-pathogenic (Gouws, 2013; Henao et al., 2022; Jordaan & Van der Waals, 2016; Wanner, 2007). Also, it has been found that non-pathogenic Streptomyces can colonize tubers that display symptoms of CS. This colonization may potentially contribute to the development of the disease (Chalupowicz et al., 2022). The study of Cui et al. (2021) reported that isolate 5A-1 exhibited pathogenicity among 20 isolated *Streptomyces* spp.

The pathogenic isolates in our study were identified as *S. caviscabies* and *S. enissocaesilis*. This identification was based on an evaluation of their morphological and biochemical characteristics, along with the analysis of their 16S rDNA gene sequences. Both pathogens are identified for the first time as causative agents of potato common scab in Egypt.

Streptomyces caviscabies was initially described as a pathogen responsible for the development of deep-pitted potato scab in Canada (Faucher et al., 1995; Goyer et al., 1996). Additionally, Corrêa et al. (2015) detected this pathogen in several potato fields in Brazil where it was associated with potato scab. In Iran, a group of *S. caviscabies* strains was isolated from scabby potatoes and these strains caused significant disease severity under both natural and greenhouse conditions (Khodakaramian & Khodakaramian, 2013). Recently, five isolates from typical CS lesions in China were identified as *S. caviscabies*. These isolates exhibited typical symptoms of CS on potatoes under greenhouse conditions (Gong et al., 2017).

The species *S. enissocaesilis* was first reported as a novel potato scab pathogen in China (Zhang et al., 2010; Zhang et al., 2009). Yang et al. (2018) investigated the species composition of pathogenic *Streptomyces* responsible for causing potato scab in Yunnan Province, China. Out of the 67 pathogenic *Streptomyces* spp., 29 were identified as *S. enissocaesilis* and caused tuber

disease with CS symptoms in greenhouse pots. Recently, *S. enissocaesilis* was used as a pathogen of potato CS to screen for highly efficient bactericidal agents *(*Pu et al*.,* 2022*)*. On the other hand, *S. enissocaesilis* has previously been documented as an effective biological control agent against a wide range of diseases and pests (Aallam et al., 2021; Abbasi et al., 2019; Boukelloul et al., 2024; Ganesan et al., 2018). In the rhizosphere, *S. galilaeus* and *S. griseoplanus* have a dual role. In addition to being a pathogen that can cause potato CS, they can also act as a potential bioagent for controlling plant diseases (Cui et al., 2021; Cui et al., 2018; Nimnoi et al., 2017). Biocontrol agent assays, particularly those involving *Streptomyces* spp., require careful attention. It is crucial to conduct radish seedling and potato slice assays to verify the pathogenicity of the tested agents used for controlling plant diseases. The emergence of new pathogen causing CS may be attributed to the horizontal gene transfer of pathogenicity genes among *Streptomyces* spp. (Armijos-Jaramillo et al., 2017; Bukhalid et al., 2002).

In Egypt, the precise date of the initial recording of potato CS disease is uncertain. However Egyptian quarantine acts and regulations indicate that it was recognized as early as 1932 (EL.Kashier, 1966). Many studies have indicated that *S. scabiei* is the main causal agent of potato CS disease in Egypt (Abd El-Hafez & El Shishtawy, 2021; Eid et al., 2022; Gabr, 1988; Galal et al., 1999; Mehiar & El-Samra, 1978). Additionally, a few other causal agents have been identified, including *S. acidiscabies* (El-Sayed*,* 2000), and *S. alkaliscabies* (Abdel-Rahman et al., 2012). The low diversity of scab-causing strains isolated from Egypt may be related to previous studies that performed only phenotypic (morphology and biochemical) identification tests, except for *S. alkaliscabies*. Therefore, phenotypic and molecular characteristics should be performed for an accurate and clear diagnosis of pathogenic strains.

Conclusion

The current study reports, for the first time, the presence of scab-causing pathogens *S. caviscabies* and *S. enissocaesilis* in Egypt. These pathogens were identified based on their phenotypic and molecular traits. Their pathogenicity was validated through potato slices and radish seedling assays, as well as a greenhouse evaluation. Further research is necessary to define the range of pathogens and develop effective control strategies.

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