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Factors Affecting Thaxtomin A Production by *Streptomyces scabies* in Egypt

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Twenty isolates of *Streptomyces scabies* were isolated from scab lesions on potato tubers collected from three different regions in Behera Governorate during the 2009-2010 growing season. The isolates of *S. scabies* recovered were totally monomorphic for the morphological and the biochemical characteristics investigated. All isolates were non motile, gram positive, exhibited grey colonies, spiral spore chain and were melanin producers. Meantime the isolates showed positive reaction for catalase activity, starch hydrolysis, casein hydrolysis and gelatine liquefaction. Also, all isolates were tolerant and exhibited growth at NaCl 5, 6 & 7% and at 20, 25 & 30°C at pH 7 & 9. None of the tested isolates were able to grow at pH 5. More variations, however, were revealed for the pathogenicity of twenty isolates. Five out of twenty investigated isolates were found to be highly virulent. The rest of tested isolates were moderately virulent. Spunta was the most tolerant potato cultivar, while cvs. Mondial and Hermis were more susceptible. The highly virulent *S. scabies* (isolate ST5) was a highly producer of Thaxtomin A, while the moderately virulent isolate (ST10) was poorly toxin producer (1.26 mg/ml medium). The optimal conditions for Thaxtomin A production were 28ºC, pH 8 and 1% cellobiose in medium. The amino acids, *i.e.* tryptophan, phenylalanine and tyrosine exhibited an *in vitro* inhibitory effect on the toxin production in the amended oatmeal bran broth medium with highest inhibitory effect was recorded for tryptophan.

Keywords: Common scab, phytotoxin, potato, *Streptomyces scabies* and Thaxtomin A.

Streptomyces spp. are group of filamentous Gram-positive saprophytic bacteria found in soil. These organisms produce a great variety of antibiotics and other secondary metabolites and few species of the genus are plant pathogens amongst them, *S. scabies* the causal agent of the potato scab (Lambert and Loria, 1989 and Faucher *et al.*, 1992). Common scab of potato occurs in all potato growing areas of the world and is induced by other species of the genus *Streptomyces* (Archuleta and Easton, 1981 and Loria *et al.*, 2003). Thaxtomin A, as a phytotoxin, capable of inducing scab-like lesions on immature potato tubers was characterized as a unique 4- nitroindol-1-3 yl containing 2.5 dioxopiprazines (King *et al*., 1989 and Lawerence *et al*., 1990). A correlation was observed between the pathogenicity of *S. scabies* isolates and their ability to produce the phytotoxin Thaxtomin A (King *et al.*, 1989 and Lerat *et al*., 2009). In addition, Delserone *et al.* (1991) found a positive correlation between *S. scabies* resistance and Thaxtomin sensitivity in different

potato cultivars. According to Kim *et al*. (1998), crude extracts of pathogenic *S. turgidiscabies* isolates produced a necrotic reaction on potato slices 24 h after application and increased in severity in the following days. However, necrosis was not observed when crude extracts of non pathogenic strains were applied onto tuber slices. Presence of Thaxtomin A from crude extracts of pathogenic *S. turgidiscabies* was identified by thin layer chromatography and high performance liquid chromatography. The pathogenicity of *Streptomyces* spp. was evaluated using potato berries where pathogenic strains caused necrotic lesions (Shigeo *et al*., 2006) and on potato slices (Natsume *et al*., 2005), while non pathogenic strains showed no symptoms. Thaxtomin A production varied generally among different media and among different *S. scabies* strains (Beauséjour *et al.*, 1999 and Wach *et al*., 2007). Natsume *et al*. (2001) found that the Thaxtomin A production decreased at pH 7.5 and 15˚C and the aerial mycelium formation was greater in case of higher pH, Ca^{+2} concentration and temperature. In addition, Lauzier *et al.* (2002) reported that tryptophan had an important inhibitory effect on the toxin biosynthesis.

Therefore, the present study was conducted to identify the role of Thaxtomin A in virulence of *Streptomyces scabies*, the causal agent of common scab of potato and to reveal the factors affecting its production.

Materials and Methods

1- Isolation and characterization of bacterial isolates associated with common scab symptoms:

During the 2009-2010 growing season, potato tubers showing common scab symptoms were collected from different fields in Behera Governorate. Bacterial isolates associated with common scab were isolated from potato tubers as described by Loria and Davis (1989). Whole tubers were surface-disinfested for 10 minutes in 0.6 % sodium hypochlorite and rinsed twice for 10 minutes in sterile distilled water (SDW). The corky layer of each lesion was removed aseptically and about 5 mm of straw-coloured tissue directly underneath the lesion was removed and triturated in 5ml SDW with a pestle and mortar. After approximately10 minutes, a drop of the suspension was plated on yeast malt extract (YME-ISP medium 2). Plates were incubated at 30°C for 10-14 days and single colonies were identified according to Shirling and Gottlieb (1966). Cultures were maintained on YME slants at 4°C for further studies.

Characteristics of all the recovered bacterial isolates, *i.e.* colony colour, spore chain morphology, production of melanin and production of diffusible pigments, were investigated according to Shirling and Gottlieb (1966) in three Petri dishes 7 and 14 days at 28°C. Spore chain morphology of mature colonies was investigated at 100x magnification. Presence and absence of melanin was recorded 7 days after growth on two types of slants of peptone yeast extract iron agar and tyrosine agar according to Shirling and Gottlieb (1966). Motility, Gram staining, catalase activity, starch hydrolysis, casein hydrolysis, gelatine liquefaction, growth tolerance to different NaCl concentrations 5, 6 & 7%, growth at 20, 25and 30°C as well as growth at different pH (5, 7 and 9) were investigated as described by Bergy's manual of determinative bacteriology (Bergy, 2004).

2- Pathogenicity tests and varietal reaction:

Certified tubers of potato cultivars Spunta, Hermis, and Mondial friendly obtained from Kafr El-Zayat international potato association were planted in a greenhouse in 30 cm-diameter pots containing peat moss and vermiculite medium. Forty five days after planting, plants were harvested to obtain the mini tubers. Potato mini tubers were harvested and surface-disinfested for 10 min. in 0.6% sodium hypochlorite and rinsed twice for 10 min. in sterile distilled water (SDW) and left to dry. Bacterial inocula were prepared as described by Leiner *et al.* (1996). Spores of Streptomyces isolates produced on colony surfaces were scrapped free and used to inoculate 50 ml volumes of tryptone yeast broth. These cultures were incubated for 8 days on a rotary shaker at 160 rpm. The inocula were added to autoclaved vermiculite (50 ml of tryptone yeast broth to 1.5 l. of vermiculite). The vermiculite inocula were added to twenty potato mini tubers of each cultivar in small plastic bags, incubated for seven days and shaken vigorously several times during that period. The control treatment was an uninoculated potato mini tuber. Mini tubers were removed from vermiculite, rinsed in water and left to dry. Percent surface area covered with necrotic lesions was estimated and individual scab index was calculated as follows: 0 = no scab, 1= 1 %, 2 = 10%, 3 = 25%, 4 = 50%, 5 = 75% and 6 > 75% of the tuber surface covered. The average scab index for each isolate was calculated according to Leiner *et al.* (1996) as follows:

$$\text{Average scab index} = \frac{\Sigma \text{ (individual scab index X tubers per index)}}{\text{Total number of tubers}}$$

Then, isolates were classified as highly virulent, *i.e.* average scab index ≥ 70%, moderately virulent, *i.e.* average scab index < 70% - ≥ 50% and weakly virulent, *i.e.* average scab index < 50%.

3. Toxin studies:

3.1. Toxin extraction and quantification:

The most highly virulent bacterial isolate as well as one of the most weakly virulent ones, identified in the pathogenicity test, were chosen for comparative studies for toxin production. One hundred and fifty micro litres spore suspension of the two tested isolates were mixed with 50 ml oatmeal bran broth, and incubated at 28ºC with shaking (200 rpm) for eight days. After centrifugation (300 rpm, 20 min), toxin was extracted from supernatants using an equal volume of ethyl acetate. The extract was concentrated by evaporation at 50ºC by rotary evaporator, and the remaining was redissolved in ethyl acetate. Toxins were then analyzed according to Babcock *et al.* (1993). The toxins were separated by thin-layer chromatography on glass plates recoated with 0.25 mm of silica gel 60, using chloroform/methanol (9:1) as migration solvents, and the yellow bands (R*f* = 0.27) indicated the purified toxin. Bands were co migrated with authentic Thaxtomin kindly supplied by R.R. King, Fredericton, Canada. Thaxtomin was quantified by UV and VIS spectroscopic analysis, which was performed with a Deuterium UV/VIS 21 D spectrophotometer using the absorption coefficient determined by King *et al.* (1992). Thaxtomin was

eluted with methanol and monitored as absorbance 380nm.Toxin in crude extracts from the two tested isolates were identified by high performance liquid chromatography (HPLC) using a Shimadzu 10A system with a symmetry C18 column (Waters, 3.9´150 mm). Thaxtomin A was eluted with a 25% acetonitrile during 20 min and monitored at 380 nm (King and Calhoun, 2009).

3.2. Duration and rate of Thaxtomin A production:

A highly toxin producer isolate was chosen to investigate factors affecting Thaxtomin A production. The oatmeal bran broth medium was prepared according to Shirling and Gottlieb (1966). Flasks (150 ml) with medium were inoculated with the tested *S. scabies* isolates ST5. Filtrates were extracted after 2,4,6,8 and 10 days of inoculation, and the amounts of Thaxtomin A were determined as previously described according to King *et al.* (1992).

3.3. The effect of temperature:

The tested isolate grown on oatmeal bran broth medium was incubated at 25, 28 and 30ºC for eight days to investigate the effect of temperature on toxin production. Five Erlenmeyer flasks were prepared for each temperature degree and the amount of toxin in each extract was quantified according to Babcock *et al*. (1993)

3.4. The effect of pH:

The effect of pH on toxin production by the tested isolate was investigated by quantifying the toxin production at different pH values ranging from 7 to 9. The pH values were adjusted by using 0.1N 1 ml of NaOH. Five Erlenmeyer flasks were prepared for each value and the amounts of toxin were quantified according to Babcock *et al*. (1993) as abovementioned.

3.5. The effect of certain carbohydrates:

The tested isolate was inoculated to oatmeal bran broth medium supplemented with 0, 0.1, 0.3, 0.5, 0.7 and 1% (w/v) of the tested sugars, *i.e.* glucose, fructose, and cellobiose, in 150 ml Erlenmeyer flasks. Five replicate flasks for each carbohydrate concentration were prepared. Flasks free of sugars served as a control. Flasks were incubated for 5 days at 28ºC and the amount of toxin in each treatment was quantified according to Babcock *et al*. (1993) as abovementioned.

3.6. The effect of amino acids on Thaxtomin A production:

Three amino acids, *i.e*. tyrosine, tryptophan and phenylalanine were tested and added as 2% (w/v) to 50 ml of oatmeal bran broth medium mixed with 150 µl spore suspension of the tested isolate. Five replicate flasks were performed for each amino acid. Oatmeal bran broth medium without amino acid served as a control. All flasks were incubated for eight days at 28ºC and the amounts of toxin were determined as abovementioned.

Statistical analysis:

The obtained data were statistically analysed using SAS program (Anonymous, 2000). Least significant differences test (LSD) was used to compare means and to rank isolates.

Results

1. Characteristics of bacterial isolates associated with common scab of potato:

Twenty isolates of *Streptomyces scabies* were recovered from potato tubers showed common scab symptoms, collected from different fields in Behera Governorate during the 2009-2010 growing season. All isolates were investigated for their different biochemical and morphological characteristics. All isolates showed grey colonies, with spiral spore chain, gram positive and were non motile. Also, all isolates exhibited positive reaction for catalase activity, gelatine liquefaction, hydrolysis of starch, growth on pH (7 and 9), growth on NaCl (5, 6 and 7%), growth at 20, 25 and 30°C of temperature, and the melanin production. However, the isolates did not produce any diffusible pigments (Figs. 1 & 2 and Table 1).

Table 1. Morphological and biochemical characteristics of *Streptomyces scabies* isolates recovered from potato tubers collected from different fields in Behera Governorate during the 2009-2010 growing season

Characteristics	ST 1	ST 2	ST 3	ST 4	ST 5	ST 5	ST 7	ST 8	ST 8	ST 10	ST 11	ST 12	ST 13	ST 14	ST 15	ST 16	ST 17	ST 18	ST 19	ST 20
Colony color	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey
Spore chain	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Melanin production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Diffusible pigment	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Motility	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gram staining	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase activity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Casein hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tolerance to Nacl 5%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 20˚C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25 C ˚	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30˚C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at pH 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = Positive reaction; - = Negative reaction; S = Spiral spore chain. Colony morphology was recorded on yeast malt extract medium 7 days after incubation at 30˚C. Melanin production was investigated on peptone yeast extract iron agar medium 14 days after incubation at 30˚C. Spore chain morphology was tested on yeast malt extract medium 7 days after incubation at 30˚C by light microscope on 100x. Presence or absences of diffusible pigments were investigated on yeast malt medium 14 days after of incubation at 30˚C.

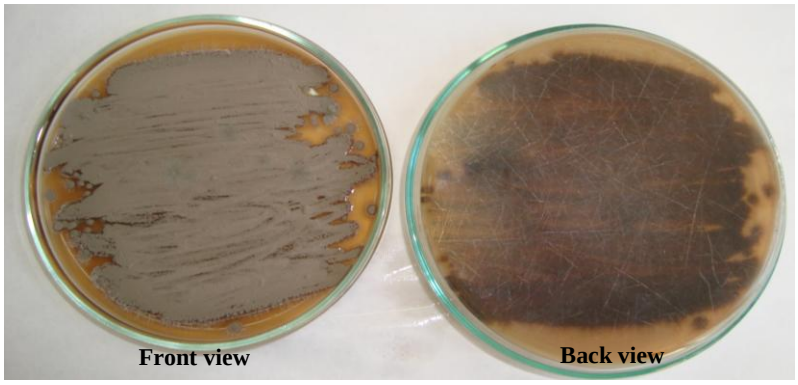

Fig. 1. Culture characteristics of *Streptomyces scabies* (isolate, ST5) showing grey colonies with melanin pigment production.

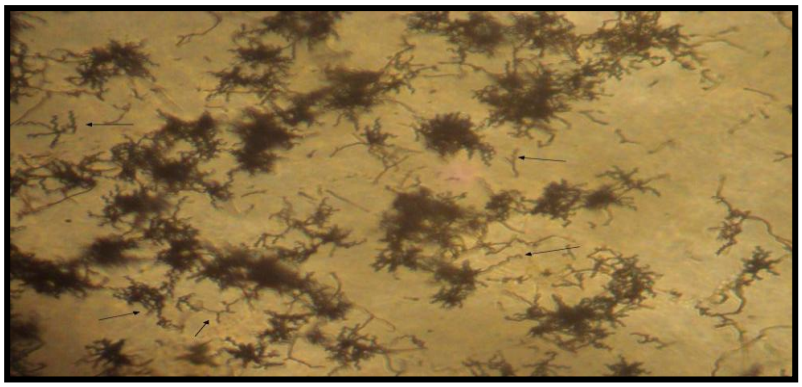

Fig. 2. *Streptomyces scabies* (isolate ST5) showing the phenotype of spiral spore chain, arrows indicate the spiral shape of sporophore.

2. Pathogenicity tests and varietal reaction:

Data in Table (2) show that there was a considerable variation between the tested isolates for their virulence on the potato cultivar tested. Five isolates out of the twenty isolates investigated were characterized as highly virulent, *i.e.* scab index ≥70%. The rest fifteen isolates, however, were moderately virulent, *i.e.* scab index <70- ≥50%. No isolates were characterized as weakly virulent, *i.e.* scab lesions index <50%. On the other hand, tested potato cultivars, *i.e*. Hermis, Mondial, and Spunta, were all susceptible to common scab lesions to different degrees. Cv. Spunta, however, was the most relatively tolerant of the three tested cultivars and exhibited 53.37% common scab index. Meanwhile, cvs. Hermis and Mondial were more susceptible and showed 73.73 and 63.28% scab indices, respectively (Table 2).

Table 2: Scab index of the recovered *Streptomyces scabies* isolates on mini tubers of three cultivars of potato under greenhouse conditions

Isolate code No.	Tested potato cultivar			Mean	Virulence*
	Hermis	Mondial	Spunta		
ST 1	62.0	52.0	42.3	52.1 [p]	M.V
ST 2	66.0	55.0	44.6	55.2 [m]	M.V
ST 3	74.6	64.6	55.6	64.93 [h]	M.V
ST 4	91.0	78.0	68.0	79.0 [c]	H.V
ST 5	94.3	85.0	74.6	84.63 [a]	H.V
ST 6	81.0	72.3	62.0	71.76 [e]	H.V
ST 7	71.6	61.3	51.0	61.3 [j]	M.V
ST 8	64.0	54.3	44.3	54.2 [n]	M.V
ST 9	72.6	64.0	54.0	63.53 [i]	M.V
ST 10	65.0	55.3	45.5	55.26 [m]	M.V
ST 11	70.0	60.3	51.0	60.43 [k]	M.V
ST 12	81.0	68.6	58.3	69.3 [g]	M.V
ST 13	63.3	52.0	42.6	52.63 [o]	M.V
ST 14	91.0	79.6	69.6	80.06 [b]	H.V
ST 15	68.3	58.0	48.3	58.2 [l]	M.V
ST 16	60.0	50.0	40.0	50.0 [r]	M.V
ST 17	76.6	66.0	56.3	66.3 [f]	M.V
ST 18	61.0	51.0	41.0	51.0 [q]	M.V
ST 19	73.3	63.3	53.6	63.4 [i]	M.V
ST 20	88.0	75.0	64.6	75.86 [d]	H.V
Mean	73.73 [a]	63.28 [b]	53.37 [c]		

* MV= moderately virulent, *i.e.* scab index < 70 % - ≥ 50 %.
HV= highly virulent, *i.e.* scab index ≥ 70 % of tuber surface.
- Each figure represents the mean of three replicates.
LSD for potato cultivars at 0.05 = 4.1
LSD for *Streptomyces scabies* isolates at 0.05 = 1.1

3. Toxin studies:

3.1. Toxin production and quantification:

Thaxtomin A produced in the present study by the tested *S. scabies* isolates, *i.e.* ST5 and ST10 showed the same R*f* as the standard Thaxtomin A used (Fig. 3). Spectrophotometer readings at 398, 395, 390 nm for standard Thaxtomin A and for extracted toxin of isolates ST5 and ST10, showed a similar spectral absorption curve for both standard toxin and the extracted toxin of both isolates. In addition, the eluted standard Thaxtomin A showed a single peak at retention time 20 min in HPLC determination. The extracted toxin of both isolates ST5 and ST 10 showed typically the same peak. Spectrophotometric curve used to quantify the toxin, showed that Streptomyces isolate ST5 produced the highest toxin concentration (2.5 mg/ml) on oatmeal broth medium while the isolate ST10 exhibited the lowest toxin production, *i.e.* 1.26 mg/ml medium (Fig. 3 and Table 3).

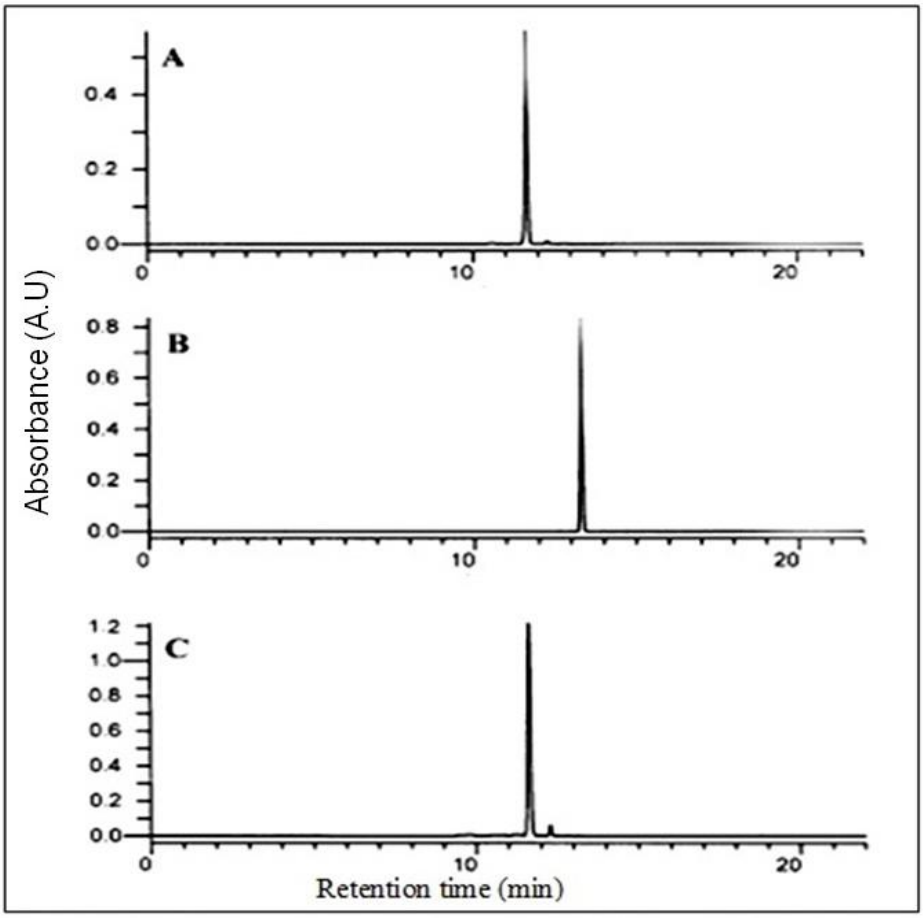

Fig. 3: HPLC chromatograms of standard Thaxtomin A (a), culture filtrate of *S. scabies*, isolate ST10 (b); and culture filtrate of *S. scabies*, isolate ST5 (c).

Table 3: Thaxtomin A production by the highly virulent and the moderately virulent isolates of *Streptomyces scabies*

Isolate code No.	Virulence	Thaxtomin A production* (mg/ml of oatmeal bran broth medium)
ST 5	Highly virulent	2.50
ST 10	Moderately virulent	1.26
LSD at 0.05		0.25

* Thaxtomin A was measured, eight days after inoculation, in extract of (OMBB) medium.
- Each figure represents the mean of five replicates.

3.2. Duration and rate of toxin production by S. isolates:

Data illustrated in Fig. (4) show that Thaxtomin A production increased with time up to eight days after inoculation with the tested isolate (ST5). Meantime, the maximum toxin production rate was occurred at 8 days and 10 days after medium inoculation with the tested *S. scabies* isolate (ST5).

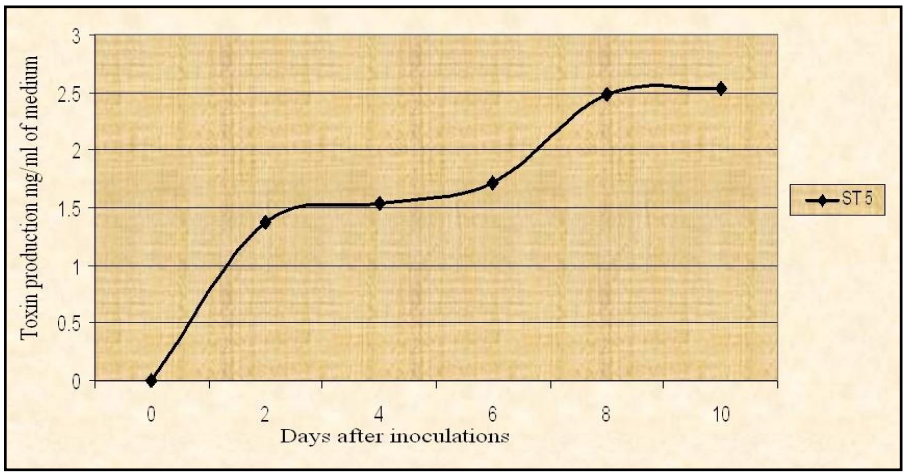

Fig. 4. Thaxtomin A production rate by *Streptomyces scabies* isolate (ST5) on oatmeal bran broth medium.

3.3. The effect of temperature:

Data presented in Table (4) and illustrated in Fig. 5 (a) show that the temperature of incubation of *S. scabies*, (isolate ST5), enhanced Thaxtomin A production of the isolate up to 28°C and recorded 1.88 mg/ml medium. However, the temperature of inoculation over this degree decreased the toxin production as recorded 0.92 mg/ml medium at 30°C.

Table 4: The effect of temperature on Thaxtomin A production of *Streptomyces scabies* (isolate ST5).

Temperature (°C)	Thaxtomin A (mg/ml oatmeal broth medium)*	
	Inoculated medium	Uninoculated medium(control)
25	1.46	0.0
28	1.88	0.0
30	0.92	0.0
LSD at 0.05	0.40	

* Thaxtomin A was measured eight days after inoculation.
- Each figure represents the mean of five replicates.

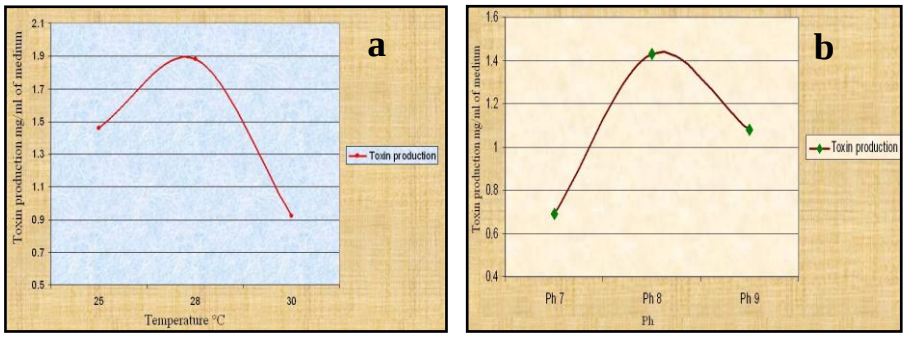

Fig. 5. Effect of temperature (a) and pH (b) on the *in vitro* Thaxtomin A production of *Streptomyces scabies* (isolate ST5) eight days after inoculation.

3.4. The effect of pH:

Data in Table (5) revealed that production of Thaxtomin A by *S. scabies* isolate ST5 was the highest at pH 8 (Fig. 5b) as the toxin production was 1.43 mg/ml oatmeal bran broth medium. However, toxin production decreased at pH 7 and pH 9 where the toxin production was 0.69 and 1.08 mg/ml of oatmeal bran broth medium, respectively (Fig. 5b & Table 5).

Table 5. The effect of pH on Thaxtomin A production of *Streptomyces scabies* (isolate ST5)

Tested pH	Thaxtomin A production* (mg/ml of oatmeal bran broth medium)	
	Inoculated medium	Control**
7	0.69	0.0
8	1.43	0.0
9	1.08	0.0
LSD at 0.05	0.25	

* Thaxtomin A was evaluated 8 days after inoculation on oatmeal bran broth medium.
** Uninoculated oatmeal bran broth medium.
- Each figure represents the mean of five replicates.

3.5. The effect of carbohydrates

Data illustrated in Fig. (6) show that cellbiose applied to the tested OMBB medium enhanced Thaxtomin A production. Thaxtomin A increased with increasing cellbiose concentration and 3.57 mg/ml Thaxtomin A was recorded with 1% cellbiose. The fructose, however, decreased Thaxtomin A production to 0.1% fructose in the medium, while the higher fructose concentrations (0.3 - 1%) exhibited a higher proportionate increase in toxin production. On the other hand, glucose application to OMBB medium inhibited the Thaxtomin A production at all concentrations tested, *i.e.* 0.1 to 1%.

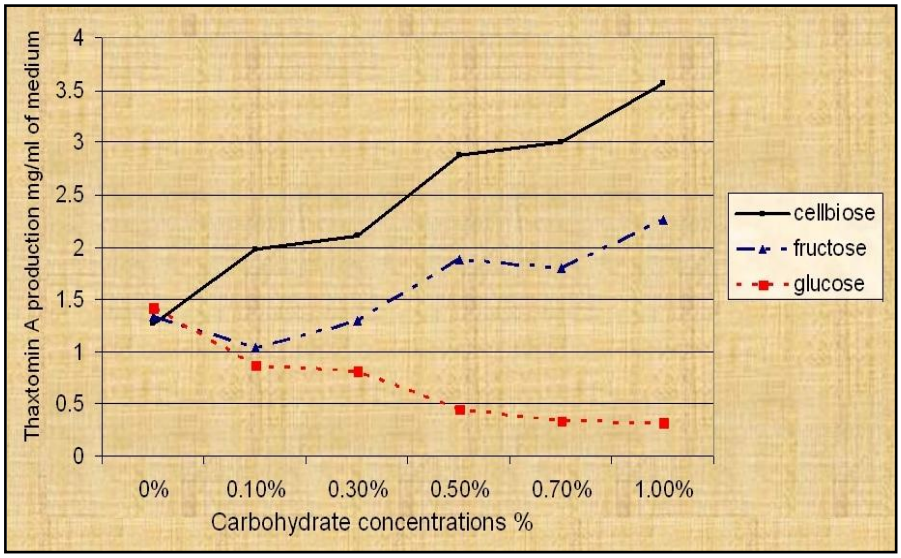

Fig. 6. The effect of carbohydrates in certain concentration on Thaxtomin A production of *Streptomyces scabies* isolate ST5 on oatmeal bran broth medium, eight days after inoculation.

3.6. The effect of amino acids:

Data in presented in Table (6) and illustrated in Fig. (7) show that the tested amino acids, *i.e.* tryptophan, phenylalanine and tyrosine applied to the OMBB medium inhibited Thaxtomin A production of the tested *S. scabies*, isolate ST5, in the medium. The tryptophan, however, exhibited the highest effect as toxin production in the medium was 0.39 mg/ml compared to 1.43 mg/ml for the unamended control. This was followed by phenylalanine and tyrosine as Thaxtomin A production was 0.63 mg/ml and 0.84 mg/ml for the two amino acids, respectively.

Table 6. Effect of certain amino acids on Thaxtomin A production of *Streptomyces scabies* isolate ST5 on oatmeal bran broth medium

Tested amino acid*	Thaxtomin A production** (mg/ml oatmeal bran broth medium)
Tryptophan	0.39
Phenylalanine	0.63
Tyrosine	0.84
Control (unamended)	1.43
LSD at 0.05	0.41

* Amino acids were added as 2% (w/v) of OMBB medium

** Thaxtomin A was measured 8 days after inoculation.

- Each figure represents the mean of five replicates.

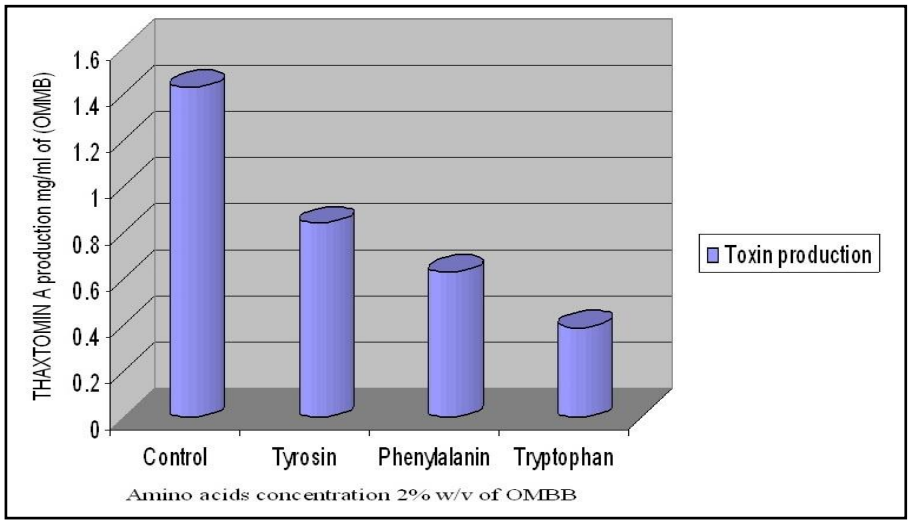

Fig. 7. Effect of certain amino acids on Thaxtomin A production of *Streptomyces scabies* isolate ST5 on oatmeal bran broth medium.

Discussion

All twenty *Streptomyces scabies* isolates recovered in the present study from Behera Governorate during 2009-2010 growing season were totally monomorphic for the morphological and biochemical characteristics investigated. All isolates were non motile, Gram positive, exhibited grey colonies, spiral spore chain and were melanin producers. Meantime the isolates showed positive reaction for catalase activity, starch hydrolysis, casein hydrolysis and gelatine liquefaction. Also, all isolates were tolerant to NaCl 5%, 6% and 7% and exhibited growth at 20, 25, 30 °C and pH 7 and 9. None of the tested isolates could grow at pH 5. However, tested isolates differed in their virulence on potato mini tubers. Five isolates out of the twenty isolates investigated were highly virulent with scab ≥ 70 %. The other fifteen isolates were moderately virulent with scab index < 70 % - ≥ 50 %. Also, obtained results showed that the cv. Spunta was the most tolerant potato cultivar tested as exhibited 53.3% scab index, while cvs. Mondial and Hermis were more susceptible and exhibited 63.2% and 73.7% scab indexes, respectively. Obtained results revealed an appositive correlation between the pathogenicity of *S. scabies* isolates and their ability to produce the phytotoxin Thaxtomin A. These results supported (Babcock *et al*., 1993 and El-Sheikh, 2010) view of the association of Thaxtomin A and *S. scabies* virulence. Plant pathogenic Streptomyces are known to synthesize Thaxtomin under two seemingly unrelated circumstances, *in vivo* within the periderm of infected potato tubers and *in vitro* on oat-based culture media. Presented data indicated that certain carbohydrate, *i.e.* cellbiose, glucose and fructose may influence Thaxtomin production. Potatoes are particularly high in starch, but starch

does not support Thaxtomin A production. The sugar content of potato tubers can be as high as 10% of the dry weight (Kadam *et al*., 1991) and it has been reported that the incidence of potato scab is correlated with the level of reducing sugars, specifically glucose, in the tuber (Goto, 1985). However, appreciable Thaxtomin A production did not found on glucose, and when oatmeal broth is supplemented with glucose, Thaxtomin production is inhibited. These findings in particular are in agreement with Loria *et al*. (1995). *Streptomycetes* are capable of utilizing a wide variety of carbohydrates and other polymers not exploited by other soil microorganisms (Madigan *et al*., 2000 and Loria *et al*., 2003), due to a repertoire of highly regulated, secreted enzymes such as cellulases and xylanases that digest these large molecules into monomers and small oligomers, such as cellobiose, a product of cellulose degradation (Madigan *et al.*, 2000). *Streptomycetes* are known to possess a cellobiose ABC-type transporter that brings external cellobiose inside the cell (Lopez Fernandez *et al*., 1995 and Antonopoulos *et al.*, 2001) that in turn, stimulates the production and secretion of cellulases. Common scab infections are initiated on immature potato tubers in which the primary cell walls contain high levels of cellbiose. Presented results indicated that cellbiose supported Thaxtomin production, and when a potato tuber supplies this carbohydrate, the bacterium may respond by producing Thaxtomin A and ultimately disease symptoms. Oats, coincidentally, seem to contain an optimal combination, lacking in potatoes, that makes oat bran a superior medium for bacterial growth and Thaxtomin A production. Alternatively, Thaxtomin production and bacterial growth that result in host colonization may be not due solely to host-supplied signals, but might also depend on the soil environment. In the present study, no signals were found to support Thaxtomin production are unique to potatoes. Starch, glucans and xylans are common plant carbohydrates and are present in most roots and because Thaxtomin seem to target cellulose biosynthesis, one would expect plant pathogenic Streptomycetes to have broad host ranges. In fact, these organisms can infect underground structures of many crops, and are neither host nor tissue specific (Loria *et al*., 1997 and 2003).

Meantime, the present study confirms the regulatory effect of tryptophan on Thaxtomin A production in *S. scabies* isolates. These results in agreement with Babcock *et al.* (1993), who demonstrated that tryptophan, had a regulatory effect on Thaxtomin production in *S. scabies* and Thaxtomin A production was inhibited in all *S. scabies* isolates growing in the presence of 2.5 mM of tryptophan. Secondary metabolism in actinomycetes is usually delayed or reduced by an excess of readily available nitrogen (Shapiro, 1989). It was observed that all amino acids caused a complete inhibition of Thaxtomin synthesis at a concentration tested in oat bran broth. However, the concentration of an amino acid necessary to cause an inhibition of Thaxtomin synthesis depends not only on the nature of the nitrogen source, but seems to vary among *S. scabies* isolates. The results also lent support to the hypothesis of King and Lawrence (1996) and King (1997), who suggested that Thaxtomin result from the condensation of *N*-methyl-4-nitrotryptophan and phenylalanine by diketopiperazine ring formation. Secondary metabolism in actinomycetes is essential to the survival of the bacteria in their natural environment. Regulation of secondary metabolism is modulated by a variety of factors so the organism can quickly adapt to its steadily changing environment (Shapiro, 1989).

Thaxtomin biosynthesis is also regulated by several factors, such as developmental stage of the bacteria (Babcock *et al.,* 1993), plant compounds (Beauséjour *et al*., 1999), glucose (Babcock *et al.,* 1993 and Loria *et al.,* 1995), and aromatic amino acids (Babcock *et al.,* 1993).

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العوامل المؤثره علي انتاج الزاكستومين أ بواسطه ستربتوميسس سكابيس في مصر

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تم عزل عشرين عزلة من البكتريا ستربتوميسس سكابيس من درنات بطاطس ظهرت عليها أعراض الجرب وذلك من مناطق مختلفة بمحافظة البحيره وذلك خلال موسم النمو ٢٠٠٩ – ٢٠١٠ وقد أظهرت جميع العزلات التي تم الحصول عليها تماثلا في خصائصها المورفولوجيه والبيوكيميائيه. كما أظهرت الدراسة ان كل العزلات كانت غير متحركة وموجبة لصبغة جرام كما أظهرت العزلات مستعمرات رمادية اللون وحوامل جرثومية حلزونية وكانت العزلات منتجة لصبغة الميلانين وأظهرت هذه العزلات نتيجة موجبة لتفاعل الكتاليز ، تحلل النشا ، تحلل الكازين ، تحلل الجيلاتين. وأظهرت الدراسة أن العزلات كانت متحملة للنمو علي تركيزات ٥% ، ٦ % ، ٧% من ملح كلوريد الصوديوم والنمو علي درجات حرارة ٢٠ ، ٢٥ ، ٣٠ درجة مئوية و كذلك علي درجات حموضة ٧ ، ٩. ولم تستطيع أي من العزلات النمو علي درجة حموضة ٥.

كما أظهرت الدراسة أختلافات بين العزلات في مقدرتها المرضية وكانت خمسة عزلات من أصل عشرين عزلة عالية المقدرة المرضية حيث أظهرت أعراض مرضيه علي الدرنات بمعدل أكبر من أو يساوي ٧٠ % بينما بقية العزلات كانت متوسطة في مقدرتها المرضية حيث أظهرت اعراض بمعدل أقل من ٧٠% الي أكبر من أو يساوي ٥٠ %.

كما كانت الأصناف المختبرة قابلة للأصابة بالمرض وبينما كان الصنف سبونتا أعلي الأصناف تحملا للأصابة بنسبة أصابة ٥٣،٣% كان الصنفين موندیال وهيرمس أكثر قابلية للأصابة بالجرب بنسبة أصابة ٦٣،٢ % و٧٣،٧ % علي التوالي.

وقد أظهرت العزلة ST5 عالية المقدرة المرضية مقدرة كبيرة علي أنتاج الزاكستومين ا بنسبة ٥،٢ مجم / مل من البيئة بينما كانت العزلة ST10 متوسطة المقدرة المرضية مقدرة ضعيفة علي أنتاج الزاكستومين ا بنسبة ١،٢٦مجم / مل من البيئة.

كما أظهرت النتائج أن افضل الظروف لأنتاج التوكسين هي ٢٨ درجة مئوية ودرجة حموضة ٨ وتركيز ١% من سكر السليبيوز .

كما وجد أن الأحماض الأمنية التربتوفان والفينيل ألانينن والتيروسين مقدرة علي تثبيط أفراز التوكسين فكان الحمض الأميني التربتوفان أعلي الأحماض الأمينية تثبيطا لأفراز التوكسين.