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CHARACTERIZATION OF STREPTOMYCES SCABIES ISOLATED FROM COMMON SCAB LESIONS ON POTATO TUBERS BY MORPHOLOGICAL, BIOCHEMICAL AND PATHOGENICITY TESTS IN CHLEF REGION IN WESTERN ALGERIA.

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Résumé

Caractérisation de Streptomyces scabies isolée à partir des lésions au niveau des tubercules de pomme de terre par des tests morphologiques, biochimiques, et de pathogénécité dans la région de Chlef dans l'ouest Algérien.

Durant l'année 2002, neuf isolats causant de symptômes de gales communes sur pomme de terre sont collectés à partir de tubercules présentant des lésions superficielles ou profondes dans différentes localités de la région de Chlef.

Les souches induisant ces symptômes ont été caractérisées par la présence de colonies crémeuses sur milieu YMA et par la formation d'un mycélium blanc qui prend une couleur brunâtre en vieillissant. L'agent étant une bactérie filamenteuse, Gram +, immobile, utilisant l'arabinose, le D fructose, le D glucose et le D rhamnose. Ces souches dégradent le xylose et l'amidon avec la production de mélanine sur un milieu à base de levure peptonné enrichie de fer (PYI).

Par ailleurs, les souches testées présentaient des caractéristiques similaires aussi bien sur le plan morphologiques que biochimiques et qui les partagent avec la souche de référence ATCC49173.

Concernant, les résultats de pathogénicité, celles-ci ont montré des symptômes typiques de la gale commune comme ceux produites par la souche de référence avec cependant des degrés d'aggressivité variables entraînant ainsi des symptômes allant de faibles lésions superficielles jusqu'à des lésions profondes et généralisées. Le postulat de Kock étant vérifié, on peut donc dire que les neuf souches testées appartiennent à l'espèce Streptomyces scabies.

Mots clefs : *Gale commune, Streptomyces scabies, Caractéristiques biochimiques, pomme de terre, Algérie.*

Abstract

In summer 2002, nine isolates causing common scab symptoms were collected from tubers presenting superficial and raised corky lesions on tubers surface in number of locations from the Chlef region in Algeria.

The common scab inducing organism were characterized by creamy colonies on yeast malt extract (YMA) and by arial mycelium which turned brown with age.

The organism was Gram positive, non motile, utilized L-arabinose, D-fructose, D-glucose and rhamnose. They degraded the xylose and starch with production of melanin on peptone yeast extract agar-iron(PYI).

Additionally, it was determined that most strains obtained from differents locations were identical in morphology and biochemical characteristics. Furthermore, these isolates shares most pattern with the reference isolate ATCC49173.

Results of pathogenicity tests showed that all isolates were pathogenic on both cultivars(Desiree and Claustar) causing symptoms with aggressiveness of strains varying from mild to moderately severe. Koch's postulates for isolates were also fulfilled.

Keywords: *Streptomyces scabies, Common scab, Potato, biochemical characteristics, Algeria.*

ملخص

في عام 2002, تم جمع 9 عزلات سببت أعراض الجرب العادي على البطاطس بعد ظهور شقوق سطحية أو عميقة على الدرنات في مختلف مناطق الشلف.

تتميز الأرامات المحدثة للأعراض بوجود مستعمرات هلامية في وسط مغذي(YMA) مع تشكيل أفطوره بيضاء تتحول بعد مدة إلى اللون البني. العامل المسبب لهذا المرض هو بكتيريا خيطية ,جرام موجب, و غير متحركة, تتغذى على الأرابينوز(arabinose), د- فركتوز (D fructose), د- جلوكوز (D glucose) و د- رامنوز(D rhamnose).

هذه الأرامات تحلل القزيلوز(xylose) و النشاء(amidon) مع إنتاج الميلانين في وسط ذو قاعدة خميرة بيبتونية مشبع بالحديد(PYI).

من جهة أخرى, أظهرت الأرامات التسع المختبرة خصائص متشابهة بما فيها المورفولوجية و البيوكيميائية الملاحظة لدى الأرومة المرجعية(ATCC49173).

فيما يخص نتائج الإمراض, بينت هذه الأرامات أعراض مثالية للجرب العادي للبطاطس مشابهة لأعراض الأرومة المرجعية, مع درجة أضرار متغيرة و ذلك بظهور تشققات سطحية تتطور إلى شقوق عميقة و معممة على درنات البطاطس.

و قد تم أيضا التحقق من فرضية كوك Kock, إذا نستطيع القول بان الأرامات التسع المختبرة تنتمي إلى فصيلة *Streptomyces scabies*

الكلمات المفتاحية: *الجرب العادي, Streptomyces scabies , خصائص بيوكيميائية, البطاطس, الجزائر*

Potatoes are an important dietary staple in Algeria. It is the second most important food crop after the cereal. It is one of the most nutritious staple crop because they are source of complex carbohydrates and they contain most of the vitamins needed for every day life.

In Algeria, the potato is widely grown and particularly in north western regions. The cultivated area used for potato crop production are around 75 000 ha, with an average yield of 16,0 tons/ha.

The agriculture statistics established that important yield improvements were achieved in the country during the last 15 years mainly due to the use of new cultivars, improved fertilizers and irrigation techniques. However, the actual yield, at least for many regions is still well below the attainable and potential yields.

In Chlef region composed of the Willaya of Ain Defla, the potato is a very important crop and can be cultivated throughout the year. However tuber yields are generally much lower than those in Northern European countries. This is because the environmental conditions but also others limiting factors relating to biotics attacks. These are mainly fungus, bacteria and virus agents. Losses due to diseases agents can occur when crop are growing, at lifting and when tubers are stored. Some diseases such as common scab , did not destroy tubers but the surface blemishes they decrease marketable value.

Potato scab is a common tuber disease that occurs throughout the potato growing areas. In fact, this disease is caused by several *Streptomyces* species. This common scab is also occasionally found on other crops, such as carrot, beet, radish and turnip(Goyer and Beaulieu,1997; Lehtonen et al, 2004).

Streptomyces scabies (Thaxter)Waksman&Henrici, a soil borne Actinomycete, is considered the principal causal agent of potato scab(Goth, 1965).

Lambert and Loria (1989), Goyer et al(1996), Lindholm et al(1997), characterized *S.scabies* as an organism that presents smooth gray spores borne in spiral chains, produces melanin and is able to utilize L-arabinose, D-fructose, D-glucose and rhamnose and xylose as a carbon source.

Although scab does not usually affect total yields(Bang, 1995; Scholte et Labruyère, 1985; Scholte, 1989), significant economic losses result from reduced marketability since the appearance is important (Hooker, 1981; Bouchek-Mechiche et al, 2000; Afek et Orenstein, 2002; Pasco et al, 2005).

In fact, no reports indicating the presence of such disease in Algeria and no systematic survey of the potato growing areas in Chlef region was made to determine the prevalence and the causal agent of the common scab.

In fact, Streptomyces scabies produces common scab in dry, neutral to alkaline soils in which potatoes are frequently cultivated. Other species, such as S.acidoscabies (Faucher et al, 1995; Goyer et al, 1996) and S. Turgidiscabies (Takeuchi et al, 1996; Miyajima et al, 1998), cause common scab in other soils conditions which are often unfavorable or constraint to S. Scabies.

The objective of the present study was to determine the occurrence of Streptomyces scabies in the major growing area in the western regions of Algeria namely the Willaya of Chlef and Ain Defla. Traits examined included pathogenicity, characterization of morphological, physiological and biochemical aspects.

MATERIALS AND METHODS

Disease survey

Surveys were conducted during the summer 2002 in various locations in Chlef region(table1). Potato tubers affected by common scab were obtained from six potato growing areas differing in soil conditions. The major cultivated varieties are Desiree, Claustar, Urgenta and Talassa. Tubers with such symptoms (fig. 1) were collected in each field from four to five plants per row. Both row and plants to be sampled were selected at random.

Figure 1 Common scab symptoms on Desiree (top) and Claustar (bottom) cultivars.

Pathogen isolation

The method used for isolation of the bacterial agent was suggested by Faucher et al(1993). Tubers with common scab symptoms were washed in running tap water. Pieces of skin tissues were macerated directly in sterile distilled water. Loopfuls of macerated tissue were streaked on YMA. The colonies appear generally after 3 days of incubation at 28C. Colonies characteristics of Actinomycetes were purified by restreaking on yeast malt extract agar as described by Goyer et Beaulieu (1997).

Cultural and morphological characteristics

Cultural characteristics of the isolates were compared on the basis of observations made after 7 days incubation on seven media namely YMA, PYI, King B, Czapeck Dox,

Sabouraud, PDA and nutrient agar. Growth rate and mycelia production was tested. Three replicates dishes of each medium were used and the experiment was repeated twice.

The disposition of spore chains was observed by light microscopy at 100X. Motility was determined on a stab inoculated semi solid media(Shirling et Gottieb, 1966; Mac faddin, 1976).

Physiological and biochemical characteristics

The nine isolates with the reference strain(ATCC49173) of *S.scabies* were identified by the following tests: Gram stain(Gregerson, 1978), melanin production on peptone yeast extract-iron agar and tyrosine agar medium; starch hydrolysis; hydrogene sulfide(H_2S) production, and nitrate reduction; gelatin liquification(Schaad et al, 2001); catalase production, oxydase reaction; urease production and 3 and 5% sodium chloride(NaCl) tolerance. Sensitivity to six antibiotics penicillin G(10UI/ml), gentamicin(20UI/ml), chloramphenicol(30UI/ml), kanamycin(25UI/ml), amoxicillin(25UI/ml) and tetracycline(30UI/ml) was tested with antibiotics sensitivity disks. The isolates were grown on Muller-Hinton medium. Utilization of carbohydrates was investigated with a basal medium(Waksman,1967). Filter sterilized solutions were added to autoclaved basal medium for a final concentration of 0.5%(w/v).

The following carbohydrates were tested:D- glucose, D-fructose, L-arabinose, galactose, mannose, rhamnose, xylose and starch. Tests were performed at 28C, all tests were repeated twice.

Pathogenicity testsPlant material

Certified potato tubers of the cultivar Desiree and Claustar known for their sensitivity towards the common scab were planted using one tuber per pot of 19-cm diameter containg salt/soil.

Production of inoculum

Colonies grown on YMA were mixed in sterile distilled water. The resultant mixture was adjusted to contain $3x10^7$ cfu/ml of colonies of each isolate. A total of 50 ml of this mixture was added to each tuber as it was placed in soil. Inoculated tubers were immediatly covered with soil. The pots were placed under conditions with 24 ± 5C and with a photoperiod of 14/10hr. Nutrients were supplemented with weekly applications of soluble fertilizer.

Scoring

Plants were harvested about two months after inoculation. All tubers in each pot were examined for common scab symptoms.

The percentage area with scab lesions was estimated with the Horsfall-Baratt rating scale (Goth et al,1993). The type of lesion consisted of five categories based on the assessment key of James(1971):

0=no scab; 1= superficial lesions less than mm in diameter; 2= superficial lesions greater than 10mm in diameter; 3= raised lesions less than 10mm in diameter; 4= raised lesions greater than 10mm in diameter; 5=pitted scab of all diameters.

RESULTS

Characterization of cultural and morphological characteristics of the pathogen.

The cultural and morphological characteristics of the isolates on various media are presented in table 1. All strains grew on most organic and synthetic media tested. The selective media was very effective for isolating the Streptomyces isolates.

Table1 Location, and symptoms elicited by nine isolates on tubers of potato genotypes from which they were collected.

Isolate	Location	Potato cultivar	Symptoms
Cs1da	El attaf	Desiree	Irregular shaped raised scabs
Cs2da	El attaf	Desiree	Continuous raised scabs
Cs3dof	oued fodda	Desiree	Continuous raised scabs
Cs4uf	Oued fodda	Urgenta	Superficial irregular lesions
Cs5dff	Ouled fares	Desiree	Raised irregular scabs
Cs6cff	Ouled fares	Claustar	Raised irregular scabs
Cs7dbk	Boukadir	Desiree	Superficial irregular lesions
Cs8ubk	Boukadir	Urgenta	Superficial irregular lesions
Cs9cmd	Madjadja	Claustar	Superficial irregular lesions

On YMA, typical colonies with convex form and entire margin whith white to creamy white color was observed. They appeared after three days of incubation, and their average size was 3-mm after 7 days of growth. The development of mycelia at the margin was observed for all the isolates after 5 days of incubation.

On peptone yeast extract agar, diffused melanoid pigment was noted for most of isolates tested. The two isolates however, that did not produce such pigment on this medium even when cultivated on tyrosine agar medium are Cs9cmd and Cs3dof.

On the other hand, the major characteristics observed on the Czapeck Dox is the high mycelia production comparatively to the other media even when compared to the YMA.

Unlike what was occured on YMA and Czapeck Dox media, all the isolates did produce a visible mycelium on PDA or King B. Furhermore, no or very poor growth of colonies was observed on nutrient agar and Sabouraud medium.

Light microscopy revealed that all strains presented aerial mycelia that were monopodially branched with spiral chains of spores.

Carbohydrates utilization

The utilization of various carbohydrates by all isolates suggest a very large pattern of carbon source assimilation (tables 2,3).

Table2 Cultural and morphological characteristics of the nine isolate common scab inducing organism on seven cultural media.

Medium	Growth	Aerial mycelium	Spore production and color
Czapeck Dox agar	Moderate growth with white color	Very abundant	White color
Peptone yeast extract agar-iron	Brown color; moderate growth	abundant	Abundant with white color
yeast malt extract agar	Well abundant with creamy color		Moderate with white color
Nutrient agar	Absent	absent	Absent
PDA	Creamy colonies with moderate growth	Absent to very poor	Very poor
King B	Moderate, with creamy color	Absent to very poor	Poor
Sabouraud	Very poor growth	Very poor	Very poor

Table 3 Carbohydrate utilization by common scab inducing organism (Results recorded after 7 days of growth).

Carbohydrates / Isolates	Cs1da	Cs2da	Cs3dof	Cs4duf	Cs5dff	Cs6cff	Cs7dbk	Cs8ubk	Cs9cmd	Reference Isolate (ATCC49173)
D-arabinose	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+
D-fructose	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+	+	+	+	+
Raffinose	-	-	+	-	+	-	-	+	-	-
Lactose	-	-	-	-	-	-	-	-	-	-
Starch	+	+	+	+	+	+	+	+	+	+
Sucrose	-	-	-	-	-	-	-	-	-	-
Maltose	+	+	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+	+	+

-: negative reaction (no bacterial growth)
+: positive reaction (bacterial growth)

Rhamnose, glucose, fructose, xylose, maltose and starch were utilized well. Sucrose and lactose were not utilized. However, raffinose was not used by most isolates.

Biochemical and physiological tests

All isolates presented non motile and Gram positive features. The optimum growth was observed at 28C. slight growth was however, noted at 35C for all the isolates tested.

On the other hand, they were negative for oxidase, urease, indole production and nitrate reducatase. All strains were positive for production of H_2S, the presence of catalase and the growth in 4%NaCl (table 4).

Table 4 Biochemical and physiological characteristics of strains inducing common scab.

Characteritics / Isolates	Cs1da	Cs2da	Cs3dof	Cs4duf	Cs5dff	Cs6cff	Cs7dbk	Cs8ubk	Cs9cmd	Reference Isolate (ATCC49173)
Gram test	+	+	+	+	+	+	+	+	+	+
otility in peptone water	-	-	-	-	-	-	-	-	-	-
litrate reductase	-	-	-	-	-	-	-	-	-	-
Covac's oxidase	-	-	-	-	-	-	-	-	-	-
elatine hydrolysis	+	+	+/-	+	+	+/-	+	+	+	+/-
catalase	+	+	+	+	+	+	+	+	+	+
H_2S production	+	+	+/-	+	+	+	+/-	+	+	+
rease production	-	-	-	-	-	-	-	-	+/-	-
ndole production	-	-	-	-	-	-	-	-	-	-
owth at 4% NaCl	+/-	+	+	+	+/-	+	+	+	+	+/-
Growth on pH=4.5	-		-	-	-	-	-	-	-	-
pH>=6.5-8	+		+	+	+	+	+	+	+	+

-: negative reaction (no bacterial growth)
+: positive reaction (bacterial growth)
+/-: reaction not so clear

The antibiogramm tests revealed that the strains varied in susceptibility to chloramphenicol, gentamicin, kanamycin and amoxillin(fig2). Most strains were resistant to penicillin G and tetracycline including the reference isolates. The isolates Cs1da, Cs8ubk and Cs9cmd had an an intermediate reaction towards the penicillin G and tetracycline antibiotics (table 5).

Figure 2 Reaction of common scab inducing organism and *S.scabies* (isolate Cs4uf) to six antibiotics.

Table 5 Reaction of common scab inducing organism to six antibiotics.

Antibiotic Denomination	Charge in (units)	Reaction Cs1da	Cs2da	Cs3dcf	Cs4duf	Cs5dff	Cs6cff	Cs7dbk	Cs8dbk	Cs9cmd	Reference Isolate (ATCC49173)
Penicillin G	10 U	I	R	R	R	R	R	R	I	I	R
Choramphenicol	30 U	S	S	S	S	R	S	S	S	S	S
Gentamycin	20 U	S	S	S	S	S	S	S	S	S	S
Kanamycin	25 U	S	S	S	S	S	S	S	S	S	S
Tetracyclin	30 U	R	R	R	R	R	R	R	R	R	R
amoxicillin	25 U	S	S	S	S	S	S	S	S	S	S

S=Sensitivity(inhibition zone around the antibiotic greater than 3-mm)
R= Resistance(no inhibition or the inhibition zone around the antibiotic is less than 3-mm).
I: intermediate reaction.

Pathogenicity tests

One purpose of this study was to confirm that isolates were effectively the common scab inducing organism. Consequently we isolated the Streptomyces from tubers with common scab disease.

Results were obtained from two potato cultivars in this regions. Symptoms are reproduced on the two cultivars with all isolates compared to the ATC49173 isolate. Scab symptoms observed were similar to that encountred in natural conditions.

Furthermore, the agressiveness tests showed that all isolates were highly aggressive. On the other hand, the pathogen was readily reisolated from lesions of tested tubers. No inoculated control plants presented symptoms.

DISCUSSION

The strains of potato scab pathogen that we studied belonged to genus Streptomyces on the basis of morphology, biochemical and physiological patterns.

In fact, the use of both the phenotypic characteristics are the principal keys to identify the genus Streptomyces as suggested by many authors (Miyajima, 1998; Takeuchi et al, 1996; Park et al, 2003). Furthermore, the use of both the phenotypic patterns and

Molecular markers are revealed to be an excellent tools for the Streptomyces species determination (Bouchek-Mechiche et al, 2000; Wanner, 2007).

In our case, all the isolates obtained from different localities of chlef region were nearly identical in their morphology and physiology characteristics.

In fact, different standard laboratory tests such as colony morphology, production of melanoid substance, production of spore on spiral chain, Gram coloration, utilisation of wide patterns of carbohydrates, their positive response towards catalase, H_2S, indole production and gelatine production. Also their tolerance towards 4% of NaCl, their sensitivity to a wide range of antibiotics and their inability to grow at pH 4.5. were used to compare the reaction of our isolates to the ATCC 49173 which was used as a reference isolate.

For pathogenicity test, the symptoms induced by our isolates were similar to that caused by the reference strain. This similarity suggests that the isolates obtained from the Chlef region are closely related to Streptomyces scabies.

Variatin in aggressivity was also observed between the isolates on both the Desiree and Clauster cultivars. In fact, differences in both the aggressivity and virulence of individual Streptomyces isolates has already been suggested by many authors(Bouchnek-Mechiche, 2000; Kreuze et al, 1999; Wanner, 2004).

At sufficient inoculum concentration, all Chlef isolates were found to be pathogenic on potato tubers with no symptoms of alterations on other parts of plants. In others studies, however, lethality of seedling and mature plants were noticed on inoculated individuals. Such symptoms was very important with high inoculum density(Wanner, 2007).

Although virulence mechanism of pathogenic Streptomyces are still not well understood, thaxtomins, a category of phytotoxins, appear to play a great role in pathogenicity. Furthermore, King et al(1991), showed a positive correlation between thaxtomin production and pathogenicity.

Concerning the Streptomyces species that might cause the common scab, many reports showed, that this disease scab can be caused by several species such as S. Acidoscabis(Lambert et Lauria, 1989b;); S. caviscabies(Faucher et al, 1995 ; Goyer et al, 1996; Bouchek- Mechiche et al, 2000) and S. turgidscabies(Takeuchi et al, 1996 ; Pasco et al, 2005). These species have great similarity in symptoms produced and in host range.

Nevertheless, Wanner(2007), suggests that more than 80% of streptomyces attacks were really attributed to S.scabies. The consideration of all the results described and discussed above enable us to affirm that S. scabies is effectivelly present in Chlef production area. Hence, much attention should be taken by(i) understanding the biology of the causal agent, (ii) identifying the abiotic factors which determine its multiplication in order to propose controlling means to reduce the impact of this disease.

REFERENCES

[1]- Afek, u., Orenstein, J., 2002- Desinfecting potato tubers using steam treatments. Canadian Journal of Plant Pathology 24: 36-39.

[2]-Bang, H., 1995- Effects of soil conditions on the prevalence of netted scab. Acta Agriculturae Scandinavica 45: 271-277.

[3]-Bouchek- Mechiche, K., Pasco, C., Andrivon, D., Jouan, B., 2000- Differences in host range, pathogenicity to potato cultivars and response to soil temperature among Streptomyces species causing common and netted scab in France.

[4]-Elesawy, A.A., Szabo, I.M., 1979- Isolation and characterization of Streptomyces scabies strains from scab lesions of potato tubers. Designation an neotype strain of Streptomyces scabies. Acta Academiae Scientifica Hungarica 26: 311-320.

[5]-Faucher, E., ¨rados, E., Goyer, C., Hodge, N., Hogue, R., Stall, R.E., Beaulieu, C., 1995- Characterization of Streptomycetes causing deep pitted scab of potato in Quebec, Canada. International Journal of Systematic Bacteriology 46: 635-639.

[6]-Goth, R.W, Haynes, K.G, Wilson, D.R., 1993- Evaluation and characterization of advanced potato clones for resistance to scab by cluster analysis.Plant Disease 77:911-914.

[7]-Goth, R.W., 1965- Puncture method for isolating bacterial blights of bean. Phytopathology. 55:930-931.

[8]-Goyer, C., Beaulieu, F., 1997- Host range of Streptomycetes strains causing common scab.PlantDisease. 81:901-904.

[9]-Goyer, c., Otrysko, B., Beaulieu, C., 1996- Taxonomic studies on Streptomycetes causing potato common scab : a review. Canadian Journal of Plant Pathology 18: 107-113.

[10]-Gregerson, T., 1978- Rapid method for distinction of Gram negative from Gram positive bacteria. Eur.J.Appl. Microbiol.Biotechnol.5 :123-127.

[11]-Hooker, W.J., 1981- Common scab. Pages 33-34 in : Compendium of potato diseases. American Phytopathological Society, St.Paul, MN.125pp.

[12]-Horsfall, J.G., Barratt, R.W., 1945- An improved grading system for measuring plant disease(Abstr) Phytopathology.35:655.

[13]-James, G., 1971- A manual of assessment keys for plant diseases. Can.Dep.Agric.Publ.1458:32-33.

[14]-King, R.R., Lawrence, C.H., Caalhoun, L.A.1991- Correlation of phytotoxin production with pathogenicity of Streptomyces scabies isolates from scab infected potato tubers. American Potato Journal 68: 675-680.

[15]-Kreuze, J.F., Suomalainen, S., Paulin, L., and Valkonen, J.P.T, 1999- Phylogenitic analysis of 16SrRNA genes and PCR analysis of the common scab, and netted scab in Finland.

[16]-Lambert, D.H., and LORIA, R., 1989a. Streptomyces scabies. Sp.nov.nom. International Journal of Systematic Bacteriology 39: 387-392.

[17]-Lambert, D.H., and LORIA, R., 1989b. Streptomyces acidoscabies. Sp.nov.nom. International Journal of Systematic Bacteriology 39: 393-396.

[18]-Lehtonen, M.J., Rantala, H., Kreuze, J.F., Bang, H., Kuisma, L., Koski, P., Virtonen, E., Vihlman, K., Valkonen, J.P.T., 2004- Occurrence and survival of potato scab pathogens (Streptomyces species) on tuber lesions : quick diagnosis based on a pcr- based assay. Plant pathology 53 : 280-287.

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

[20]-Mac faddin, J.F., 1976- Biochemical tests for identification of medical bacteria. Williams&Wilkins.Co., Baltimore, MD.307p.

[21]-Miyajima, K., Tanaka, F., Takeuchi, T., Kuninaga, S.1998- Streptomyces turgidiscabies sp.nov. International Journal of Systematic Bacteriology 48: 495-502.

[22]-Park, D.H., Kim J.S., Kwon, S.W., Wilson, C., Yu, Y.M., Hur, J.H., Lim, C.K., 2003- Streptomyces Luridiscabiei sp.nov., Streptomyces punisciscabiei sp.nov. and Streptomyces niveiscabiei sp.nov., which cause potato common scab disease in Korea. International Journal of Systematic and Evolutionary Microbiology 53: 2049-2054.

[23]-Schaad, N.N., Jones, J.B., Chun, W., eds. Laboratory guide for the identification of plant pathogenic bacteria.American Phytopathological Society, St.Paul, MN.

[24]-Scholte, K., 1989- The effect of netted scab (streptomyces spp.) and Verticillium dahliae on growth and yield of potato. Potato Research 32: 65-73.

[25]-Scholte, K., labruyère, R.E., 1985- Netted scab, A new name fo an old disease in Europe. Potato Research 28: 443-448.

[26]-Shirling, E.B., Gottlieb, D., 1966- Methods for characterization of Streptomyces species. International Journal of Systematic Bacteriology 16: 313-340.

[27]-Takeuchi, T., Sawada, H., Tonaka, F., Madsuda, I.1996- Phylogenetic analysis of Streptomyces spp. causing potato scab based on 16 S.rRNA sequences. International Journal of Systematic Bacteriology 46: 476-479.

[28]-Wanner, L.A., 2004-Field isolates of Streptomyces differ in pathogenicity and virulence on radish. Plant disease 88:785- 796.

[29]-Wanner, L.A., 2007-A new strain of Streptomyces causing common scab in potato. Plant Disease, 91: 352-359.