The Role of Thaxtomin A and Necrosis Protein in Virulence of *Streptomyces scabies* that Cause Potato Common Scab Disease

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ABSTRACT

Three isolates of *Streptomyces scabies* (St.1, St.2, and St.3) were examined for virulence on potato mini tubers, necrosis initiation on potato slices, inhibition of radish seedling length, production of thaxtomin A, and the presence of thaxtomin A (*txtA*) and *nec1* genes. The three isolates, St.1, St.2, and St.3, were classified as highly virulent, moderately virulent, and weakly virulent, respectively. The St.1 isolate showed the highest scab severity index on potato mini tubers and necrosis on potato slices, while the St.3 had the lowest, and St.2 had intermediate values. The St.1 reduced radish seedling length by 82.35% and produced the highest toxin level (3.1 mg/ml), while the St.3 isolate reduced seedling length by 63.5% and produced the lowest level of toxin (1.18 mg/ml). The three isolates were positive for the presence of thaxtomin A (*txtA*) gene and produced a specific gene band at 500bp. However, St.1 and St.2 isolates were able to create a particular band at 700bp associated with the necrosis gene (*nec1*), while the isolate St.3 lacked this band. Based on the total protein profile analysis, the three *S. scabies* isolates clustered into two groups which diverged at a similarity index of 0.78. The St.2 and St.3 virulent isolates were located in the same group with 0.86 similarity, while the highly virulent isolate St.1 was located in a separate group. Current findings highlight the correlation between the presence of thaxtomin A and necrosis virulence factors and *S. scabies* pathogenicity.

Key words: Potato common scab – *Streptomyces scabies* – thaxtomin A – *nec1* gene.

INTRODUCTION

Potato is the third most important crop in Egypt, with about 5.08 million tons of production, making it Africa's largest potato producer (FAO STAT, 2019). Potato is challenged by many pathogenic bacteria, including *Streptomyces scabies*, that cause potato common scab disease. However, only a limited number of *Streptomyces* spp. are pathogenic and capable of infecting the underground parts of numerous plant species such as potato, sweet potato, beet, turnip, carrot, radish, and peanut (Wanner and Kirk, 2015; Li et al., 2019a). *Streptomyces scabies* is the most prevalent species and causes potato common scab, a severe and economically significant disease widely spread in potato growing regions in Egypt (El-Sayed et al., 2001; El-Sheikh, 2010; El-Sheikh et al., 2012; Abd El-Rahman et al., 2018).

Streptomyces species are gram-positive filamentous bacteria that live in soil as saprophytes and can produce a diverse range of bioactive metabolites. *Streptomyces* plant pathogenicity is mediated by the production of phytotoxic secondary metabolites. Thaxtomins, a main phytotoxin, elicits cell hypertrophy, cause stunting and death of seedlings, and a key factor in developing potato common scab disease (Fry and Loria, 2002; Loria et al., 2006; King and Calhoun, 2009; Bignell et al., 2014; Wanner and Kirk, 2015; Li et al., 2019b). Several analogues of thaxtomins were identified in *Streptomyces* spp., with thaxtomin A the predominant in *S. scabies* (Loria et al., 2008; King and Calhoun, 2009; Liu et al., 2021). Pathogenicity of *S. scabies* strains was positively linked with the production of thaxtomin A phytotoxin (El-Sayed et al., 2000; El-Sheikh, 2010; El-Sheikh et al., 2012). The crude extracts from nonpathogenic *S. scabies* strains did not release the toxin and did not affect the potato slices, while the crude extracts from pathogenic *S. scabies* strains induced necrotic lesions (El-Sheikh, 2010). Also, *S. scabies* mutants lacking thaxtomin A showed a decrease or loss of pathogenicity (Healy et al., 2000; Joshi et al., 2007a), demonstrating that thaxtomin A is an essential virulence factor.

Full virulence of pathogenic *Streptomyces* spp. is not solely dependent on thaxtomin production, but harmonization between multiple virulence products, including secondary metabolites and secreted proteins, were required for pathogenicity (Loria et al., 2008). Some of the virulence compounds secreted by *Streptomyces* spp., such as the Nec1 protein that inhibits plant defensive responses. The *nec1* gene, which codes necrosis protein synthesis (Nec1), was the first virulence gene cloned from plant pathogenic *streptomycetes* (Bukhalid and Loria, 1997). The *nec1* gene sequence is similar in many strains of *S. scabies*, *S. turgidiscabies*, and *S. acidiscabies* (Bukhalid et al., 2002). The *S. turgidiscabies* Car8 strain with a *nec1* deletion mutant failed to colonize in the root meristem of radish roots, but the wild-type strain severely colonized (Joshi et al., 2007b). The *nec1* deletion mutant might produce the same levels of thaxtomin as the wild type, implying that the mutant may lack the ability to compete with the

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plant defense system but can produce a proper amount of thaxtomin A (Healy et al., 2000). The genes involved in the thaxtomins biosynthesis (*txtAB*), and other virulence genes like the *nec1* and a tomatinase (*tomA*) genes are clustered on a large pathogenicity island (PAI). The PAI might be mobilized and transferred to nonpathogenic *streptomycetes*, resulting in the rise of new pathogenic species (Loria et al., 2006; Lerat et al., 2009; Fyans et al., 2016). Based on the above mentioned, current study aimed to evaluate the pathogenicity of three *S. scabies* isolates, associate the virulence of these isolates with thaxtomin A production and the presence of the thaxtomin A (*txtA*) and the necrosis (*nec1*) genes, as well as characterize the protein profile of these isolates using SDS-PAGE analysis.

MATERIAL AND METHODS

1. Plant and *Streptomyces* source:

Potato tubers of cv. Spunta was obtained from the International Potato Center (CIP), Kafr El-Zayat, Egypt. Three *Streptomyces scabies* isolates, St.1, St.2, and St.3, differed in virulence, were obtained from *S. scabies* cultures collection, Plant Pathology Dept., Faculty of Agriculture, Damauhour University.

2. Virulence assays of selected *S. scabies* isolates:

Common scab severity index on potato mini tubers:

Potato mini tubers of cv. Spunta was harvested 45 days after planting, washed with distilled water, surface-disinfested in 2 % sodium hypochlorite for 10 min, rinsed twice in sterile distilled water, and left to dry. The *Streptomyces* inocula were prepared as described by Leiner et al. (1996). Inocula suspensions were prepared at approximately 1 x 10^7 spore/ ml tryptone yeast broth medium, and 50 ml of inoculum of each isolate were mixed with 1.5 L of autoclaved vermiculite (Balcony garden, Egypt). This mixture was added to 20 potato mini tubers in small plastic bags, incubated for 14 days, and shaken many times during the incubation period (El-Sheikh et al., 2012). Clean potato mini tubers were served as control. Three replicates (plastic pages) per treatment were used. Fourteen days after inoculation, mini tubers were rinsed in water, left to dry, and rated for disease severity. The percentage of surface area covered by necrotic lesions was utilized to evaluate the scab index as follows: 0 = no scab; 0.5 = less than 1%; 1 = 1 to 5%; 2 = 6 to 10%; 3 = 11 to 25%; 4 = 26 to 50%; 5 = 51 to 75%; and 6 = 76 to 100% tuber surface coverage (Beaudoin et al., 2021). The percentage of scab severity index was calculated for each tested isolate as follows:

Scab severity index (%) = {Σ (Numerical value of scab index X No. tubers per index)/ (Total number of tubers X Maximum numerical value)} x100

When the scab severity index was < 50%, the isolate was classified as weakly virulent, moderately virulent when the scab severity index was < 70% - ≥ 50%, and highly virulent when the scab severity index was ≥ 70% (El-Sheikh et al., 2012).

Virulence of *S. scabies* isolates on potato tuber slices:

Potato tuber slice assay was used to confirm the necrotizing ability of the tested *S. scabies* isolates, according to Loria et al. (1995), with some modifications. Healthy potato tubers of cv. Spunta were surface-disinfested as previously described, and slices were cut out with a sterile scalpel. The potato tuber slices (15 per treatment) were placed onto moist sterile filter paper in plastic containers, and each slice was inoculated with 25µl of *S. scabies* mycelial suspension, whereas the control slices received 25µl of sterile water. After that, the plastic containers were wrapped with parafilm and incubated in the dark at 22±2°C. The diameters of necrotic lesions (cm) were measured on the inoculated potato tuber slices after 14 days of incubation. The assay was repeated twice.

Inhibition of radish seedlings growth by culture filtrate of *S. scabies* isolates:

The effect of *S. scabies* isolates on radish seedlings was assessed based on Leiner *et al.* (1996). Radish seeds were surface disinfested in 0.26% NaOCl for five min., then rinsed twice in sterile water and germinated on moisture filter paper for 24 hours in the dark. Uniform germinated seeds were then selected and placed in a Petri dish with 10ml of 1% agar water. Each dish was inoculated with 200µL of *S. scabies* culture filtrate, and the same amount of oatmeal bran broth medium was used as uninoculated control. Seedlings were grown at 24ºC with a 16-h photoperiod for seven days. Three replicates per treatment, each with 20 seedlings, were used. Seven days after inoculation, relative seedlings growth (seedlings length, mm) compared to the control was recorded.

Index of radical damage evaluation:

Each seedling was evaluated after seven days using the following index: 1= Radical is healthy; 2= Radical has necrotic flecks; 3= Radical has large necrosis areas; 4= Radical is deformed; 5= radical is dead, and average damage index was calculated for each isolate.

3. Thaxtomin A production by *S. scabies* isolates:

Spore suspension (150µL) of each isolate was mixed with 50 ml oatmeal bran broth and incubated for eight days at 28ºC with shaking (200 rpm). The cultures were centrifuged at 4500g for 10 min (Universal 32R, Hettich Zentrifugen, Germany), after which the supernatants were used to extract the toxin using an equal volume of ethyl acetate. The extract was evaporated to dryness in a rotary evaporator at 50ºC before redissolved in ethyl

acetate. The toxins were then analyzed according to Goyer et al. (1998). Thaxtomin was eluted with methanol, measured with a UV–visible spectrophotometer at 380 nm (Jenway, Model 6305, Bibby Scientific Limited, UK), and quantified using the absorption coefficient determined by King et al. (I992).

4. Molecular detection of Thaxtomin A (*txtA*) and *nec1* virulence genes in *S. scabies* isolates.

***S. scabies* DNA extraction:**

The *S. scabies* isolates were cultured on oatmeal bran broth and incubated at 28ºC for five days on a thermal shaker (200 rpm). Cultures were centrifuged at 6000g for 10 minutes, and 200-300 mg of the pellet of each isolate was used to extract DNA using the Qiagen Kit for DNA extraction (QIAGEN) according to the manufacturer's protocol. The extracted DNA was dissolved in 50 µL of TE buffer (10 mM Tris-HCl, pH 8.0 + 1.0 mM EDTA, pH 8.0). The concentration and purity of the obtained DNA were spectrophotometrically determined according to Sambrook et al. (1989) and adjusted to 50 ng/µl.

The PCR reaction for detection of *txtA* and *nec1* virulence genes:

Sequences of primers used to amplify *txtA* gene for, TxtA-F, was 5′-TGCGGTTCCGGTCTG-CTGCTCTC -3′ and 5′-GTTGTCGTACCCGCCCCGTTTGA-3′ for TxtA-R (Wang and Lazarovits, 2004), and for amplification of *nec1* gene was, Nec1-F, 5′-ATGAGCGCGA-ACGGAAGCCCCGGA-3′ and Nec1-R, 5′-GCAGGTCGTCACGAAGGATCG-3′ (Wanner, 2004). PCR amplifications were performed in a 25 µl reaction mixture with 12.5 µl of 2x GoTaq green master mix (Promega Corp.), 1 µl of each forward and reverse primer (100 Pmol/µl), 100 ng of DNA, and 9.5 µl of RNase free water. Amplification was performed in a thermal cycler (MJ Research PTC-200 Gradient PCR) programmed for one cycle of 5 min at 95˚C and 45 cycles, each consist 1 min at 95˚C, 1 min at 61˚C for TxtA, or 55˚C for Nec1 primers, 2 min at 72˚C, and a final extension step of 5 min at 72˚C. The PCR products were resolved in 1% agarose gel in 0.5X TBE buffer aside 1000 bp ladder (Invitrogen- life technologies). Gels were stained in ethidium bromide and visualized with a gel documentation system (ChemiDoc MP imaging system, BIO-RAD). The size and presence of the expected 500-bp *txtA*, or 700-bp *nec1* fragments were verified.

5- Protein analysis for *S. scabies* isolates using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE):

The *S. scabies* isolates were grown on 100 ml of Yeast Malt extract media at 28± 2ºC for 14 days under shaking (150 rpm). Bacterial cells were harvested by centrifugation (3 min. at 1800g), washed with 0.1 M phosphate buffer (pH 7), frozen at -20ºC, and soluble protein was extracted as described by Howard and Brown (2001). SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed for total proteins according to Laemmli (1970). Total proteins were electrophoretic run aside with standard protein marker on polyacrylamide resolving gel (12.5%) and a 4% stacking gel and stained with 0.1% Coomassie Brillant Blue R-250. Protein profiles were recorded as a value of 1 or 0 for the presence or absence of the band. The unweighted pair group method with arithmetic averages (UPGMA) was used to determine the similarity between the *S. scabies* isolates and to perform cluster analysis (Sneath and Sokal, 1973).

6- Statistical analysis:

The data were analyzed using GraphPad PRISM ver. 8.4.3 (GraphPad Software, San Diego, California, USA) and Tukey's HSD test was used to compare means at a probability level of ≤ 0.05. Data were expressed as mean ± SE.

RESULTS AND DISCUSSION

RESULTS

1. Virulence of tested *S. scabies* isolates:

Scab severity index on potato mini tubers:

significant differences in virulence among the tested isolates were observed (Fig. 1 and 2). The St.1 isolate caused severe scab symptoms on mini tubers with the highest scab severity index (72.3%). The necrotic lesions were brown to black, superficial, extended more than 5 mm around the tuber periderm, and the scabbed areas collapsed. On the other hand, the lowest scab severity index (18.3%) was found after inoculating the mini tubers with St.3 isolate. The lesions produced by this isolate were restricted to the lenticels, and the necrotic tissue was about 1mm, while the scabbed areas were scattered with no change in the tissue color. Meanwhile, St.2 recorded an intermediate scab severity index (53.6%) (Fig. 1A and 2). According to the scab severity index on potato mini tubers, the St.1, St.2, and St.3 isolates of *S. scabies* were classified as highly virulent, moderately virulent, and weakly virulent, respectively (Fig 1 and 2).

Necrosis initiation on potato tuber slice:

Varying degrees of necrosis were observed when mycelial suspensions of the three *S. scabies* isolates were applied to potato tuber slices (Fig.1B and Fig.2). After five days of inoculation with the highly virulent isolate St.1, necrosis started to appear and progressed during the incubation period. Severely necrotic tissue collapsed during the assay, leaving a dark black area on the tuber slice surface with a necrotic lesion diameter of

2.1 cm. Following a similar pattern, the moderately virulent isolate St.2 produced necrosis after seven days of inoculation. The necrotic tissue was a pale brown, and a slightly sunken area on the tuber slice surface was observed with a necrotic lesion diameter was around 1.67 cm. On the other hand, the weakly virulent isolate St.3 produced necrosis after eight days of inoculation and showed only slight symptoms, whereas the tissue around the inoculation area was pale yellow with no collapse in the inoculated tissue, and the damaged area was about 0.87 cm in diameter (Fig.1B and Fig.2).

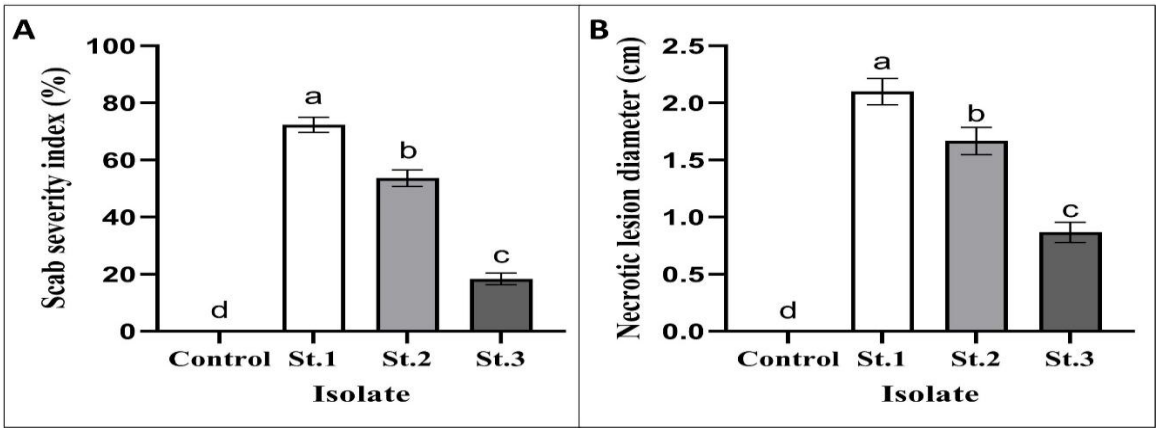

Fig. 1. Comparison of three tested isolates of *S. scabies* (St.1, St.2, and St.3) for the percentage of scab severity index on potato mini tubers (A) and necrosis production on potato tuber slice (B). Uninoculated potato mini tubers and slices received 25µl of sterile water were served as controls. Means followed by different letter (s) are significantly different by Tukey's HSD test (P ≤ 0.05). Data are means of three replicates ± SE

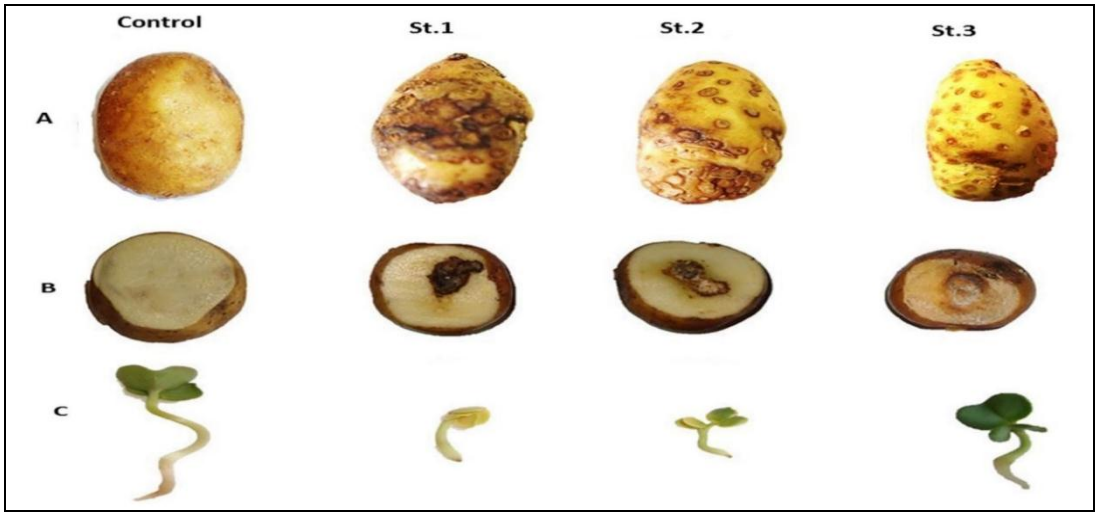

Fig. 2. Virulence assays of tested isolates of *S. scabies* (St.1, St.2, and St.3) on potato cultivar Spunta. A: potato mini tubers scab index, B: different necrotic reaction on potato tuber slices, and C: radish seedling length inhibition and damage

Inhibition of radish seedling length and seedling index of damage:

Inhibition of radish seedling growth and radical damage was evaluated after seven days of inoculation with *S. scabies* isolates (Fig.2 and 3). The control radish seedlings had 85mm in length and standard radical vigor. The highly virulent isolate St.1 had a seedling length of 15mm with 82.35% reduction in length compared with control and a 3.2 radical damage index, with multiple dead or deformed radicals. The moderately virulent isolate St.2 showed 80% inhibition of seedling length, with a seedling length of 17mm and a radical damage index of 2.4. Large necrotic areas with some deformations were the most common signs of radical damage. A different pattern was noticed for the weakly virulent isolate St.3, which had a seedling length of 31mm and 63.5% length inhibition and a radical damage index of 1.46 with minor necrotic flecks and no dead or deformed plants (Fig. 2 and 3).

Production of thaxtomin A

Thaxtomin A production by the tested isolates was evaluated after eight days of inoculation on the growth medium (Fig. 4). Significant differences were found between the three tested isolates. The weakly virulent isolate St.3 had the lowest toxin production of 1.18 mg/ml, whereas the highly virulent isolate St.1 had the highest toxin production of 3.1 mg/ml. Meanwhile, the moderately virulent isolate St.2 produced a moderately toxin amount at 2.26 mg/ml of oatmeal broth medium (Fig. 4).

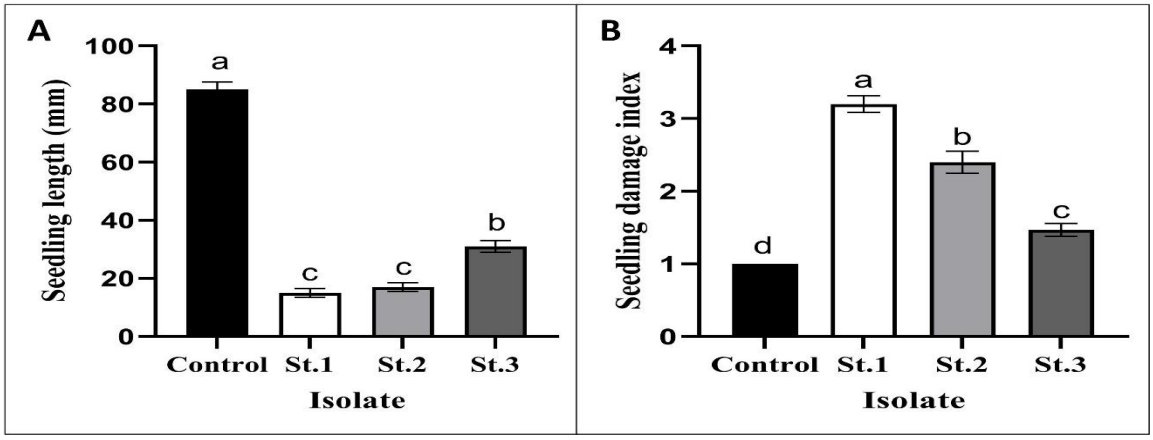

Fig. 3. Comparison between the three isolates of *S. scabies* (St.1, St.2, and St.3) for radish seedling length (A) and seedling radical damage index (B). The control treatment was only treated with oatmeal bran broth medium. Index of radical damage was evaluated after 7 days of seedlings inoculation, whereas 1= Radical was healthy; 2= Radical with necrotic flecks; 3= Radical with large necrosis areas; 4= Radical deformed; 5= Radical dead. Means followed by different letter (s) are significantly different by Tukey's HSD test (P ≤ 0.05). Data are means of three replicates ± SE

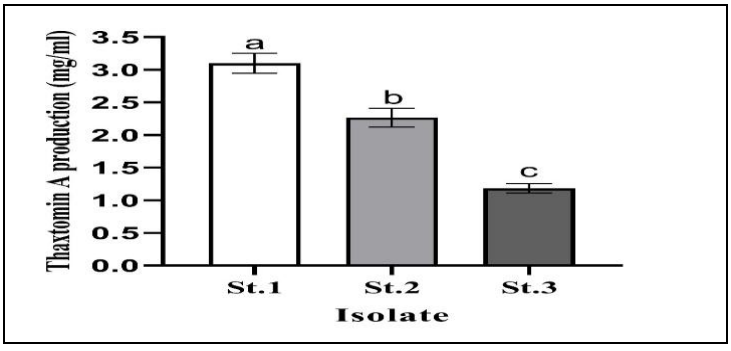

Fig. 4. Comparison between the three isolates of *S. scabies* (St.1, St.2, and St.3) for thaxtomin A production. Thaxtomin A was measured after 8 days of inoculation on oatmeal bran broth medium and represented as mg/ml of medium. Means followed by different letter (s) are significantly different by Tukey's HSD test (P ≤ 0.05). Data are means of three replicates ± SE

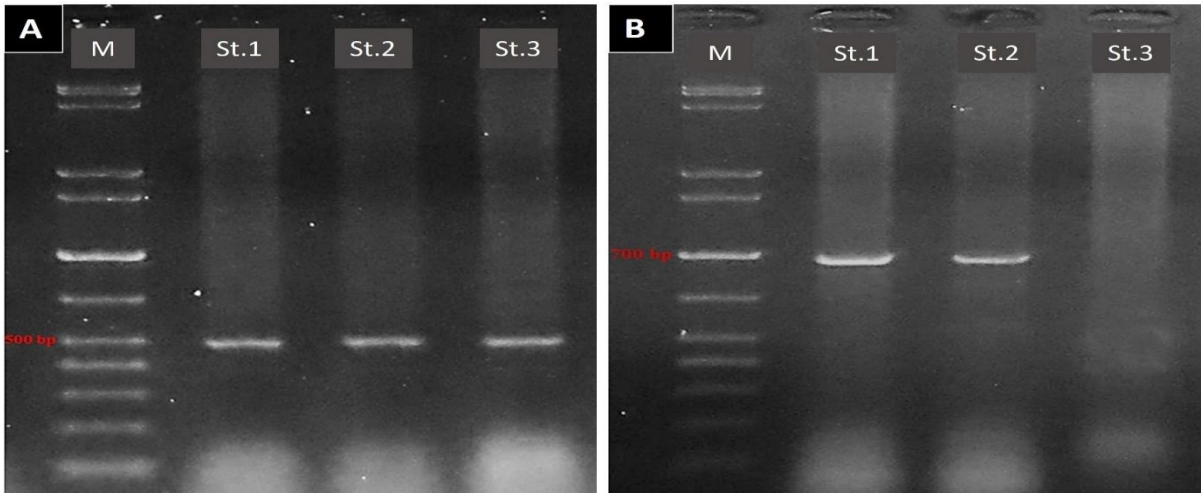

Fig. 5. Gel electrophoresis of PCR products generated by specific amplification of genomic DNA of the three tested isolates of *S. scabies* corresponding to the thaxtomin A (*txtA*) (A) and *nec1* (B) genes. M, DNA marker 100bp; Lanes St.1, St.2, and St.3 represent the *S. scabies* isolates

2. Molecular characterization of the virulence factors *txtA* and *nec1* genes using specific primers:

The thaxtomin A (*txtA*) and *nec1* virulence genes were successfully detected in the tested isolates of *S. scabies* using a specific primer PCR technique. As shown in Fig. 2, it was clear that all of the three tested isolates were able to produce a particular band of *txtA* gene of approximately 500bp (Fig. 5A). However, the highly (St.1) and moderately (St.2) virulent isolates were able to produce a specific band at 700bp related to the *nec1* gene. While, the weakly virulent isolate St.3 failed to generate this bad, indicating that this isolate might lack this gene (Fig. 5B).

3- Total Protein analysis of *S. scabies* isolates:

The protein banding patterns of the SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of the examined isolates of *S. scabies* were shown in Fig. 6. The highly virulent St.1 isolate was recognized with 11 protein bands with a molecular weight of 15 - 65kDa. While the weakly (St.3) and moderately (St.2) virulent isolates showed 12 and 14 bands, respectively, in the molecular weight between 8-65kDa. Protein bands at 29kDa were missing from the weakly pathogenic St.3 but induced in St.1 and St.2 isolates. Also, a unique band was induced at 26kDa on St.2 isolate. Similar protein profiles were identified for St.2 and St.3 isolates except for the bands at 26kDa and 29kDa (Fig. 6).

Similarity matrix and phylogenetic analysis:

The SDS-PAGE protein banding patterns of the three isolates of *S. scabies* were used to estimate a similarity matrix based on the UPGMA algorithm. The results in Table (1) illustrated that the tested isolates were different since no pairs were found with 100% similarity. The lowest level of similarity was between St.1 and St.3 isolates (0.77), while the highest similarity was between St.2 and St.3 isolates (0.86). On the other hand, St.1 and St.2 shared 0.79 similarities. The three *S. scabies* isolates clustered into two groups that diverged at a similarity index of 0.78. The moderately (St.2) and the weakly (St.3) virulent isolates were located in the same group, while the highly virulent isolate St.1 was located in a separate group (Fig. 7).

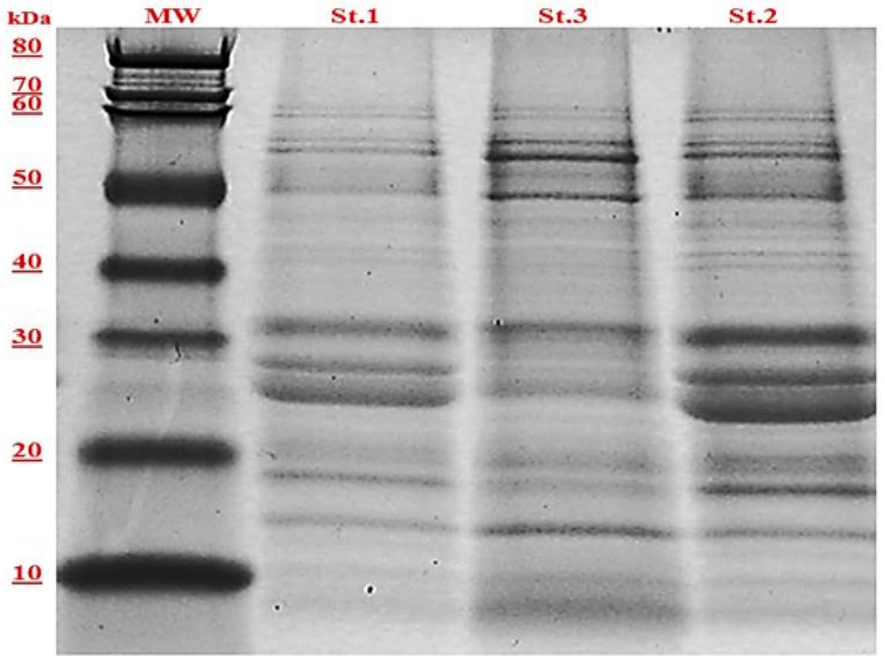

Fig. 6. SDS-Page of protein banding pattern of three *S. scabies* isolates with different degrees of virulence. MW, protein molecular weight standard; Lanes St.1, St.2, and St.3 represent the *S. scabies* isolates

Table 1. Similarity matrix based on SDS-PAGE protein profiles of *S. scabies* isolates

Isolate	St.1	St.2	St.3
St.1	1.00	-	-
St.2	0.79	1.00	-
St.3	0.77	0.86	1.00

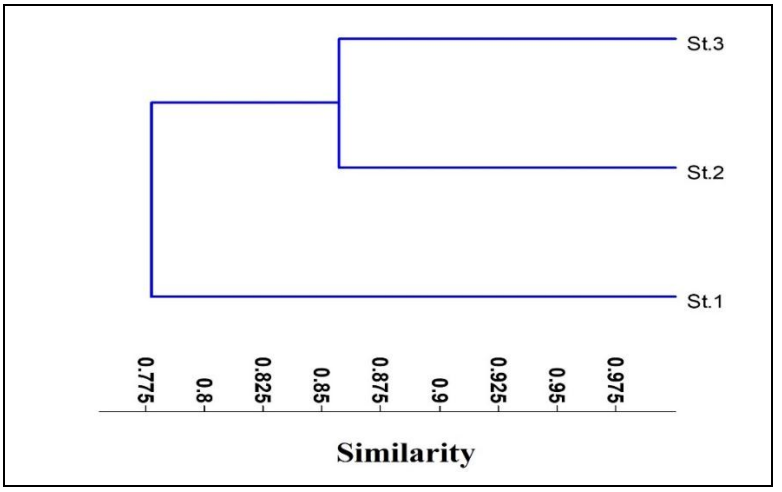

Fig. 7. Tree dendrogram of SDS-PAGE protein profiles constructed using UPGMA method showing the phylogenetic relationship of the tested isolates of *S. scabies* (St.1, St.2, and St.3)

DISCUSSION

The common scab of potato crops caused by *Streptomyces* spp. occur all over the world. Severe scab infection could decrease the tuber yield (Hiltunen et al., 2009). However, in most cases, the disease was just superficial and reduces the crop's quality and market value rather than its yield, resulting in major significant losses for farmers (Sharma et al., 2014; Wanner and Kirk, 2015). The symptoms development in potato depends on various factors, including the potato cultivar, ambient conditions, and the virulence and pathogenicity of *Streptomyces* spp. (Bouchek-Mechiche et al., 2000; Wanner, 2009; Dees et al., 2012).

The virulence bioassays of selected *S. scabies* isolates showed significant variations in the virulence between the tested isolates based on the scab severity index on potato mini tubers and the necrosis production on potato tuber slices. The scab severity index of potato mini tubers and necrosis diameters on potato slices were highest in the St.1 isolate and lowest in the St.3 isolate. St.2 had an intermediate level. The three tested *S. scabies* isolates, St.1, St.2, and St.3, were classified as highly virulent, moderately virulent, and weakly virulent, respectively. These results were in harmony with earlier studies that found variations in virulence among *Streptomyces* spp., notably *S. scabies* isolates (Wanner, 2004; Dees et al., 2013; Karagöz and Kotan, 2017; Shi et al., 2019). In former research conducted in Elbeheira Governorate of Egypt, El-Sheikh et al. (2012) identified 20 isolates of *S. scabies* with five that were highly virulent and 15 were moderately virulent based on scab index on potato mini tubers. Also, Hussein et al. (2019) isolated 12 isolates of *S. scabies* that differed in their virulence on potato plants.

Radish seedling bioassay is a standard and rapid test for identifying plant pathogenic *Streptomyces* isolates (Wanner, 2004). An isolate would be classified plant pathogenic if it produces considerable stunting and scab lesions of the radish seedlings (Wanner, 2004). This assay was successfully distinguished between the isolates used in this study, with the highly virulent isolate St.1 reduced seedling length by 82.35%, while the weakly virulent St.3 isolate reduced seedling length by 63.5%, and moderately virulent isolate St.2 reduced seedling length by 80%. *S. scabies* generally causes shoot and root length decrease, tissue swelling, and tissue chlorosis and necrosis, particularly at the root tip of radish seedlings (El-Sayed, 2000; Bignell et al., 2014; Jourdan et al., 2016). Pathogenicity of seedlings is mainly mediated by thaxtomin phytotoxins production (Leiner et al., 1996; Loria et al., 1997; Loria et al., 2006; Bignell et al., 2014).

Thaxtomin is an essential agent for the pathogenicity of *Streptomyces* spp., and it increases the ability of *S. scabies* to induce common scab disease (Loria et al., 2006; Johnson et al., 2007; King and Calhoun, 2009; Wanner, 2009; Dees et al., 2013; Jourdan et al., 2016). Thaxtomin A is the main form of thaxtomins produced by *S. scabies* (Loria et al., 2008; Liu et al., 2021). The symptoms of *S. scabies* in potato tubers and radish seedlings were identical to those elicited by thaxtomin A, demonstrating that these phytotoxins may be responsible for this species' pathogenicity (Loria et al., 2006). There were significant variations in the production of thaxtomin A between the three isolates investigated in this study. The highly virulent St.1 isolate produced the most significant amount of toxin (3.1 mg/ml), whereas the weakly virulent St.3 isolate produced the least amount (1.18 mg/ml); meanwhile, the moderately virulent isolate St.2 produced 2.26 mg/ml of toxin. The positive linkage between the virulence level of *S. scabies* isolates and the secreted amount of to thaxtomin A observed in this study agreed with the results reported in prior reports (King et al., 1991; El-Sayed, 2000; El-Sheikh, 2010; El-Sheikh et al., 2012). Mechanistically, thaxtomin A inhibit cellulose synthesis, allowing bacteria to penetrate plant tissues during host colonization resulting in plant cell hypertrophy and seedling stunting (Scheible et al., 2003; Loria et al., 2006; 2008). However, other phytotoxins, phytohormones, and proteins may play a role in the severity and progression of the disease (Bignell et al., 2014; Fyans et al., 2016; Li et al., 2019a; Liu et al., 2021).

Using the specific primer-PCR approach effectively detects the presence of the virulence factor genes in the selected *Streptomyces* spp. isolates (Wanner, 2004; Cao et al., 2012; Fyans et al., 2016). The obtained results showed that the three tested isolates were positive for the presence of thaxtomin A (*txtA*) gene and produced a specific gene band at 500bp. These results were consistent with many previous reports that detect *txtAB* operon on most pathogenic *Streptomyces* spp., particularly *S. scabies* (Wang et al., 2004; Wanner, 2004, 2006; Qu et al., 2008; Dees et al., 2013, Jourdan et al., 2016). The thaxtomin A phytotoxin is a key pathogenicity determinant in *Streptomyces* spp. (Healy *et al.*, 2000; Bignell et al., 2014; Li et al., 2019a). On the other hand, the necrosis gene (*nec1*) was found only in the highly (St.1) and moderately (St.2) virulent isolates with a specific gene band at 700bp. The weakly virulent isolate St.3 failed to produce the same band, indicating that this isolate may lack this gene. Unlike thaxtomin gene, the *nec1* gene is not essential for pathogenicity since some pathogenic *Streptomyces* isolates lack this gene (Bukhalid *et al.*, 2002. Wanner,

2006, Flores-Gonzáles et al., 2008; Wanner, 2009; Bignell et al., 2014; Fyans et al., 2016). The *nec1* gene encodes a protein that causes necrosis in plant tissues and has a role in the infection by suppressing plant defenses (Bukhalid *et al.*, 1998, 2002; Joshi et al., 2007b). Using PCR-based approaches to detect the *txtAB* operon and *nec1* gene is reliable for detecting *Streptomyces* spp. in soil and biological samples (Cullen and Lees, 2006; Flores-Gonzáles et al., 2008; Qu et al., 2008).

The SDS-PAGE method was an efficient method for determining the phylogenetic relations between the various species of the genus *Streptomyces* (Manchester *et al.*, 1990; Özdemir et al., 2013). SDS-PAGE of total protein was used to characterize the three tested *S. scabies* isolates. Eleven, fourteen, and twelve protein bands were recognized with the highly (St.1), the moderately (St.2), and the weakly (St.3) virulent isolates, respectively in the molecular weight between 8- 65kDa. The lowest level of similarity was between St.1 and St.3 isolates (0.77), while the highest similarity was between St.2 and St.3 isolates (0.86). The three S. scabies isolates clustered into two groups, whereas the moderately (St.2) and the weakly (St.3) virulent isolates were located in the same group, while the highly virulent isolate St.1 was located in a separate group.

CONCLUSION

In conclusion, the outcomes of this research may help in the understanding of the virulence of *S. scabies* the causative agent of the potato common scab, in order to aid in the development of disease management strategies. This study has found a positive correlation between the presence of two virulence factors (thaxtomin A and necrosis proteins or genes) and *S. scabies* pathogenicity. Further investigation of how to inhibit or restrict the role of these virulence factors is required for managing the potato common scab disease.

REFERENCES

Abd El-Rahman, A.F., A.A. El-kafrawy, O.A. Abd El-hafez and R.E.A. Abd El-Ghany. 2018. Evaluation of some fungicides effectiveness in control of blackleg and common scab of potato. Egyptian J. of Agricultural Research. 96(4): 1307-1324.

Beaudoin, N., I. Isayenka, A. Ducharme, S. Massie, A. Gagnon and R. Hogue. 2021. Habituation to thaxtomin A increases resistance to common scab in 'Russet Burbank' potato. PLoS ONE. 16(6): e0253414.

Bignell, D.R.D., J.K. Fyans and Z. Cheng. 2014. Phytotoxins produced by plant pathogenic Streptomyces species. J. Appl. Microbiol. 116:223-23.

Bouchek-Mechiche, K., L. Gardan, P. Normand and B. Jouan.2000. DNA relatedness among strains of *Streptomyces* pathogenic to potato in France: description of three new species, *S. europeiscabiei* sp. nov. and *S. stelliscabiei* sp. nov. associated with common scab, and *S. reticuliscabiei* sp. nov. associated with netted scab. Int. J. Syst. Evol. Microbiol. 50:91-99.

Bukhalid, R.A. and R. Loria. 1997. Cloning and expression of a gene from *Streptomyces scabies* encoding a putative pathogenicity factor. J. Bacteriol .179:7776-7783.

Bukhalid, R.A., TTakeuchi, D. Labeda and R. Loria. 2002. Horizontal transfer of the plant virulence gene, *necl*, and flanking sequences among genetically distinct Streptomyces strains in the diastatochromogenes cluster. Appl. Environ. Microbiol. 68:738-744.

Cao, Z., G. Khodakaramian, K. Arakawa and H. Kinashi. 2012. Isolation of borrelidin as a phytotoxic compound from a potato pathogenic *Streptomyces* strain. Biosci. Biotechnol. Biochem. 76:353-357.

Cullen, D.W. and A. K. Lees. 2007. Detection of the *nec1* virulence gene and its correlation with pathogenicity in *Streptomyces* species on potato tubers and in soil using conventional and real-time PCR. J. Appl. Microbiol. 102:1082-1094.

Dees, M., A. Sletten and A. Hermansen. 2013. Isolation and characterization of *Streptomyces species* from potato common scab lesions in Norway. Plant Pathol. 62:217-225.

Dees, M.W. and L.A. Wanner. 2012. In search of better management of potato common scab. Potato Res. 55:249-268.

El-Sayed, A.E. 2000. Production of thaxtomin A by two species of *Streptomyces* causing potato scab. Folia Microbiol. 45:415-422.

El-Sayed, A.E., G. El-Didamony and K. Mansour. 2001. Isolation and characterization of two types of actinophage infecting *Streptomyces scabies*. Folia Microbiol. 46(6):519-526.

El-Sheikh, M.A. 2010. Common scab of potato as affected by soil mineral elements and tuber chemical constituents. J. Agric. and Envi. Sci. Egypt. 9(2):1-24.

El-Sheikh, M.A., S.A. El-Kazzaz, E. El-Argawy and M.H. Ghozlan. 2012. Factors affecting thaxtomin A production by *Streptomyces scabies* in Egypt. Egypt. J. Phytopathol. 40(2):131-147.

FAO STAT. 2019. Food and Agriculture Organization of the United Nations. FAOSTAT Statistical Database. http://faostat.fao.org/default.aspx.

Flores-Gonzáles, R., I. Velasco and F. Montes.2008. Detection and characterization of *Streptomyces* causing potato common scab in Western Europe. Plant Pathol. 57:162-169.

Fry, B.A. and R. Loria. 2002. Thaxtomin A: evidence for a plant cell wall target. Physiological and Molecular Plant Pathology. 60:1-8.

Fyans, J.K., L. Bown and D.R.D. Bignell. 2016. Isolation and characterization of plant-pathogenic *Streptomyces* species associated with common scab-infected potato tubers in Newfoundland. Phytopathology. 106(2):123-131.

Goyer, C., J. Vachon and C. Beaulieu. 1998. Pathogenicity of *Streptomyces scabies* mutants altered in thaxtomin A production. Phytopathology. 88(5):442-445.

Healy, F.G., M. Wach, S.B. Krasnoff, D.M. Gibson and R. Loria. 2000.The *txtAB* genes of the plant pathogen *Streptomyces acidiscabies* encode a peptide synthetase required for phytotoxin thaxtomin A production and pathogenicity. Mol. Microbiol. 38:794-804.

Hiltunen, L.H., T.O.janperä, H. Kortemaa, E. Richter, M.J. Lehtonen and J.P.T. Valkonen. 2009. Interactions and biocontrol of pathogenic *Streptomyces* strains co-occurring in potato scab lesions. J. Appl. Microbiol. 106:199-212.

Howard, G.C. and W.E. Brown. 2001. Modern Protein Chemistry: Practical Aspects. ISBN, CRC Press, USA.

Hussein, M.A., M.M.Hassan and A.E. Elfarash. 2019. Variability and genetic diversity among *Streptomyces scabiei* infected potato cultivars with common scab from Egypt. Archives of Agriculture Sciences J. 2(3): 57-72.

Johnson, E.G., M.V. Joshi, D.M. Gibson and R. Loria. 2007. Cello-oligosaccharides released from host plants induce pathogenicity in scab-causing *Streptomyces* species. Physiol. Mol. Plant Pathol. 71:18-25.

Joshi, M., X. Rong, S. Moll, J. Kers and C. Franco. 2007b. *Streptomyces turgidiscabies* secretes a novel virulence protein, Nec1, which facilitates infection. Mol. Plant Microbe. Interact. 20:599-608.

Joshi, M.V., D.R.D. Bignell, E.G. Johnson, J.P. Sparks, D.M. Gibson and R. Loria. 2007a. The AraC/XylS regulator TxtR modulates thaxtomin biosynthesis and virulence in *Streptomyces scabies*. Mol. Microbiol. 66:633-642.

Jourdan, S., I.M. Francis, M.J. Kim, J.J.C. Salazar and S. Planckaert. 2016. The CebE/MsiK transporter is a doorway to the cello-oligosaccharide-mediated induction of *Streptomyces scabies* pathogenicity. Sci. Rep. 6:27144.

Karagöz, K. and R. Kotan. 2017. Identification and characterization of some *Streptomyces* species isolated from symtomatic potatoes in Erzurum Province of Turkey. Eastern Anatolian J. of Sci. 3 (1):27-37.

King, R.R. and L.A. Calhoun. 2009. The thaxtomin phytotoxins: Sources, synthesis, biosynthesis, biotransformation and biological activity. Phytochemistry. 70:833-841.

King, R.R., C. H. Lawrence and A. Larry. 1992. Chemistry of phytotoxins associated with *Streptomyces scabies* the causal organism of potato common scab. J. Agric. Food Chem. 40(5): 834–837.

King. R.R., C.H. Lawrence and M.C. Clark. 1991. Correlation of phytotoxin production with pathogenicity of *Streptomyces scabies* isolates from scab infected potato tubers. Am. Potato J. 68:675-80.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227:680-685.

Leiner, R.H., B.A. Fry, D.E. Carling and R. Loria. 1996. Probable involvement of thaxtomin A in pathogenicity of *Streptomyces scabies* on seedlings. Phytopathology. 86:709-713.

Lerat. S., A-M. Simao-Beaunoir and C. Beaulieu. 2009. Genetic and physiological determinants of Streptomyces scabies pathogenicity. Molecular Plant Pathology. 10(5): 579-585.

Li, Y., J. Liu, D. Adekunle, L. Bown, K. Tahlan and D.R.D. Bignell. 2019b.TxtH is a key component of the thaxtomin biosynthetic machinery in the potato common scab pathogen *Streptomyces scabies*. Mol. Plant Pathol. 20:1379-1393.

Li, Y., J. Liu, G. Díaz-Cruz, Z. Cheng and D.R.D. Bignell. 2019a. Virulence mechanisms of plant-pathogenic *Streptomyces* species: an updated review. Microbiology. 165:1025-1040.

Liu, J., L-F. Nothias, P.C. Dorrestein, K. Tahlan and D.R.D. Bignell. 2021. Genomic and metabolomic analysis of the potato common scab pathogen. *Streptomyces scabiei*. ACS Omega. 6:11474-11487.

Loria, R., D.R.D. Bignell, S. Moll, J.C. Huguet-Tapia and M.V. Joshi. 2008.Thaxtomin biosynthesis: the path to plant pathogenicity in the genus *Streptomyces*. Antonie van Leeuwenhoek. 94:3-10.

Loria, R., J. Kers and M. Joshi. 2006. Evolution of plant pathogenicity in *Streptomyces*. Annu. Rev. Phytopathol. 44:469-487.

Loria, R., R.A. Bukhalid, B.A. Fry and RR. King. 1997. Plant pathogenicity in the genus *Streptomyces*. Plant Dis. 81: 836-846.

Loria, R., R.A. Bukhalid, R.A. Creath, R.H. Leiner, M. Olivier and J.C. Steffens.1995. Differential production of thaxtomins by pathogenic *Streptomyces* species *in vitro*. Phytopathology. 85: 537-541.

Manchester, L., B. Pot, K. Kersters and M. Goodfellow.1990. Classification of *Streptomyces* and *Streptoverticillium* species by numerical analysis of electrophoretic protein patterns. Systematic and Applied Microbiology. 13:333-337.

Özdemir, K., Ö.E. Berber and M. Atalan. 2013. Determination of total cell protein profiles of *Streptomyces* species. J. of Experimental Biology and Agricultural Sciences 1(3):186-190.

Qu, X., L. A. Wanner and B. J. Christ. 2008. Using the txtAB operon to quantify pathogenic *Streptomyces* in potato tubers and soil. Phytopathology. 98:405-412.

Sambrook, J., E. F. Fritsch and J. Maniatis. 1989. Molecular cloning, a laboratory Manual. 2nd edition. Cold spring harbor Laboratory Press, NY, USA.

Scheible, W.-R., B. Fry, A. Kochevenko, D. Schindelasch, L. Zimmerli, S. Somerville, R. Loria and C.R. Somerville. 2003. An Arabidopsis mutant resistant to thaxtomin A, a cellulose synthesis inhibitor from *Streptomyces* species. Plant Cell. 15:1781-1794.

Sharma, A., S. Gautam and S. Saxena. 2014. *Streptomyces*. Encyclopedia of Food Microbiology (Second Edition), Pages 560-566. Editor(s): Carl A. Batt, Mary Lou Tortorello. Academic Press.

Shi, W., L. Mingcong, W. Guangshan, T. Renmao, L. Cuiping, W. Bing, L. Rongshan, S. Chunyu, C. Xiuli, Z. Bo and G. Zheng. 2019. The occurrence of potato common scab correlates with the community composition and function of the geocaulosphere soil microbiome. Microbiome. 7:14.

Sneath, P.H.A. and R.R. Sokal. 1973. Numerical Taxonomy: the Principles and Practice of Numerical Classification. W. H. Freeman, Baltimore.

Wang, A. and G. Lazarovits. 2004. Enumeration of plant pathogenic *Streptomyces* on postharvest potato tubers under storage conditions. Canadian J. of Plant Pathology. 26(4):563-572.

Wanner, L.A. 2004. Field isolates of *Streptomyces* differ in pathogenicity and virulence on radish. Plant Dis. 88:785-796.

Wanner, L.A. 2006. A survey of genetic variation in *Streptomyces* isolates causing potato common scab in the United States. Phytopathology. 96:1363-1371.

Wanner, L.A. 2009. A patchwork of Streptomyces species isolated from potato common scab lesions in North America. Am. J. Potato Res. 86:247-264.

Wanner, L.A. and W.W. Kirk. 2015. *Streptomyces* – from basic microbiology to role as a plant pathogen. Am. J. Potato Res. 92:236-242.

الملخص العربي

دور الزاكستومين أ وبروتين النيكروزس في القدرة المرضية *لإستربتوميسس سكابيس* المسبب للجرب العادي في البطاطس

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تم إختبار القدرة المرضية لثلاثة عزلات من بكتيريا إستربتوميسس إسكابيس علي درنات البطاطس الصغيرة، إحداث موت للانسجة في شرائح البطاطس، تثبيط نمو بادرات الفجل، إنتاج سم الزاكستومين أ، وكذلك وجود الجين المسئول عن انتاج الزاكستومين أ (*txtA*) و جين *nec1*. تم تصنيف العزلات St.1 و St.2 و St.3 علي انها عالية القدرة المرضية و متوسطة القدرة المرضية ومنخفضة القدرة المرضية، علي التوالي. أظهرت العزلة St.1 أعلي نسبة جرب علي درنات البطاطس الصغيرة و موت لانسجة شرائح البطاطس بينما العزلة St.3 كانت لديها اقل هذه النسب. العزلة St.2 كانت لديها قيم متوسطة. العزلة St.1 ثبطت طول الجذر في بادرات الفجل بنسبة ٨٢,٣٥ % وانتجت أعلي مستوي من الزاكستومين أ (٣,١ مجم/ميليلتر) بينما العزلة St.3 خفضت طول الجذر في بادرات الفجل بنسبة ٦٣,٥ % وانتجت أقل مستوي من الزاكستومين أ (١,١٨ مجم/ميليلتر). الثلاث عزلات كانت موجبة لوجود جين الزاكستومين أ (*txtA*) وأظهرت حزمة محددة للجين عند حوالي ٥٠٠ قاعدة مزدوجة. من ناحية أخري، العزلتين St.1 و St.2 أظهرتا حزمة محددة معبرة عن جين *nec1* عند ٧٠٠ قاعدة مزدوجة ، بينما العزلة St.3 كانت تفتقد لهذه الحزمة. إعتماداً علي تحليل صورة البروتين الكلي تم توزيع الثلاث عزلات من إستربتوميسس إسكابيس علي مجموعتين تفترقا عند مؤشر تشابه ٠,٧٨. العزلتان St.2 و St.3 كانتا في نفس المجموعة بنسبة تشابه ٠,٨٦ بينما العزلة St.1 كانت في مجموعة منفصلة. تسلط النتائج المتحصل عليها الضوء علي العلاقة بين وجود سم الزاكستومين أ و جين النيكروزس و قدرة إستربتوميسس إسكابيس علي احداث الجرب في البطاطس.

الكلمات المفتاحية: الجرب العادي في البطاطس – إستربتوميسس إسكابيس – الزاكستومين أ – جين *nec1*