Eastern Anatolian Journal of Science
Volume III, Issue I, 27-37
ISSN: 2149-6137

Identification and Characterization of Some *Streptomyces* Species Isolated from Symtomatic Potatoes in Erzurum Province of Turkey

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Abstract

Potato common scab is a significant disease that reduces the quality of potato tubers in worldwide. The goal of this study was to identify and characterize *Streptomyces* species, isolated scab lesions in potato production fields of Erzurum province, one of the important potato production areas of Turkey. Potato tubers with scab symptoms were collected from potato production fields, isolations were made and pathogenicity tests were carried out. Strains with positive pathogenicity reactions were identified according to classical and molecular methods. Polymerase chain reactions (PCR) were carried out with specific primers to assess the presence of TxtAB, Nec1, and TomA, genes considered common to the pathogenicity island (PAI) of *Streptomyces* sp. 47 of pathogenic strains were determined as *S. scabiei*, 15 strains as *S. bottropensis*, eight strains as *S. stelliscabiei* and four strains as *S. europoeiascabiei.* The species, *S. stelliscabiei*, *S. bottropensis* and *S. europaeiscabiei* heretofore had not been reported as pathogens of potato in Turkey. In addition, all identified strains are reported for the first time as present in the Eastern Anatolia Region of Turkey. Our testes revealed that the isolates mostly have the *Nec1*, *TomA* genes and *TxtAB* but Some of the isolated strains have lacking of the three genes, based on our PCR tests.

Key words: Scab, *Streptomyces*, Turkey, PCR, PAI

Received: 10.01.2017
Revised: 10.03.2017
Accepted:12.03.2017
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Cite this article as: K. Karagöz and R. Kotan, Identification and Characterization of Some Streptomyces Species Isolated from Symtomatic Potatoes in Erzurum Province of Turkey, Eastern Anatolian Journal of Science, Vol. 3, Issue 1, 27-37, 2017.

Introduction

Streptomycetes are spore-forming gram positive bacteria found in soil in large numbers and are specialized bacterial group with high G+C content. Some of these can produce many kinds of antibiotics (KIESER et al. 2000), and some are pathogenic on plants. *Streptomyces* species can infects tuber and root crops, including beet, radish, turnip, peanut, sweet potato and carrot. Additionally, they can harm seedlings of some monocotyledonous or dicotyledonous plants (WANNER 2009). Most important damages of *Streptomyces* species occur on potato and they cause significant quality reduction of tubers by infesting them with a disease called common scab.

The disease lead to different symptoms on potato like netted scab, pitted scab or surface scab (STEAD and WALE 2004). Symptom types is depend on plant varieties, infection time, virulence of pathogen and environmental factors (WANNER et al. 2011). The symptoms generally come up injured part of tubers. Nonetheless, injuring of tubers is not necessary for symptoms become aware because pathogens can introduce to tuber via lenticels. Further, symptoms may observed on the stolon (WANNER 2009).

Farmers generally don't harvest potato tubers which influenced by common scab. Besides losses of yield, these tubers serve as inoculums source for next vegetation period. In this context; Infected tubers can be considered more effective than soil's inoculums for transferring of disease (PAVLISTA 1996) and besides infected tubers are thought to play a key role in transferring new scab forms and formation of more virulent strains (LORIA 2001).

There are different strategies for control of disease involving chemical, biological, and cultural means, or use resistant varieties. But generally, commercially unimportant varieties have resistance and none of them are fully resistant (ZADINA et al. 1975). Additionally, resistance of a variety can be variable depending on species or strains of pathogens, moisture and pH of soil (HAYNES et al. 1997). Actually in most cases,

knowledge about the pathogen species is very important to develop control strategies.

Plant pathogenicity in the genus is based on production of the toxin, thaxtomin (LORIA et al. 2006; WANNER 2009; DEES et al. 2013). Thaxtomin cause plant cell hypertrophy in expanding plant tissues (LORIA et al. 2006). Nec1 and TomA genes are also present in a wide range of common scab-inducing Streptomyces strains. But they are not necessarily required for pathogenicity (WANNER 2009). The Nec1 gene encodes a protein that induces necrosis in plant tissue and TomA encodes a virulence factor homologous to tomatinase, an enzyme that belongs to saponinases found in plant pathogenic fungi (KERS et al. 2005; LORIA et al. 2006).

Many studies have been being conducted about on scab-causing *Streptomyces* in different countries. Phytopathogenic *Streptomyces* species are present particularly in USA and Canada(LAMBERT and LORIA 1989b, a; GOYER et al. 1996; WANNER 2007b; ST-ONGE et al. 2008; JIANG et al. 2012), France (BOUCHEK-MECHICHE et al. 2000), Japan (MIYAJIMA et al. 1998), Korea (PARK et al. 2003), United Kingdom (THWAITES et al. 2010), Uruguay (LAPAZ et al. 2012), Norway (DEES et al. 2013), Germany (LEIMINGER et al. 2013), Spain and Netherlands (FLORES-GONZALEZ et al. 2008), Iran (CAO et al. 2012), Algeria (BENCHEIKH and SETTI 2007), Finland and Switzerland (LETHONEN et al. 2004). Presence of scab disease was known in Turkey since Bremer's macroscopic studies in 1948. In recent years some research were also conducted in Turkey (KARAHAN 2006). However, there isn't enough research and information about scab-causing *Streptomyces* species in Turkey. Current study was designed for identification and characterization of scab-causing *Streptomyces* species.

Materials and Methods

Sampling and isolation

Naturally infected potato tubers were collected from different potato production fields in Erzurum Province of Turkey at harvest time. Tubers were put in sterile polystyrene bag and were brought to laboratory. Samples firstly were rinsed sdH_2O (sterile distilled water) to remove soil particles and were dried on blotter. Then tubers were sterilized in NaOCl (1%) for 1 minute and treated with sdH_2O. Scab lesions were excised by scalpel and crushed in 1 ml sdH_2O using porcelain mortar and pestle. Crushed extract was suspended in 5 ml sdH_2O and 50 µl of this suspension was inoculated on water agar plates with glass rod. Petri dishes were incubated at 30 °C for a week (LAMBERT and LORIA 1989b). Grown colonies were checked under microscope, then colonies resembling *Streptomyces* were transferred to yeast malt extract (YME) agar plates and incubated at 30 °C for 15-20 days. Then single colonies transferred YME agar plates again to obtain pure cultures.

Pathogenicity tests

First pathogenicity tests were conducted on potato discs. Potato tubers, cv. Marfona were peeled and sterilized in 0,05% $Ca(ClO)_2$ and 0,1% $CaCO_3$ for 3 min with stirring, and were rinsed twice in sdH_2O. Disks (2 cm^2 X 0,5 cm thick) excised from tubers were placed in Petri plates. A piece of medium, strains grown on OM (oat meal) agar plates, was cut and was placed upside down on the disks. Then Petri plates were incubated at 22 °C for 6 days, formation of shallow necrotic lesions and deep pitted lesions were accepted positive pathogenicity. Uninoculated OM pieces were used as control and all tests were repeated three times (CONN et al. 1998).

Another pathogenicity test was conducted on mini tubers. Potato seedlings, cv. Agria, were firstly seeded in field and routine cultivation processes were performed. When the potato plants closed to flowering period, stem cuttings were prepared and were transferred to pots including sterilized sand. Then, it was waited for mini tuber formation.

The strains were grown on YME agar plates at 28 ± 2 ºC for 15 – 20 days. Spores were transferred to 50 ml Oat Meal Broth (OMB) and incubated at 28 ± 2 ºC, 200 rpm for 7 days (LIU et al. 1996). They were centrifuged and washed three times with sdH_2O. Bacterial suspensions were approximately prepared at 1 x 10^{7-8} spore / ml with sdH_2O by serial dilutions. 500 µl of Bacterial suspensions were inoculated to the mini tubers and the tubers were covered with sand again. Inoculated plants were incubated at 28 ± 2 ºC for 21 days. Formation of brownish or black superficial, slightly raised or pitted, and pitted lesions were considered as positive pathogenicity (LORIA and KEMPTER 1986). All tests were repeated three times.

Defining morphological and biochemical traits

Spore chain morphology was examined by light microscopy. Aerial spore mass colors were determined by morphological examination on YME. Production of melanoid pigments detected on PYI (peptone yeast iron) and TYR (tyrosine) agar. Agar slants were inoculated with bacterial suspensions, after 2 and 4 days pigmentations were checked. Formation of greenish brown to black diffusible pigment was recorded as

positive. Uninoculated tubes were used as control (SHIRLING and GOTTLIEB 1966).

The pH sensitivity was determined on media containing (per liter) 10,0 g of dextrose, 0,5 g of L-asparagine, 15,0 g of agar, and 40 mM phosphate as the monopotassium, dipotassium, and disodium salts to adjust the pH in 0,5-U intervals from 3,5 to 8,5 (LAMBERT and LORIA 1989a). The inhibitory effect of selected compounds were determined on modified Bennett agar containing (per liter) 1 g of beef extract, 1 g of yeast extract, 2 g of tryptone, 10 g of glycerol, and 15 g of agar. Medium was prepared and cooled by 50 °C, then toxic compounds including penicillin G (10 IU ml^{-1}), streptomycin sulfate (20 µg ml^{-1}), sodium chloride (5,6 and 7%, w/v), phenol (1 %, v/v) and crystal violet (0,5 µg ml^{-1}) were added (WILLIAMS et al. 1983)and growth at 37 ^{o} C was tested on YME. Bacterial suspensions were spread on the medium in a single line by sterile swab, then growth was evaluated 14 days later (LAMBERT and LORIA 1989a).

Carbon sources, which the strains can use, were also determined. Filter-sterilized L-arabinose, glucose, fructose, D-mannitol, sucrose, raffinose, rhamnose, D-xylose and ether sterilized Myo(I)- Inositol were added to basal mineral salt agar medium (cooled to 60°C) at concentrations of 1%. Then bacterial suspensions were inoculated on the agar medium and incubated at 28°C for 2 weeks. Basal mineral salt agar without carbon source was used as control, all tests were repeated 3 times (SHIRLING and GOTTLIEB 1966).

Extraction of DNA and PCR conditions

Sporulated strains on YME agar plates were transferred to 30 ml trypticase soy broth (TSBA) and were incubated on thermo shaker at 26-28^{o}C, 200 rpm for 3 days. Cultures were centrifuged at 7500 x g for 10 minutes. Then 0,1 g pellet was crushed with liquid nitrogen by porcelain mortar and pestle. Then DNA was extracted as previously described (KUMAR et al. 2010).

PCR was performed in reaction mix containing 10 mM Tris-HCl (pH 9,0), 50 mM KCl, 1,5-2 mM M_gCl_2 (Sigma P2192), 0,1% Triton X-100 (Sigma T8787), 25 pmol each primer (Methabion), 2,5 U Taq DNA polymerase (Sigma T1806), 200 µM dNTP mix (Sigma D7295), 25 ng template DNA and water in final volume of 50 µl for Scab1m / Scab2m, Stel3 / Aci2, Stel3 / T2st2, Nf / Nr and Tom3 / Tom4 primer pairs (WANNER 2006). PCR was performed for TxtAB with Stx1a / Stx1b primer pairs. Briefly; 1 µM primers at final volume and 2,5 µl template DNA were added tubes containing Qiagen (201443) Taq PCR master mix (Flores-Gonzalez et al., 2008).

PCR conditions of Tom3 / Tom4, Scab1m / Scab2m, Stel3 / Aci2, Stel3 / T2st2 and Nf / Nr primer pairs are initial denaturation step at 95 °C 3 min, 40 cycles consisting of denaturation at 95 °C for 20 s., annealing at temperature given (Table1) for 30 s., extension at 72 °C for 2 min., and final extension step at 72 °C for 4 min. PCR condition of Stx1a / Stx1b primer pairs are initial denaturation step at 95 °C for 5 min., 35 cycles consisting of denaturation at 95 °C for 30s., annealing at 60 °C for 15 s., extension at 72 °C for 1 min., and final extension step at 72 °C for 10 min. Annealing temperatures and $MgCl_2$ concentrations are presented in (Table 1). Eppendorf master personal thermal cycler was used for all PCR applications. The amplified DNA was run 1,5 % agarose gel.

Table 1. Primer pairs

Target	Primers	AT °C	$MgCl_2$ (mM)	PS (bp)
S. scabiei and *S.europaeiscabiei*	Scab1m (5' CGACACTCTCGGGCATCCGA 3') Scab2m (5' TTCGACAGCTCCCTCCCTTAC 3')	60	1,0	1278
S. stelliscabiei	Stel3 (5' GAAAGCATCAGAGATGGTGCC 3') T2st2 (5' CGACAGCTCCCTCCCCGTAAG 3')	60	1,5	476
S. bottropensis	Stel3 (5' GAAAGCATCAGAGATGGTGCC 3') Aci2 (5' CGACAGCTCCCTCCCACAAG 3')	60	1,0	475
Nec1	Nf (5' ATGAGCGCGAACGGAAGCCCCGGA 3') Nr (5' GCAGGTCGTCACGAAGGATCG 3')	60	2,0	700
TomA	Tom3 (5' GAGGCGTTGGTGGAGTTCTA 3') Tom4 (5' TTGGGGTTGTACTCCTGCTC 3')	59	1,5	392
TxtAB	Stx1a(5' GTGGACCGTGGAGCATCT 3') Stx1b(5' CAGTTCGGCGTAACTCAGC 3')	60	1,5	402

AT; annealing temperature, PS; Product size

Results

Pathogenicity tests of all strains were evaluated as positive according to the both of the methods (Tuber slice and mini tuber). 47 out of 74 Pathogenic strains identified as *S. scabiei* have grey, spiral spore chains (Figure 1) and ability of melanin pigment production on PYI and TYR. All these strains can use nine ISP sugars as carbon source. Eight strains identified as *S. stelliscabiei* have grey, spiral spore chains and ability of melanin pigment production on PYI and TYR. All of nine ISP sugars were used by these strains. 15 strains identified as *S. bottropensis* have red substrate mycelium, grey and spiral spore chains. All of these strains have ability of producing melanoid pigment on PYI and TYR. They also used all of nine ISP sugars as a carbon source. Four out of 74 strains were also defined as *S. europaeiscabiei*. These strains have grey, spiral spore chains and ability of melanin pigment production and can use nine ISP sugars as carbon source Morphological and biochemical characteristics of the strains are presented in Table 2.

Molecular tests were also performed for identification and characterization of strains. As a result of viewing electrophoresis gel, expected bands, which showing the specific 16S regions were obtained for all the strains (Figure 2). PCR Analyses also showed that strains can lack some of target genes in PAI. *Nec1*, *TomA* and *TxtAB* genes are exist 72, 71 and 70 out of 74 scab-causing strains, respectively. Results are presented in Table 2.

Figure 1. Spiral spore chains of *S.scabiei* KS 592(40X)

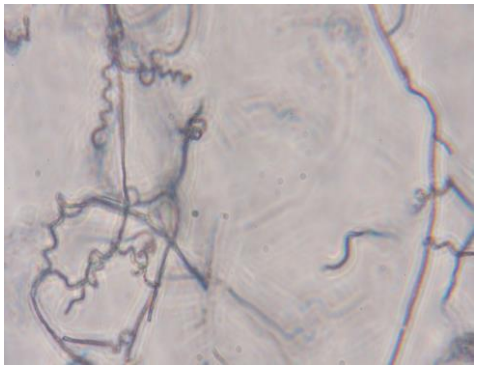

Figure 2. PCR results

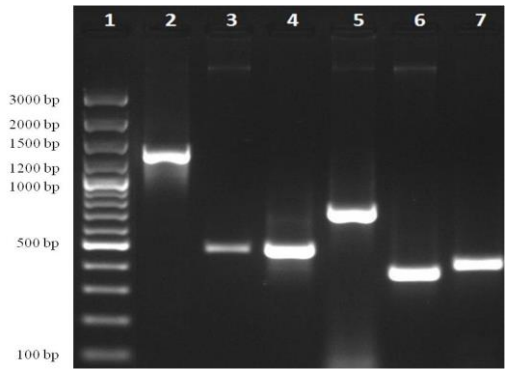

Figure 2: Lane1: Marker, Lane 2: *S.scabiei* and *S.europaeiscabiei*, Lane 3: *S. stelliscabiei*, Lane 4*: S. bottropensis*, Lane 5: *Nec1*, Lane 6: *TomA*, Lane 7: *TxtAB*

Table 2. Morphological and biochemical characteristics of isolated strains

Strain number	identification result	Spore color	Spore morphology	Melanoid pigment production		Streptomycin	Penicillin	Chrystal violet	phenol	NaCl (%)			Minimum growth of pH
				PYI	TYR					5	6	7	
KS170	*S. stelliscabiei*	G	S	+	+	-	+	-	-	+	+	+	5,5
KS176	*S. stelliscabiei*	G	S	+	+	-	+	-	-	-	-	-	5,5
KS186	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS195	*S. bottropensis*	G	S	+	+	-	+	-	+	-	-	-	4,0
KS196	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS197	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS198	*S. scabiei*	G	S	+	+	-	-	-	-	+	-	-	5,0
KS225	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS252	*S.europaeiscabiei*	G	S	+	+	-	+	-	+	+	+	-	5,0
KS294	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS295	*S. scabiei*	G	S	+	+	-	-	-	-	+	-	-	5,0
KS297	*S. stelliscabiei*	G	S	+	+	-	+	-	-	-	-	-	5,5
KS300	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS302	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS303	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS306	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS307	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS317	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS318	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS319	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS461	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS463	*S. scabiei*	G	S	+	+	-	-	-	-	+	+	-	5,0
KS464	*S.europaeiscabiei*	G	S	+	+	-	+	-	+	-	-	-	5,0
KS466	*S. stelliscabiei*	G	S	+	+	-	+	-	-	-	-	-	5,5

Spor color; G: grey, Spore chain; S: spiral, Phenol: 1%, Chrystal violet: 0,5 µg ml^{-1}, Streptomycin 20 µg ml^{-1}, Penicillin 10 IU m

Table 2. Continue

Strain number	identification result	Spore color	Spore morphology	Melanoid pigment production		Streptomycin	Penicillin	Chrystal violet	phenol	NaCl (%)			Minimum growth of pH
				PYI	TYR					5	6	7	
KS469	*S. scabiei*	G	S	+	+	-	-	-	-	+	-	-	5,0
KS470	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS471	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS475	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS476	*S. scabiei*	G	S	+	+	-	-	-	-	+	+	-	5,0
KS479	*S. scabiei*	G	S	+	+	-	-	-	-	+	-	-	5,0
KS480	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS481	*S. scabiei*	G	S	+	+	+	-	-	-	-	-	-	5,0
KS486	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS488	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS489	*S.europaeiscabiei*	G	S	+	+	-	+	-	-	-	-	-	5,0
KS493	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS494	*S. stelliscabiei*	G	S	+	+	-	+	-	-	-	-	-	5,5
KS497	*S. scabiei*	G	S	+	+	-	-	-	-	+	-	-	5,0
KS499	*S. scabiei*	G	S	+	+	-	-	-	-	+	-	-	5,0
KS500	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS503	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS505	*S. scabiei*	G	S	+	+	+	-	-	-	+	+	-	5,0
KS507	*S. scabiei*	G	S	+	+	-	-	-	-	+	-	-	5,0
KS509	*S. bottropensis*	G	S	+	+	-	+	-	+	-	-	-	4,0

Spore color; G: grey, Spore chain; S: spiral, Phenol: 1%, Chrystal violet: 0,5 µg ml^{-1}, Streptomycin 20 µg ml^{-1}, Penicillin 10 IU

Table 2. Continue

Strain number	identification result	Spore color	Spore morphology	Melanoid pigment production		Streptomycin	Penicillin	Chrystal violet	phenol	NaCl (%)			Minimum growth of pH
				PYI	TYR					5	6	7	
KS510	*S. bottropensis*	G	S	+	+	-	+	-	+	+	-	-	4,0
KS511	*S. scabiei*	G	S	+	+	-	-	-	-	+	+	-	5,0
KS514	*S. bottropensis*	G	S	+	+	-	+	-	+	+	-	-	4,0
KS515	*S. bottropensis*	G	S	+	+	-	+	-	+	-	-	-	4,0
KS518	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS521	*S. scabiei*	G	S	+	+	-	-	-	-	+	-	-	5,0
KS523	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS526	*S. stelliscabiei*	G	S	+	+	-	+	-	-	+	+	+	5,5
KS528	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS529	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS530	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS535	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS536	*S. scabiei*	G	S	+	+	-	-	-	-	+	+	-	5,0
KS545	*S.europaeiscabiei*	G	S	+	+	-	+	-	-	-	-	-	5,0
KS546	*S. scabiei*	G	S	+	+	-	-	-	-	+	-	-	5,0
KS548	*S. stelliscabiei*	G	S	+	+	-	+	-	-	-	-	-	5,5
KS553	*S. scabiei*	G	S	+	+	-	-	-	-	+	+	-	5,0
KS554	*S. bottropensis*	G	S	+	+	-	+	-	-	+	+	-	4,0
KS555	*S. scabiei*	G	S	+	+	-	-	-	-	-	-	-	5,0
KS560	*S. bottropensis*	G	S	+	+	-	+	-	+	-	-	-	4,0

Spore color; G: grey, Spore chain; S: spiral, Phenol: 1%, Chrystal violet: 0,5 µg ml^{-1}, Streptomycin 20 µg ml^{-1}, Penicillin 10 IU

Table 2. Continue

Strain number	identification result	Spore color	Spore morphology	Melanoid pigment production		Streptomycin	Penicillin	Chrystal violet	phenol	NaCl (%)			Minimum growth of pH	Growth at 37 ºC
				PYI	TYR					5	6	7		
KS573	*S. bottropensis*	G	S	+	+	-	+	-	+	+	+	-	4,0	+
KS592	*S. scabiei*	G	S	+	+	-	-	-	-	+	+	-	5,0	+
KS593	*S. bottropensis*	G	S	+	+	-	+	-	+	+	-	-	4,0	+
KS618	*S. bottropensis*	G	S	+	+	-	+	-	+	+	+	-	4,0	+
KS620	*S. bottropensis*	G	S	+	+	-	+	-	+	+	-	-	4,0	+
KS627	*S. stelliscabiei*	G	S	+	+	-	+	-	-	+	+	+	5,5	+
KS629	*S. bottropensis*	G	S	+	+	-	+	-	+	+	+	-	4,0	+
KS636	*S. bottropensis*	G	S	+	+	-	+	-	+	+	-	-	4,0	+
KS646	*S. bottropensis*	G	S	+	+	-	+	-	+	-	-	-	4,0	+
KS647	*S. bottropensis*	G	S	+	+	-	+	-	+	-	-	-	4,0	+

Spore color; G: grey, Spore chain; S: spiral, Phenol: 1%, Chrystal violet: 0,5 µg ml^{-1}, Streptomycin 20 µg ml^{-1}, Penicillin 10 IU ml

Discussion

Potato scab is a worldwide disease caused by different *Streptomyces* species. *S.scabiei* is considered as leading causal agent of scab disease (HOSAKA et al. 2000) and varies scab-causing *Streptomyces* species have been reported from different locations around of the world (BOUCHEK-MECHICHE et al. 2000; PARK et al. 2003; FLORES-GONZALEZ et al. 2008; DEES et al. 2013). In a former research, conducted in Central Anatolia Region of Turkey, existence of *S. scabiei* and *S. reticulliscabiei* strains were reported and most of the strains were identified as *S. scabiei*. But the strains just identified with morphological and biochemical tests data. Molecular methods weren't used for identification (KARAHAN 2006). Current research showed that there are different scab-causing *Streptomyces* species in Turkey. Existence of *S. bottropensis*, *S. stelliscabiei* and *S. europaeiscabiei* in potato have firstly reported in Turkey with this study and Identification results were supported by morphological, chemical and molecular tests. Additionally, all identified strains have firstly reported in Eastern Anatolia Region of Turkey. In our research, *S. scabiei* was defined as causal agent of scab disease in Eastern Anatolia Region of Turkey. When the former research, which conducted in Central Anatolia Region of Turkey, is considered, it is possible to say that *S. scabiei* is the main causal agent of common scab disease in Turkey. In former researches, *S. eurpaeiscabie* and *S. turgidiscabies*, *S. euroscabiesi* , *S. scabiei* and *S. europaeiscabiei* were defined as predominant pathogens in Norway (DEES et al. 2013), Germany (LEIMINGER et al. 2013) and some part of USA (WANNER 2006) respectively. It is well known that Turkey has been importing potato seeds from different countries including, USA, French, Germany, UK, Denmark, Belgium and a few more. Therefore, different pathogens can be seen in Turkey.

Morphological and biochemical analyze result of *S. scabiei* (LAMBERT and LORIA 1989b), *S. stelliscabiei*, *S.europaeiscabiei* (BOUCHEK-MECHICHE et al. 2000) and *S.bottropensis* (YAMAZAKI et al. 1992; LEIMINGER et al. 2013) strains were mostly fitted data in the literature with some exceptions. Chemicals and antibiotics resistance of strains showed some variations.

For molecular identification of strains, PCR was conducted with specific 16S primer pairs, which designed scab-causing *Streptomyces* species before (WANNER 2006, 2009). The primer pairs were also used successfully in this work. *S. scabiei* and *S.stelliscabiei* strains were easily individuated because *S. stelliscabiei* strains were only amplified by Stel3/T2st2 primer pair and they have no reaction to Scab1m/2m. Stel3/Aci2 primer pair also provided distinctiveness for *S. bottropensis* strains which have already different morphological characteristics. But scab1m/scab2m primer pair don't provide identification of *S. scabiei* and *S. europaeiscabiei* in PCR because scab1m sequence is perfectly homologous to *S. scabiei* and has 1 bp mismatch to *S. europaeiscabiei* (WANNER 2006). However, it is reported that *S. scabiei* and *S.europaeiscabiei* strains have different penicillin resistance (BOUCHEK-MECHICHE et al. 2000). When this data evaluated along with PCR result, identification of *S. europaeiscabiei* was done. Additionally, identification results of *S. scabiei* and *S. europaeiscabiei* strains were verified by 16S rDNA analysis. Sequence data of S. *scabiei* KS196 and *S. europaeiscabiei* KS464 was deposited in GenBank as representative isolates with KR422360 and KR422361 accession numbers, respectively.

According to PCR analyses results, strains largely have *TxtAB*, *Nec1*, and *TomA* genes in PAI. But lacking of the one or more of these genes was determined in some strains. Two strains (*S.scabiei* KS481 and *S.bottropensis* KS 636) have lacking *Nec1* genes. *TomA* gen wasn't detected in three strains (*S. stelliscabiei* KS170, *S. scabiei* KS518 and *S. scabiei* KS553). It is known that different pathogenic *Streptomyces* species can have lacking of Nec1 or TomA (FLORES-GONZALEZ et al. 2008; WANNER 2009). Actually, most of researchers suggested that Nec1 and TomA genes are related with pathogenicity but they aren't primary determinant of pathogenicity. Some pathogen strains lacking one or two of these genes were reported (WANNER 2009; DEES et al. 2013; LEIMINGER et al. 2013). Besides of this, existence of Nec1 and *TomA* genes were also reported in non-pathogenic strains (WANNER 2009).

Ability of thaxtomin production was suggested as primary pathogenicity determinant of scab-causing *Streptomyces* species in many studies (WANNER 2007a; FLORES-GONZALEZ et al. 2008; WANNER 2009; LEIMINGER et al. 2013). In former studies, researchers collected potatoes with different symptoms (etc. superficial, raised or pitted); they tested many streptomyces isolates for evaluating of PAI. Hereby, it is showed that pathogenic strains mostly have TxtAB operon with negligible exceptions. One of these study present that all of 100 scab-causing strains have TxtAB (WANNER 2006). In another study from Germany, researchers isolated 295 *Streptomyces* strains from potato. 265 out of these were identified as pathogenic

and researchers reported that all of pathogenic strains have TxtAB operon (LEIMINGER et al. 2013). On the other hand, it is reported that some scab-causing *Streptomyces* species may not have TxtAB operon (LORIA et al. 2006; FLORES-GONZALEZ et al. 2008; WANNER et al. 2011). Consequently, many researchers have consensus about on scab-causing strains mostly have ability of thaxtomin but this may not be already operative. We think that TxtAB negative may produce another thaxtomin analogue. However, these strains may lack TxtAB operons or false negative results may occur with PCR. When literature knowledge is considered, all possibilities are possible. It is needed that chemical thaxtomin production analyses for certain provision. It was thought to being done this analysis with another research.

In consequently; existence of different scab-causing *Streptomyces* species were assigned in Turkey and PAI of these strains were also characterized. According to our data and observation, more scab-causing *Streptomyces* species are exist in Turkey. In our opinion, more research was needed on this topic to define pathogen range and to develop control strategies.

Acknowledgement

This paper is a part of doctoral thesis and funded by Ataturk University, Scientific Research Council (BAP) with BAP 2012/231 project number. Some parts of this paper were presented in Turkey V. Plant Protection Congress as abstract.

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