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Role of Protein Profiling in Explaining Aggressiveness of *Streptomyces Scabies* to *Solanum tuberosum*

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ABSTRACT

Scabby potato tubers with raised, netted and pitted lesions were collected from different places in Egypt. Sixteen isolates were recovered, checked for pathogenicity and identified as *Streptomyces scabies*. The isolates were similar in morphological, physiological and biochemical determination differing in disease propensities. In vitro studies the colony color was grey for all isolates and production of melanoid pigments was observed. The pathogenic potential of isolates was tentatively different in the developed grading system. Protein electrophoretic studies revealed high molecular weight bands in all isolates, though 6 isolates with low detected protein (60-70 kd) and disease index ranging between (92% -99 %) indicating their role in pathogenic potential . The study may conclude that SDS-PAGE needs greeter work with deeper depth for possible correlation between a distinct protein in the pathogen and severity of infection and scab lesions type on potato tubers.

Keywords: common scab, *Streptomyces scabies*, Protein electrophoretic, SDS-PAGE.

1. Introduction

Potato (*Solanum tuberosum* L.) is the main staple food in many countries as it is a great source of proteins, carbohydrates, vitamins, minerals, fiber contents and stored energy (Enciso-Rodriguez 2018). China is the largest potato producer with 99.5 million tons/year which represents 25.02% from worldwide production, followed by other countries such as Russia, India and the United States. Egypt is considered among the major producers and exporters of potato crop in Africa, its production was approximately about 4.5 million tones in 2017. (Assad *et al*., 2018 and FAOSTAT, 2019).

Common scab (CS) is a serious disease with quality constraints in the production of potato throughout the world. In the late 19th century, the causal agent of potato common scab was first identified in North America (Thaxter, 1892), while a review of the recent scientific literature reveals that this disease is now present worldwide, wherever potatoes are grown.

The disease is caused by a great genus *Streptomyces*, in which only a few of species cause the disease (Hiltunen *et al.,* 2009; Dees and Wanner, 2012 and Lin *et al.,* 2012). *Streptomyces* spp. are aerobic, filamentous, Gram-positive prokaryotes belonging to the order Actinomycetales, suborder Streptomycineae, family Streptomycetaceae and genus Streptomyces Kutzner (1981). *Streptomyces* such as *S. scabies*, *S. ipomoeae*, *S. turgidiscabies* and *S. acidiscabies* cause many symptoms on potato and other hosts as beet, radish , parsnip, carrot and peanut crops. Symptoms of common scab include deep pitted and raised scab-like lesions which reduce the market quality and consumption values of the diseased tuber crops (Loria *et al*., 2006 and Hiltunen *et al.,* 2011). Scab symptoms caused by pathogenic species were indistinguishable from each other and symptoms were not species-specific Dees *et al.,* (2013). And the severity of symptoms seems to be influenced by inoculum level in the soil, cultivar susceptibility and/or the virulence of the infecting species (Cullen & Lees, 2007).

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S. scabies considered the most important plant pathogen in the genus Streptomyces worldwide. This pathogen can cause erumpent, superficial, or pitted lesion types, but erumpent lesions are most common, and most lesions have a raised, rough, corky appearance. This pathogen also causes scab of root crops such as radish, turnip, and carrot in some countries Hooker, (1981). *S. scabies* infects peanut, resulting in a disease called "pod wart of peanut" Kritzman *et al.,* (1996) that is characterized by necrotic, raised lesions on peanut hulls in South Africa (DeKlerk *et al.,* 1996).

Streptomyces species are phenotypically and genotypically diverse, differing in numerous physiological and morphological characteristics (Lambert and Loria, 1989; Lindholm *et al.,* 1997; Loria *et al.,* 1997; Bouchek-Mechiche *et al.,* 1998; Bramwell *et al.,* 1998; Kreuze *et al.,* 1999).
Leiminger *et al.,* 2012 reported that Morphological, physiological and molecular characterization remains an integral part of species identification. Morphological characterization is based on the colony colors and spore when grown on yeast malt extract agar medium (Tashiro *et al.,* 2012). Physiological testing involves growth at different pH levels, utilization of different sugars production of melanin in the presence of tyrosine, and resistance to various antibiotics (Lindholm *et al.,* 1997; Bouchek-Mechiche *et al.,* 1998). Schick & Klinkowski (1962) asserted that the formation of pigments is associated with pathogenicity in actinomycetes. Takeuchi *et al.,* (1996) described the occurrence of a new potato scab-causing species, *S. turgidiscabiei*, which was found in the eastern Hokkaido, Japan, and which did not produce melanin or other diffusible pigments. Dees *et al.,* (2013) reported that Norwegian scab isolates related to *S. turgidiscabiei*, which caused symptoms but were unable to produce melanin. Melanin production is associated with pathogenicity, but it is neither essential nor invariable (Lambert & Loria 1989; Gregory & Vaisey, 1956).

Plant pathogenicity in the genus of *streptomyces* is based on production of the toxin thaxtomin , this toxin induces the symptoms characteristic to common scab (King *et al.,* 1989; Lawrence *et al.,* 1990; Healy *et al.,* 2000; Bignell *et al.,* 2010), as thaxtomin induces plant cell hypertrophy in expanding plant tissues (Fry & Loria, 2002; Scheible *et al.,* 2003).Also pathogenicity of *S. scabies* is mainly caused by the production of toxins known as thaxtomins, which induce cell death in plant cells and tissues (Goyer *et al.,* 1998; Healy *et al.,* 2000; Duval *et al.,* 2005).*S. scabies* cause disease on seedlings of monocot and dicot plants in laboratory studies . Disease symptoms include decrease of shoot and root length, radial swelling, and tissue chlorosis and necrosis. Seedling pathogenicity appears to be mediated by thaxtomin A production. This toxin also causes a reduction in seedling growth and dramatic radial swelling of roots and shoots of seedlings (Leiner *et al.,* 1996), Seedling pathogenicity by *S. scabies* was first described by Hooker and Kent (Hooker *et. al*., 1946).

One-dimensional (1D)-polyacrylamide gel electrophoresis (PAGE), also known as sodium dodecyl sulphate (SDS)-PAGE, is the core analytical separation technique in proteomic studies, since Tiselius' pioneering back study in 1937.

Paradis *et al*. (1994) stated that analysis of electrophoretic patterns of soluble proteins on SDS PAGE is a reliable method for studying the diversity among microbial isolates. They also found high similarity within electrophoretic profiles of deep-pitted scab-inducing *Streptomycetes*. Isolates of *S. scabies*, non-pathogenic isolates phenotypically similar to *S. scabies*, could be subdivided into two groups with a low degree of affiliation. However, pathogenic and non-pathogenic strains were found in the two groups.

Aly *et al.* (2001) compared protein patterns of five isolates of *F. oxysporum f.sp. vasinfectum* (FOV) and a nonpathogenic isolate of *F. oxysporum* by PAGE and SDS-PAGE.

The present study was oriented to test for how far the SDS-PAGE can be reliable in determining the pathogenicity of *S.scabies* isolates from potato tubers according to their protein banding patterns.

2. Materials and Methods

Source of common scab organism:

Samples of naturally infected potato plants with tubers showing well-developed deep, raised and superficial scab symptoms were collected freshly from different localities of potato production districts in Beheira, , Giza, Damietta and Ismailia Governorates in Egypt Fig.(1).

A **B** **C**

Fig. 1: Scab symptoms on naturally infected potato tuber showing**,** A: superficial lesions (Russet scab) B: raised lesions. C: pitted scab.

The causal agents of potato common scab disease was isolated from various infected tubers according to differently recognized lesion after surface disinfection as described by Loria and Davis (1989), onto oat meal agar OMA medium or medium amended with tyrosin (5g/L) (OMA-T) medium. .The plates were incubated at 28°C for 7 days. The appeared colonies which phenotypically characteristic of *Streptomyces*, were transferred to yeast malt extract agar (YME) (Loria, 2001) after approximately 6 days. To obtain pure cultures subsequent transfer to fresh medium was done for use in the further trails.

Pathogenicity tests

Pathogenicity on radish

A selection of 16 Streptomyces isolates were subsequently tested for pathogenicity on radish (*Raphanus sativus*),seedlings as previously described by (Flores-Gonzalez *et al.,* 2008). Seeds of radish were disinfected in 0.5% sodium hypochlorite solution for 1 min and then rinsed several times in sterilized distilled water S.D.W. Thereafter, the seeds were placed onto 1.5% water agar (WA) plates and then incubated at room temperature for 24 hrs. to germinate. The germinated seeds were placed individually in glass tubes (25 mm diameter) containing 10ml of 1% WA. After that 300 µL of each culture suspension were added to inoculate seeds. Three replicates of each isolate were used. The inoculated tubes were kept at room temperature and examined daily.

After growth the appearance of the diseased seedlings was recorded. The pathogenic isolate which causes stunting of the seedlings were determined. As radial swelling was considered. Treatment with S.D.W. was used as a negative control. Experiment was performed twice in duplicate.

Pathogenicity test on potato plants and disease assessment

Pots experiment under greenhouse conditions

The pathogenicity of 16 isolates of *S. scabies* was tested on planted potato tubers of (cv. Spunta) ,which were kindly provided by potato brown rot project (PBRP), ARC, Egypt. Healthy potato tubers were planted in 30-cm-diameter pots containing sterile 1:1 soil/sand mixture. They were surface sterilised by soaking in 0.5% sodium hypochlorite for 5 min and then rinsed twice in sterilized water before planting. Fifty millilitre (10^6 CFU/ml) was taken from each isolate culture, and was added to each pot before planting. Pots were arranged in a randomised complete block design with five replicates. Control tubers were treated similarly with tap water. Tubers were harvested and scored after 90 days from planting.

Disease incidence (DI)

(DI %) was determined by counting number of lesions in 1 cm² of tuber surface was determined using a 0 to 5 scale as described by Shihata, (1974)of tuber surface in different treatments according to the scab proposed by Shihata, (1974).

$$\text{Potato Disease incidence (DI) \%} = \frac{0A+1B+2C+3D+4E+5F}{5T} \times 100$$

Where: 0=symptomless tubers, 1 = trace – 10% tuber surface is scabbed, 2= 11 – 20 % tuber surface is scabbed, 3= 21 –30 % tuber surface is scabbed, 4 =31 – 40 % tuber surface is scabbed, 5 = more than

40 % tuber surface is scabbed A, B, C, D, E and F are the number of tubers corresponding to the numerical grades respectively. T = is the total number of tubers, i.e. T = A+ B+ C+ D+ E+ F.

Identification of Pathogen

Morphological, physiological and biochemical characteristics of *Streptomyces* isolates were considered:

According to International *Streptomyces* Project (ISP) the morphological and physiological characterization were preformed (Shirling and Gottlieb 1966 and Loria *et al.*2010). To asses colony and spore color, YME (ISP2) agar was used, and to determine production of melanin Tyrosine agar (ISP7) was used. Cultures of water agar were used to determine spore-chain type by direct microscopic examination. Sugar utilization was recorded as presence or absence of growth on two Petri dishes of basal mineral salts agar amended with 1% of the sugars: (D– fructose, D- glucose, D- mannitol, D-raffinose, D- xylose, L-arabinose, cellibiose, sucrose and inositol).

Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Total cellular protein of the 16 samples were analyzed by denaturing polyacrylamide gel electrophoresis (12%) as described by Laemmli (1970); commonly known as SDS-PAGE. A precautionary step was taken by running the gel at 100 volts for 30 min. After completion of the run, the gel was stained with Coomassie Brilliant Blue R-250 for an overnight followed by destaining . Then the detained gel was photographed and analyzed using gel documentation system.

Celluar protein banding pattern

Total cellular protein for 16 samples were analyzed by denatured polyacrylamide gel electrophoresis (PAGE-SDS) as described by Laemmli, (1970). The isolates were inoculated in 5 ml Glucose yeast malt (GYM) broth at 37°C with continuous shaking at 150 rpm. Cells were then collected by centrifugation at 6,000 rpm for 10 minutes. The pellet was suspended in an appropriate volume of distilled water equivalent to its size. A 50µl of cell suspension was mixed with an equal volume of 2X buffer (0.125M Tris-HCI, pH 6.8, 4% SDS, 20% glycerol, 10% β-M.E.), boiled for 3 min in a water bath and immediately transferred to an ice-water bath before being loaded onto the gel.

Electrophoresis of protein gel

The gel apparatus (Bio-Rad Laboratories, California, USA) was assembled and the lower and upper chambers were filled with the tank buffer. Loading of protein samples was done by Hamilton syringe (10 µl). Pre stained molecular weight protein marker from Bio-Rad, was applied to the gel. Electrophoresis was carried out at about 100 volts (≈ 20-30 mA) in 1X Tris/glycine-SDS-running buffer for 1.5-2 hours.

Staining and destaining of the protein gel

The gel was stained in 50 ml of staining solution (0.125% coomassie blue R-250, 50% methanol and 10% acetic acid) with shaking (40 rpm) for 6 hours at 37ºC, and then washed once with distilled water, detained by detaining solution (40% methanol and 7% acetic acid) for at least two hours, photographed and analyzed.

Statistical analysis

Analysis of variance was carried out using MSTAT-C programme. The least significant difference (LSD) at $p \leq 0.05$ was applied to cheek differences among treatments (Gomez & Gomez 1984).

3. Results

Morphological, Physiological and Biochemical Characteristics of *Streptomyces Spp*.

Sixteen *Streptomyces sp*. isolates were obtained from naturally infected potato tubers. Showed that all isolates had grey spores that formed in spirl chains and the spore wall was smooth. Produced a melanin pigment on tyrosine agar. All isolates utilized Larabinose, D-Fructose, D-Glucose, D-Manitol,

Rhamnose, Sucrose, D-Xylose, i-Inositol, Raffinose. *S. scabies* does not grow at pH 4.5 Lambert and Loria, (1989).

The isolates showed similar characteristics, conforming to those of S. *scabies* strain type as reported by Lambert and Loria (1989) (Table 1).

Table 1: Symptom specification and regions of pathogenic isolates .

Isolate No.	Type of Scab	Governorate	Region
CS-1	Superficial	Beheira	Badr
CS-2	Superficial	Beheira	Badr
CS-3	Raised spot	Beheira	Badr
CS-4	Raised spot	Beheira	El Nubaria
CS-5	Raised spot	Beheira	El Nubaria
CS-6	Raised spot	Beheira	El Nubaria
CS-7	Raised spot	Beheira	El Nubaria
CS-8	Superficial	Beheira	El Nubaria
CS-9	Superficial	Beheira	El Nubaria
CS-10	Superficial	GIZA	Manshet Radwan
CS-11	Raised spot	GIZA	Manshet Radwan
CS-12	Superficial	Damietta	Kafr Saad
CS-13	pitted	Damietta	Kafr Saad
CS-14	Raised spot	GIZA	Manshet Radwan
CS-15	Superficial	Ismailia	El-Saleheya
CS-16	Raised spot	Ismailia	El-Saleheya

Pathogenicity tests

Pathogenicity assay on radish

All sixteen isolates of *S. scabies* were tested for pathogenicity on radish seedlings. The radish inoculated seeds treatments showed hypertrophy or did not even germinate. By comparison with control. (Fig. 2).

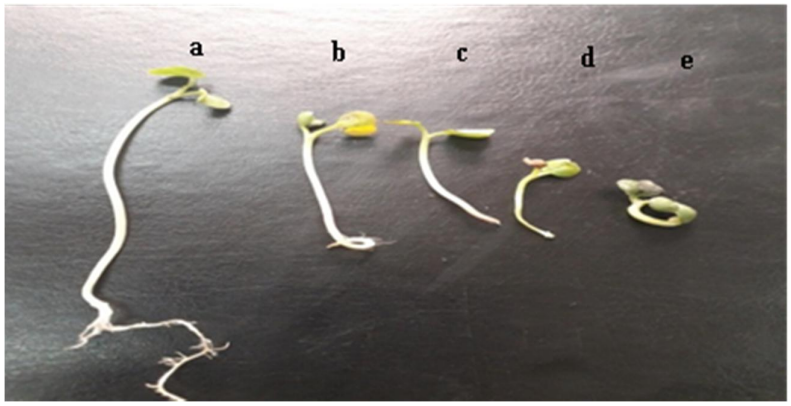

Fig. 2: Symptoms caused by 4 isolates of *Streptomyces scabies* on radish.(a) Germinated seed was not treated. (b) inoculated with *S. scabies* isolate 12 and(c) isolate 15 or isolate 6 (d) and isolate 8 (e).

Pathogenicity test on potato

Pathogenicity of isolated bacteria was tested on cv. Spunta potato plants. Data in Table 2 shows that all 16 isolates were pathogenic to potato plants and varied in their pathogenicity

Isolate (CS- 8) caused the highest DI followed by isolates (CS- 6, CS- CS-16, CS-4, CS- 5and CS-11) which were in grade 3 (90-100), while isolates (CS-2, CS-3, and CS- 7) exhibited the lowest DI and grade 1 from (50-70).

Table 2: The disease incidence (DI %) for 16 pathogenic isolates of *S. scabies* ascendingly.

Isolate No.	Mean % of (DI)	Grade
CS-8	99.78	
CS-6	95.5	
CS-16	95	
CS-4	93.5	Grade3
CS-5	92	(90-100)
CS-11	92	
CS-12	86.67	
CS-15	86.15	
CS-14	81.1	Grade2
CS-10	78	(70-90)
CS-1	77.7	
CS-13	77.14	
CS-9	76	
CS-7	61.42	
CS-3	56	Grade1
CS-2	51.42	(50-70)
LSD at 0.05	2.23	

Table 3: Reaction of sixteen pathogenic isolates by utilization test of sugars following International *Streptomyces* Project (ISP), aspects.

	Carbon utilization								
Isolate No.	**L-arabinose**	**D-fructose**	**D – glucose**	**D-Mannitol**	**Rhamnose**	**Raffinose**	**D-Xylose**	**Sucrose**	**i-Inositol**
CS-1	+	+	+	+	+	-	+	+	++
CS-2	+	+	+	+	+	+	+	+	++
CS-3	+	+	+	+	+	++	+	+	+
CS-4	+	+	+	+	+	+	±	±	+
CS-5	+	+	+	+	+	±	+	+	++
CS-6	+	+	+	+	+	+	+	+	+
CS-7	+	+	+	+	+	++	+	+	+
CS-8	+	+	+	+	+	++	++	+	+
CS-9	+	+	+	+	+	+	±	+	++
CS-10	+	+	+	+	+	+	+	+	+
CS-11	+	+	+	+	+	±	±	+	++
CS-12	+	+	+	+	+	±	+	+	+
CS-13	+	+	+	+	+	+	+	±	+
CS-14	+	+	+	+	+	+	++	+	+
CS-15	+	+	+	+	+	+	+	+	+
CS-16	+	+	+	+	+	±+	±	+	+

(++) Strong utilization of sugar, (+) Moderate utilization (±) Weak utilization.

Physiological tests of isolated pathogen

Data presented in Table 4 indicated that all tested isolates were gram positive, gelatin liquefaction positive, starch hydrolysis positive, catalase test positive, casein hydrolysis positive , no growth at both 4 °C and 40 °C, reduced nitrate to nitrite. All isolates gave negative V.P. and M.R. tests and also negative indole formation.

On the basis of morphological, cultural, physiological and pathological characteristics of the isolated pathogen, it was concluded that all tested isolates could be *Streptomyces scabies.*

Determination of total protein profile in *streptomyces scabies* isolates

The protein profile Fig (3) revealed a total 258 bands between 200-10 kDa. The Binary data matrix was generated taking 1 as presence and 0 as absent. The maximum number of bands were produced from the isolates 9, 14 and 15 bands. They recorded 18 bands each. The isolates 1, 2, 7, 13 and 16 produced 16 bands each. The minimum number of bands (13) was produced by the isolates 4, and 5. The similarity index presented in Table (5) showed the maximum similarity (100%) between the following isolates: 1&2, 2&7, 3&6, 4&5, 9&14, 15 and 13&15. However the minimum similarity (84%) between the isolate 4 and the isolates 9, 14 and 15, the isolate 5 with 14&15 and the isolate 9 with both isolates 14&15.

Table 4. Morphological and physiological characteristics of the isolated pathogenic of *Streptomyces scabies*

	Isolate numbers															
Tests	**CS-1**	**CS-2**	**CS-3**	**CS-4**	**CS-5**	**CS-6**	**CS-7**	**CS-8**	**CS-9**	**CS-10**	**CS-11**	**CS-12**	**CS-13**	**CS-14**	**CS-15**	**CS-16**
Spore chain type on (YME)	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral
Color of colony	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Grey	Grey
Gram staining	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth 4 ° C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth 40 ° C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Casein hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melanin production on Tyrosine agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole formation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V.P. test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M.R. test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

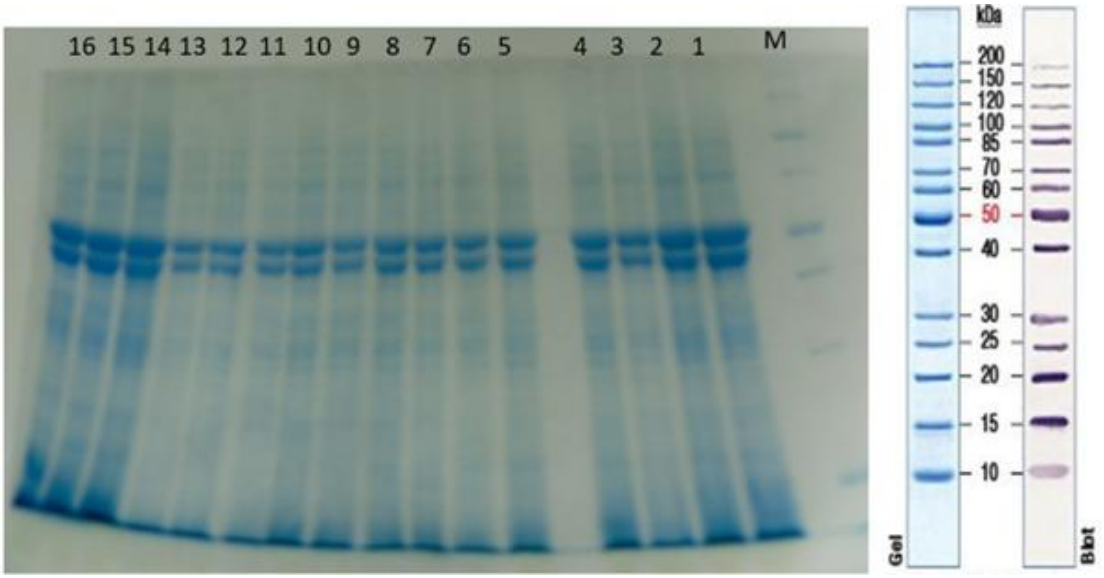

Fig. 3: SDS-PAGE protein banding patterns for the 16 isolates, lane M represents the protein marker**.**

Table 5: similarity index for the 16 isolates based on their protein banding pattern.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1.00															
2	1.00	1.00														
3	0.94	0.94	1.00													
4	0.87	0.87	0.93	1.00												
5	0.87	0.87	0.93	1.00	1.00											
6	0.94	0.94	1.00	0.93	0.93	1.00										
7	1.00	1.00	0.94	0.87	0.87	0.94	1.00									
8	0.97	0.97	0.90	0.90	0.90	0.90	0.97	1.00								
9	0.97	0.97	0.91	0.84	0.84	0.91	0.97	0.94	1.00							
10	0.91	0.91	0.97	0.90	0.90	0.97	0.91	0.88	0.94	1.00						
11	0.88	0.88	0.87	0.93	0.93	0.87	0.88	0.90	0.91	0.90	1.00					
12	0.91	0.91	0.90	0.90	0.90	0.90	0.91	0.94	0.94	0.94	0.97	1.00				
13	0.94	0.94	0.88	0.87	0.87	0.88	0.94	0.97	0.97	0.91	0.94	0.97	1.00			
14	0.97	0.97	0.91	0.84	0.84	0.91	0.97	0.94	1.00	0.94	0.91	0.94	0.97	1.00		
15	0.97	0.97	0.91	0.84	0.84	0.91	0.97	0.94	1.00	0.94	0.91	0.94	0.97	1.00	1.00	
16	0.94	0.94	0.88	0.87	0.87	0.88	0.94	0.97	0.97	0.91	0.94	0.97	1.00	0.97	0.97	1.00

The cluster analysis for the 16 isolates was grouped in two main groups between 0.888 and 0.9 (Fig.4). The 1st group begins at a distance between 0.912 and 0.924, then branched into two parts. The first part included the isolates 4 and 5, while the 2nd part gave a sub-cluster which was branched into two parts one included the isolates 3 and 6 and the other one included the isolate 10 alone. The 2nd group started between the same distance as the first group but in a slightly higher level. Two main sub-clusters were branched from this group. The first one was at a distance approximately 0.969 and included the isolates 11 and 12 separately. The second main sub-cluster started at a distance between 0.948 and 0.96 was divided into two sub sub-clusters. The 1st was at 0.971 distance branched knot two parts including three isolates i.e. 13 and 16 together and isolate 8 alone. Moreover, the 2nd sub sub-cluster started at distance 0. 972 was branched into two groups, one of them included isolates 9, 14 and 15. The other group included the last three isolates i.e. 7, 2 and 1.

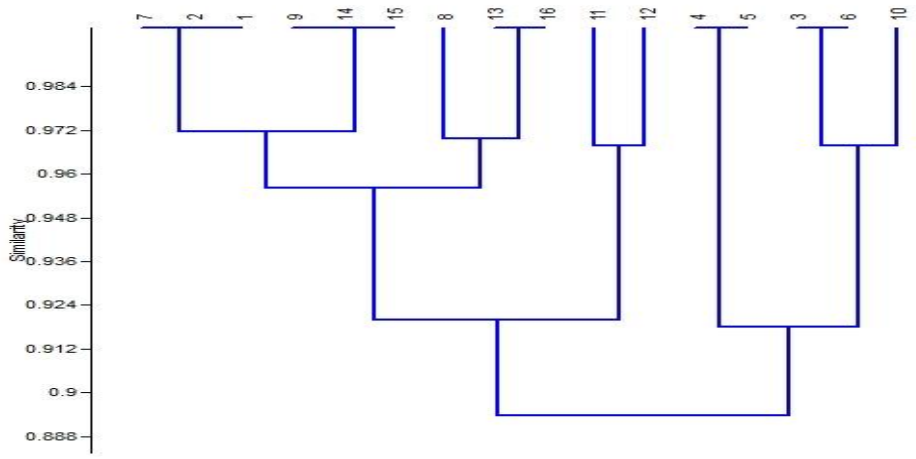

Fig. 4: Dendogram based on cluster analysis of Streptomyces isolates

4. Discussion

Sixteen isolates were isolated from scabbed potato tubers, showing graduations in severity as well as in type, ranging from superficial to raised or pitted lesions. . Pathogenicity test showed that all isolates are pathogenic to potato tubers of Spunta cv. Significant variances in the virulence was shown between the tested isolates. Where isolate (CS- 8) caused the highest DI followed by isolates (CS- 6, CS- CS-16, CS-4, CS- 5and CS-11) which were in grade 3, (90-100), while isolates (CS-2, CS-3, and CS- 7) exhibited the lowest DI and grade 1 from (50-70) .Results are in line with those obtained by several researches (Shihata, 1974; Faucher *et al.,* 1993; Lorang *et al.,* 1995 and Galal *et al.,* 1999).
Identification of the pathogenic bacterial isolates was applied through detecting its morphological, biochemical and physiological characters. All isolates had grey spores that formed in spiral chains and the spore wall was smooth. Produced a melanin pigment on tyrosine agar and were phenotypically similar to *S. scabies* .It is clear from results of pathogenicity tests that the *Streptomyces* isolates caused reduction of the length of radish seedlings due to the virulence of tested isolates. These results are in agreement with Liu *et al.,* (2004). The obtained data was combined with those obtained in the pathogenicity tests and compared with earlier findings of (Faucher *et al.,* 1993; Lorang *et al.,* 1995; Galal *et al.,* 1999 and Wanner, 2004). Data indicated that all tested isolates are *S. scabies* .

Although SDS-PAGE is considered an effective tool in determining taxonomical relations between the various species of genus Streptomyces Özdemir *et al.,* (2013) also in classification and diagnosis of various bacteria as revealed by many researchers (Berber *et al.,* 2003, Berber *et al.,* 2004; Berber and Yenidünya 2005). It is worth mentioned that SDS-PAGE was effective in differentiation of *Bacillus sphearicus* strains into six subtypes (Berber and Cokmus, 2001). Unfortunately in our study SDS-PAGE could not differentiate between the different isolates of *S. scabies* with regard to pathogenicity incidence. It was noticed that the isolates number 2, 7 and 1 was grouped together according to dendogram Fig. (4) gave 51.42 (grade 1), 61.42, and 77.7 (grade2) DI %, respectively, while the isolates 8, 16 and 13 which were in the same sub cluster recorded varied DI% i.e. 99.78, 95 (grade 3) and 77.14 % (grade 2).

Our results were similar to those observed by Omar *et al.,* 2009 who mentioned that there were no relationship between protein profiles and pathogenicity of isolates.

Regarding to *Streptomyces scabies* isolates, high molecular weight bands protein of unknown function was highly detected in all the isolates . Moreover, the isolates number 8, 6, 16, 4, 5 and 11 showed significantly low detected proteins with KDa (60 and 70 kd) and their scabies index ranges from 99 till 92 (Table 2), those low molecular bands may play a role in the pathogenicity of the isolates. In general our study concluded that SDS-PAGE can not be relied on alone in differentiation of *S. scabis* isolates from different origins but must be supported by further studies and tools.

References

Aly, A.A., E.M. Hussein, S.M.E. Zayed and H.A. Eisa, 2001. Lack of relationships between protein electrophoretic patterns of *Fusarium oxysporum* f. sp. vasinfectum isolates and their pathogenicity of cotton. J. Agric. Sci. Mansoura Univ., 26:4886-4896.

Assad, Y.H., S.M. Makled, and Eman F. Elkodosy, 2018.Analytical study of egyptianpotato exports, the most important markets of the europianunion. Arab university of Agriculture Science, 26(1):61-73.

Berber, I., E. Atalan, and C. Cokmuş, 2004. The influence of pesticides on the spore viability, toxin stability and larvicidal acitivity of Bacillus sphearicus 2362 starin. Fresenius Environmental Bulletin, 13: 424-429.

Berber, I., and C. Cokmus, 2001. Characterization of Bacillus sphaericus strains by Native-PAGE. Bulletin of Pure & Applied Sciences, 20: 17-21.

Berber, I., C. Cokmuş, and E. Atalan, 2003. Characterisation of Staphylococcus species by SDS-PAGE of whole cell and extracelluler proteins. Microbiology, 72: 42-47.

Berber, I., and E. Yenidünya, 2005. Identification of Alkaliphilic Bacüllus Species Isolated from Lake Van and Its Surroundings by Computerized Analysis of extracellular Protein Profiles. Turkish Journal of Biology, 29: 181-188.

Bignell, D.R.D., J.C. Huguet-Tapia, M.V. Joshi, G.S. Pettis, and R. Loria, 2010. What does it take to be a plant pathogen: genomic insights from Streptomyces species. Antonie Van Leeuwenhoek. International Journal of General and Molecular Microbiology, 98: 179–94.

Bouchek-Mechiche, K., C. Guerin, B. Jouan, and L. Gardan, 1998. Streptomyces species isolated from potato scabs in France: numerical analysis of "Biotype-100" carbon source assimilation data. Research in Microbiology, 149: 653-663

Bramwell P.A., P. Wiener, A.D. Akkermans, and E.M. Wellington, 1998**.** Phenotypic, genotypic and pathogenic variation among streptomycetes implicated in common scab disease. Letters in Applied Microbiology, 27: 55-260.

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. Journal of Applied Microbiology, 102: 1082–94.

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

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

DeKlerk, A., A. McLeod, R. Faurie, W.J. Swart, and F.D.N. Denner, 1996. Identification and toxin production of Streptomyces isolates responsible for common scab in the northern Cape Province of South Africa. Proc. Eur. Assoc. Potato Res. Triennial Conf., 13th, Veldhoven, the Netherlands

Duval, I., V. Brochu, M. Simard, C. Beaulieu, and N. Beaudoin, 2005. Thaxtomin A induces programmed cell death in *Arabidopsis thaliana* suspension-cultured cells. Planta 222, 820–831. doi: 10.1007/s00425-005-0016-z.

Enciso-Rodriguez, F., D. Douches, M. Lopez-Cruz, J. Coombs, and G. de Los Campos, 2018. Genomic selection for late blight and common scab resistance in tetraploid potato (Solanum tuberosum). G3 Gene Genomes Genet., 8: 2471–2481.

FAOSTAT, 2019. Food and Agriculture Data. Crops. Available at http://www. fao.org/ faostat/en /#data /QC

Faucher , E., B. Otrysko , E. Paradis , N.C. Hodge , R.E. Stall, and C. Beaulieu, 1993. Characterization of Streptomyces causing russet scab in quebec. Plant Dis., 77:1217-1220.

Flores-Gonzalez, R., I. Velasco, and F. Montes, 2008. Detection and characterization of Streptomyces causing potato common scab in Western Europe. Plant Pathology, 57: 162–9.

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

Galal, A.A., S.H. Gad El-Hak, Y.T. Abd El-Mageed. and N.S. Youssef, 1999. Some Studies on Potato Common Scab Disease of pathological Studies. Egypt J. Microbiol., 34 (1):181- 199.

Gomez, K.A., and A.A. Gomez, 1984. Statistical procedures for agricultural research. 2nd ed. New York (NY): Willey, 680.

Goyer, C., J. Vachon, and C. Beaulieu, 1998. Pathogenicity of Streptomyces scabies mutants altered in thaxtomin A production. Phytopathology 88: 442–445. doi:10.1094/PHYTO.1998.88.5.442

Gregory, K.F., and E.B. Vaisey, 1956. Pathogenicity of tyrosinase deficient mutants of *Streptomyces scabies*. Canadian Journal of Microbiology, 2: 65–71.

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. Molecular Microbiology, 38: 794–804

Hiltunen, L.H., M. Alanen, I. Laakso, A. Kangas, E. Virtanen, and J. Valkonen, 2011. Elimination of common scab sensitive progeny from a potato breeding population using thaxtomin A as a selective agent. Plant Pathol., 60: 426–435.

Hiltunen, L.H., T. Ojanperä, H. Kortemaa, E. Richter, M. Lehtonen and J. Valkonen, 2009. Interactions and biocontrol of pathogenic *Streptomyces* strains co-occurring in potato scab lesions. Journal of Applied Microbiology, 106:199-212.

Hooker, W.J., and G.C. Kent, 1946. Infection studies with Actinomyces scabies. Phytopathology 36:388-389.

Hooker, W.J., 1981. Common scab. Pages 33- 34 in: Compendium of Potato Diseases. American Phytopathological Society, St. Paul, MN

King, R.R., C.H. Lawrence, M.C. Clark, and L.A. Calhoun, 1989**.** Isolation and characterization of phytotoxins associates with Streptomyces scabies. Journal of the Chemical Society, Chemical Communications, 13: 849–50.

Kreuze J.F., S. Suomalainen, L. Paulin, and J.P.T. Valkonen, 1999. Phylogenetic analysis of 16S rRNA genes and PCR analysis of the nec1 gene from Streptomyces spp. causing common scab, pitted scab, and netted scab in Finland. Phytopathology, 89: 462-469

Kritzman, G., A. Shani-Cahani, B. Kirshner, Y. Riven, Z. Bar, J. Katan, and A. Grinstein, 1996. Pod wart disease of peanut. Phytoparasitica, 24:293-304.

Kutzner H.J., 1981. The family Streptomycetaceace. In: Starr M.P., Stolp H., Truper H.G., Balows A., Schlegel H.G. (eds). The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria, p. 2028-2090. Springer-Verlag, Berlin, Germany

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227(5259): 680-685.

Lambert D.H., and R. Loria, 1989. Streptomyces scabies sp. nov. International Journal of Systematic Bacteriology, 39: 387-392

Lawrence, C.H., M.C. Clark, and R.R. King, 1990. Induction of common scab symptoms in aseptically cultured potato-tubers by the vivotoxin, thaxtomin. Phytopathology 80: 606–8.

Leiminger, J., M. Frank, C. Wenk, G. Poschenrieder, A. Kellermann, and A. Schwarzfischer, 2012. Distribution and characterization of Streptomyces species causing potato common scab in Germany. Plant Pathology, 62: 611-623.

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.

Lin, L., H.M. Ge, T. Yan, Y.H. Qin and R.X. Tan. 2012. Thaxtomin a-deficient endophytic *Streptomyces* sp. Enhances plant disease resistance to pathogenic *Streptomyces scabies*. Planta. 236:1849-1861.

Lindholm P., H. Kortemaa, K. Haahtela, M. Kokkola, M. Salkinoja-Salonen, and J.P.T. Valkonen, 1997. Streptomyces spp. isolated from potato scab lesions under Nordic conditions in Finland. Plant Disease, 81: 1317-1322.

Liu, D., W. Zhao, and D. Xiao, 2004. Overview of potato scab in China. International Publication of Streptomyces Scabies, 180- 188.

Lorang, J. M., D. Liu, N.A. Anderson , and J.L. Schottel, 1995. Identification of potato scab inducing and suppressive species of Streptomyces. Phytopathology, 85(3):261–268.

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

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

Loria R, 2001. Diseases caused by bacteria. In: Stevenson W, Loria R, Franc GD, Weingarnter DP, eds, Compendium of Potato Diseases. St Paul, MN, USA: APS Press, 14–5.

Loria R., C.A. Clark, R.A. Bukhalid and B.A. Fry, 2010. Gram positive bacteria. Pages 236-249 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria . N.W. Schaad, J.B. Jones, and W.Chun, eds. American Phytopathological Society, St. Paul, MN.

Loria, R. and J.R. Davis, 1989. Streptomyces scabies In: Schaad,N.W. ed., Laboratory guide for identification of plant pathogenic bacteria. 2nd edition APS Press, St. Paul.Minn., USA.

Omar, M.R., Eman A.M. Osman., Kh.K. Kasem and Amal A. Asran, 2009. Characterization of *Fusarium moniliforme* involved in cotton seedling damping-off by pathogenicity and electrophoretic protein profiles. J. Agric. Sci. Mansoura Univ., 34 (5): 5177 – 5188.

Özdemir K., İ. Berber, E. Öğün and M. Atalan, 2013. Determination of total cell protein profiles of Streptomyces species. Journal of Experimental Biology and Agricultural Sciences, 1 (3).

Paradis E., E. Faucher and C. Beaulleu, 1994. Comparison of electrophoretic protein profiles within streptomycete strains causing common scab of potato in Quebec. page 416 in : Plant Pathogenic Bacteria, Versailles (France),June9-12,1992 Ed. INRA, Paris 1994 (LesColloques,n066) .

Scheible, W.R., B. Fry and Kochevenko *et al.,* 2003. An Arabidopsis mutant resistant to thaxtomin A, a cellulose A et synthesis inhibitor from Streptomyces species. The Plant Cell, 15: 1781–94.

Schick, R., and M. Klinkowski, 1962. Die Kartoffel – Ein Handbuch. Berlin, Germany: Deutscher Landwirtschaftsverlag.

Shihata, Z.A., 1974. Studies on common scab disease of potato M.Sc. Thesis 108, Fac. Agric., Assiut Univ., Egypt.

Shirling, E.B. and D. Gottlieb, 1966. Methods for characterization of Streptomyces species. Int. J. Syst. Bacteriol., 16:313-340.

Takeuchi, T., H. Sawada, F. Tanaka, and I. Matsuda, 1996. Phylogenetic analysis of Streptomyces spp. causing potato scab based on 16S rRNA sequences. International Journal of Systematic and Evolutionary Microbiology, 46:476–9.

Tashiro, N., K. Manabe, A. Saito, and K. Miyashita, 2012. Identification of potato scab-causing Streptomyces sp. occurring in strongly acidic soil conditions in Saga Prefecture in Japan. Journal of General Plant Pathology, 78: 353-359.

Tiselius, A., 1937. A new apparatus for electrophoretic analysis of colloidal mixtures. Trans Faraday Soc. 33:524–531.

Thaxter, R., 1892. Potato scab. Conn. Agric. Exp. Stn., 1891: 153–160.

Wanner, L.A., 2004. Field isolates of Streptomyces differ in pathogenicity and virulence on radish. Plant Dis., 88(8):785–796