Available online: www.notulaebiologicae.ro
Print ISSN 2067-3205; Electronic 2067-3264
Not Sci Biol, 2016, 8(2):151-155. DOI: 10.15835/nsb.8.2.9819
Notulae Scientia Biologicae
Original Article

Comparison between Pathogenic *Streptomyces scabies* Isolates of Common Scab Disease

Mohamed HOSNY[1], Kamal A. ABO-ELYOUSR[2]*,
Mahmoud R. ASRAN[1], Farag A. SAEAD[2]

[1]*Sohag University, Faculty of Agriculture, Plant Pathology Department, Sohag, Egypt; mrgr_2007@yahoo.com; masran2209@yahoo.com*
[2]*Assuit University, Faculty of Agriculture, Plant Pathology Department, Assuit, Egypt; kaaboelyousr @agr.au.edu.eg (*corresponding author); proffarag@yahoo.com*

Abstract

Streptomyces scabies (Thaxter) causes destructive and serious damages to many vegetable field crops, including potato. Fourteen pure isolates were obtained from naturally diseased potato tubers showing symptoms of common scab disease, collected from different localities of Sohag governorate, Egypt. All tested isolates were identified as *S. scabies* (Stc) according to morphological and biochemical tests. Isolate Stc 10 exhibited the highest activity of polyphenoloxidase enzyme, followed by isolate Stc 11, while isolate Stc 2 produced the lowest activity of this enzyme. Concerning the peroxidase activity, the isolates varied in their production; Stc 11 exhibited the highest activity enzyme, followed by isolate Stc 2, whereas isolate Stc 10 produced the lowest activity of enzyme. In regard with Tyrosine Amonnia Lyase (TAL) activity, isolate Stc 2 exhibited the highest activity, followed by isolate Stc 10, whereas isolate Stc 11 exhibited the lowest activity. Agarose gel electrophoresis of the PCR amplification products revealed a band representing the expected 279 bp DNA fragment in each DNA extracted from the highly pathogenic isolates Stc 10 and 11. The results demonstrated that PCR amplification of the *nec1* gene could be used as a reliable marker for detecting pathogenic *Streptomyces* isolates on potato tubers.

Keywords: common scab, gene virulence, Streptomyces, potato, tuber

Introduction

There are many diseases that attack potato in the field crop among which common scab is one of the bacterial diseases that register high economic loses. *Streptomyces scabiei* (Thaxter) Lambert and Loria (syn. *Streptomyces scabies*) is the main causal agent of common scab, a widely-distributed disease that causes important economic wastes in potato production (Lambert and Loria, 1989; Goyer *et al.*, 1996). Common scab symptoms consist of superficial to deep corky lesions on the tubers. Plant pathogenic *Streptomyces* spp. can survive in soil or plant debris for over a decade (Kritzman and Grinstein, 1991).

Streptomyces scabies strains were recently placed into three distinct genomic species based on DNA relatedness and 16S rDNA sequence, and assigned as either *S. scabies*, *S. europaeiscabiei* and *S. stelliscabiei* (Bukhalid *et al.*, 2002). The *nec1* gene encodes a secreted necrogenic protein with an uncharacterized plant cell target and was shown to confer a pathogenic phenotype on the nonpathogenic *Streptomyces lividans* (Bukhalid *et al.*, 1998). The G + C content (54%) of *nec1* is a typical relative to high G + C coding region, characteristic of *Streptomyces* strains, suggesting it was acquired through horizontal transfer from another toxin. The *nec1* gene is also structurally and functionally conserved among unrelated pathogenic *Streptomyces* species and absent in nonpathogens from a wide geographic origin (Bukhalid *et al.*, 2002; Park *et al.*, 2003). In addition, there exists a strong correlation between thaxtomin A production and the presence of *nec1* in pathogenic strains of *S. scabies, S. acidiscabies* and *S. turgidiscabies* (Bukhalid *et al.*, 1998). However, the *nec1* protein is not required for thaxtomin production and therefore it represents an independent virulence factor (Kers *et al.*, 2005).

The hereby work aimed to isolate and identify the common scab pathogen, and to study the variation between the isolates of the pathogen by production of some enzymes, as evidence of the gene virulence.

Materials and Methods

Source of Streptomyces scabies, the pathogen of common scab disease

Isolates of *Streptomyces scabies* from diseased potato tubers harvested from Sohag Governorate, Egypt, were used. The pathogenicity of these isolates was previously tested by authors and they varied in their pathogenicity; from 14 isolates identified (Stc 1...14), in the hereby study, three isolates (Stc 2, 10, 11) with different degree in pathogenicity were used (Hosny *et al.*, 2014).

Identification of pathogenic bacteria by morphological and physiological characteristics

The isolated bacterial isolates proved to be pathogenic and cause common scab to potato tubers; they were identified according to their morphological, cultural and physiological characteristics, as recommended by Krieg and Holt (1984), Schaad (1992), Holt *et al.* (1994).

Received: 20 Apr 2016. Received in revised form: 13 June 2016. Accepted: 14 June 2016. Published online: 17 June 2016.

The following morphological, physiological and biochemical characteristics were tested: shape of cell, motility, sporulation, color of colonies, gram staining, gelatin liquefaction, starch hydrolysis, catalase test, esculin hydrolysis, growth at 4 °C, growth at 40 °C, H_2S production, levan production, methyl-red test (MR), phenyl alanine deminase, casein hydrolysis, urease and action on carbon compounds.

Comparison between pathogenic isolates

Certain enzyme production

Peroxidase activity

One ml of ten days old bacterial culture was properly mixed in 2 ml sodium phosphate buffer 0.1 µ at pH7.1. The homogenate was filtered through Whatman No.1 filter paper. The suspension was centrifuged at 6,000 rpm at 4 °C for 20 min and stored at -18 °C until use.

One tenth ml of the filtrate was added to 0.5 ml sodium phosphate buffer 0.1 µ at pH = 7.1, 0.1 ml H_2O_2 1% and 0.3 ml pyrogallol 0.05 µ. The mixture was completed to 3 ml using distilled water and color density was read in absorbance spectrophotometer model Miltonroy spectronic 601, at 425 nm every 30 seconds for 10 reads (Kochba *et al*., 1977).

Peroxidase activity was calculated as mg/g fw (fresh weight).

Phosphate buffer preparation was done with eight and nine tenth gram di-sodium hydrogen dissolved in 250 ml distilled water in a volumetric flask (solution A) and 3.9 gm sodium hydrogen phosphate was dissolved in 250 ml distilled water in another flask (solution B). Solution A was added to solution B to have pH7.1.

Polyphenoloxidase activity

Enzyme samples were extracted as mentioned before in peroxidase activity extraction. One tenth extracted sample was added to 0.5 ml sodium phosphate buffer 0.1 ml at pH = 7 and 0.5 ml catechol 0.001 N. The mixture was completed to 3 ml using distilled water and color density was read in spectrophotometer (Miltonroy spectronic 601) at 495 nm every 30 seconds for 10 reads (Lisker *et al*., 1983).

Polyphenoloxidase activity was calculated as mg/g fw (fresh weight).

Tyrosine Amonnia Lyase (TAL) activity

Enzyme samples were extracted as mentioned before in peroxidase activity extraction. TAL activity was assayed by monitoring p-hydroxycinnamic acid formation at 310 nm using a double beam spectrophotometer (Uvikon, Kontron, Herts, UK) in 10 mM Tris buffer at 35 °C. The recommended pH was determined between the pH values 6 and 10. All reactions were performed in a total volume of 1 ml (Kyndt *et al*., 2002).

Tyrosine Amonnia Lyase activity was calculated as mg/g fw (fresh weight).

Gene virulence

Extraction of microbial DNA from pure cultures

Following the growth of *Streptomyces* spp. on Yeast Malt Extract (YME) medium at 25 °C for 5–7 days, a 4 mm^2 plug of growth was used to inoculate 2 ml Tryptone Soya Broth (TSB) containing 10% sucrose. Cultures were incubated at 25 °C at 100 rev min^{-1} for 2–5 days following which 1.5 ml of culture was removed and centrifuged at 11,550 g for 4 min to pellet cells. Supernatant was discarded and the pellet was re-suspended in 0.5 ml sterile quarter strength Ringer's solution (QSR) (Oxoid UK catalog No. BR52) and vortexed. Suspensions were centrifuged at 11,550 g for 2 min to pellet cells and the QSR solution was discarded. The cell pellet was re-suspended in 1.0 ml CTAB extraction buffer (2% CTAB, 1.5 mol l^{-1} NaCl, 0.2 mol l^{-1} Tris, 25 mmol l-1EDTA; pH 8.0) and the suspension transferred to a 2 ml Eppendorf tubes containing 0.1-0.2 g of 1.0 mm glass beads.

Cells were lyses by physical disruption in a Mini-Bead Beater-8 (Bio-Spec Products, Bartlesville, OK, USA) at a medium setting for 1 min. Tubes were centrifuged (11,550 g for 2 min) to pellet cell debris, and the supernatant was transferred to new 2 ml Eppendorf tubes and then extracted with an equal volume of chloroform, mixed and re-centrifuged (11,550 g for 5 min). DNA in the aqueous phase was precipitated with 0.3 mol l^{-1} sodium acetate (pH 5.2) and an equal volume of isopropanol, for 1 h at room temperature. The DNA was pelleted by centrifugation (11,550 g for 5 min), washed in 70% ethanol, re-pelleted, re-suspended in 100 µl TE (10 mmol l^{-1} Tris–HCl and 1 mmol l^{-1} EDTA, pH 8.0) and purified by Sephadex G-75 spin column chromatography using the method of Cullen and Hirsch (1998).

DNA was quantified using a TKO 100 Mini-Fluorometer (Hoefer Scientific Instruments, San Francisco, CA, USA) following the manufacturer's instructions and the quality was checked by agarose gel electrophoresis.

Design of nec1-specific PCR primers

A set of 20-mer primers was designed to target the coding sequence of the virulence *nec1* gene of *S. scabies*. The expected amplicon size amplified by the (Nec-NF1) PCR primer was 279 bp (Table 1). The conventional PCR as previously designed by Bukhalid *et al.* (1998) was followed.

PCR methods and reagents

PCR amplification of DNA samples was based on an initial denaturation at 95 °C (2 min), followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 65°C for 1 min, extension at 72°C for 90 s and a final elongation at 72°C for 5 min in a reaction volume of 25 µl using a GeneAmp PCR System 9600 thermal cycler (Applied Biosystems). PCR products were analyzed by electrophoresis on 2% agarose gels (Sambrook *et al*., 1989).

Statistical analysis

Analysis of variance (ANOVA) was carried out using MSTAT-C program. The Least Significant Difference (L.S.D) at $P \leq 0.05$ was applied to detect differences among treatments (Gomez and Gomez, 1984).

Table 1. PCR primer designed for specific detection of the *nec1* gene of pathogenic *Streptomyces scabies*

Primer	Sequence (5' to 3')	Size of product (bp)
NecNF1	CACTCTTGGAGATCTCATGC	279

Results

Identification of the causal pathogens

Identification of the isolated pathogenic bacteria was carried out using the morphological and physiological characteristics.

Data presented in Table 2 indicated that all tested isolates were of filamentous (or spiral) shape, non-motile, sporing, gram positive, gelatin liquefaction positive, starch hydrolysis positive, urease negative, catalase test positive, esculin hydrolysis positive antibiotic positive, no growth at both 4 °C and 40 °C, H_2S production negative, levan production negative, methyl-red test negative, phenyl alanine deminase test negative, casein hydrolysis positive.

Accordingly, data in Table 3 indicated that all tested isolates produced acid and did not produce gas from glucose, fructose, sucrose, lactose, maltose, manitol, dextrin and anhydrous dextrose.

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

Comparison between pathogenic isolates

Certain enzymes production

This experiment was conducted to study the variation of enzyme production, Peroxidase (PO), Polyphenoloxidase (PPO) and Tyrosine amonnia lyase (TAL) by three isolates: isolates Stc 11 and 10, the highly pathogenic isolates, compared with isolate No. 2 as the lowest pathogenic isolate.

Results of the current experiment are represented in Table 4 and indicated that isolate Stc 11 exhibited the highest activity of peroxidase enzyme, followed by isolate Stc 2, whereas isolates No. 10 exhibited the lowest activity.

Results also indicated that isolate Stc 10 exhibited the highest activity of polyphenoloxidase enzyme, followed by isolate Stc 11, whereas isolate Stc 2 showed the lowest activity of enzyme. Concerning TAL activity, isolate Stc 2 exhibited the highest activity followed by isolate Stc 10, whereas isolate Stc 11 exhibited the lowest activity.

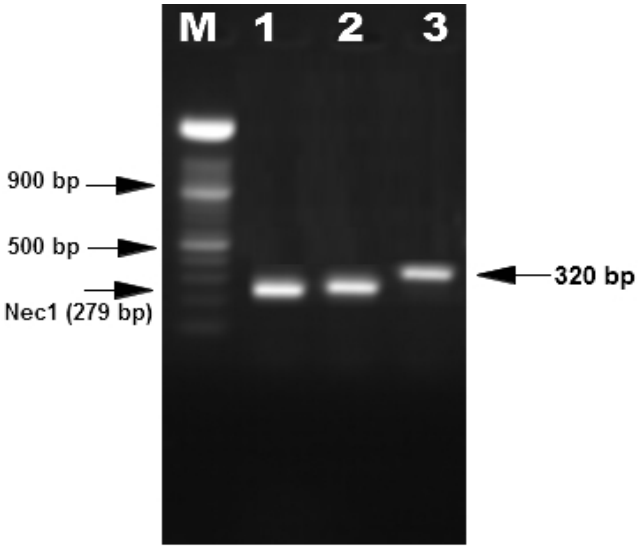

Fig. 1. Detection of *nec1* gene in *Streptomyces scabies* isolates by PCR. Lane M: DNA marker; 1: isolate No. (10); 2: isolate No. (11); 3: isolate No. (2) (using primer *NecNF1*; 279 bp)

Table 2. Morphological and physiological characteristics of the isolated pathogenic isolates of *Streptomyces scabies*

Tests	Isolates No. (Stc)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Shape of cell	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral
Motility	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sporulation	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Color of colonies	white	white	white	white	white	white	white	white	gray	white	white	white	white	white
Gram staining	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esculin Hydrolysis	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Growth 4°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth 40°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Levan production	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl-red test (MR)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenyl alanine deminase	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Casein hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: + = positive reaction; - = negative reaction

Table 3. Fermentation of carbon compounds by the isolated pathogenic isolates of *Streptomyces scabies*

Carbon compound		Isolates No. (Stc)													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Glucose	Acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Gas	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fructose	Acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Gas	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	Acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Gas	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactose	Acid	+	-	+	+	+	+	+	+	+	+	+	+	+	+
	Gas	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Maltose	Acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Gas	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Manitol	Acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Gas	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dextrin	Acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Gas	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anhydrous dextrose	Acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Gas	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: + = positive reaction; - = negative reaction

Table 4. Peroxidase, Polyphenoloxidase and Tyrosine amonnia lyase activity in three *S. scabies* isolates

Isolate (Stc)	Peroxidase activity (mg/g fw/min)	Polyphenoloxidase activity (mg/g fw/min)	Tyrosine amonnia lyase (TAL) (mg/g fw/min)
2	0.441 b	0.022 b	0.125 a
10	0.321 c	0.030 a	0.123 a
11	0.784 a	0.025 b	0.013 b
L.S.D	0.0195	0.0052	0.0091

Different letters indicate significant differences among treatments within the same column according to L.S.D (Least Significant Difference test 5%).

Pathogenic isolates comparison by PCR technique

Streptomyces scabies isolates Stc 10 and 11 which had been previously confirmed as highly pathogenic isolates, and isolate Stc 2 which had been previously confirmed as low-pathogenic one, were selected and checked by PCR method. Agarose gel electrophoresis of the PCR amplification products revealed a band representing the expected 279 bp DNA fragment in each DNA extracted from the highly pathogenic isolates Stc 10 and 11.

Discussion

Results reported herein indicated that the fourteen bacterial isolates obtained from naturally diseased potato tubers collected from different localities of Sohag governorate proved to be pathogenic and able to infect potato tubers, causing common scab symptoms. They were identified as *Streptomyces scabies*. These results are in agreement with those noted by several researchers who reported that *S. scabies* was responsible for common scab of potato (Lambert and Loria, 1989; Goyer and Beaulieu, 1997; Bencheikh and Setti, 2007; Manome *et al*., 2008).

The experiment was conducted to determine the role of certain enzymes in the virulence of *Streptomyces scabies* isolates. The activity of Peroxidase (PO), Polyphenoloxidase (PPO) and Tyrosine Amonnia Lyase (TAL) enzymes in the highest pathogenic isolates Stc (10 and 11) compared with the lowest disease index isolate Stc 2.

Results of this experiment indicated that the tested isolates varied in their production of the analyzed enzymes. Isolate Stc 11 exhibited the highest activity of peroxidase enzyme, followed by isolate Stc 2, whereas isolates Stc 10 exhibited the lowest activity. Also, isolate Stc 10 exhibited the highest activity of polyphenoloxidase enzyme, followed by isolate Stc 11, then isolate Stc 2. Finally, isolate Stc 2 exhibited the highest activity of TAL enzyme, followed by isolate Stc 10, whereas isolate Stc 11 exhibited the lowest activity. These results may indicate that there was no correlation between the activity of these enzymes and the severity of common scab disease attack. Such results are in disagreement with those reported by Shihata (1974) who mentioned that highly pathogenic isolates were less active in B-glucosidase, Polyphenoloxidase and Peroxidase enzymes, followed by moderately pathogenic isolates and finally weakly pathogenic isolates.

PCR techniques were developed in the current study for the specific detection of the virulence gene *nec1* of pathogenic *Streptomyces* isolates on potato tuber samples. This assay was based on the design of *Nec1* (279 bp) primer by using PCR assays which were able to distinguish pathogenic *Streptomyces* species *nec1* from low pathogenic ones.

Data hereby obtained demonstrated a clear correlation between pathogenicity of *Streptomyces* isolates and the detection of the *nec1* gene by conventional PCR. These results were similar with thise reported by Bukhalid *et al.* (2002). The low-pathogenic isolate Stc 2 of *Streptomyces* lacked the *nec1* gene.

The previous work carried out by Bukhalid *et al.* (2002) reported that the sequence of *nec1* was identical in all pathogenic strains, irrespective of their geographic origin, isolation host, or level of DNA relatedness to the *Streptomyces scabies* type strain, thus being a eliable marker for detecting pathogenic *Streptomyces* isolates on potato tubers. Such an assay could also have practical implications as a rapid substitute for pathogenicity tests involving the use of potato tubers (Schaad, 1988).

Conclusions

The current results demonstrated that PCR amplification of the *nec1* gene could be used as a reliable marker for detecting pathogenic *Streptomyces* isolates on potato tubers.

References

Bencheikh M, Setti B (2007). Characterization of *Streptomyces scabies* isolated from common scab lesions on potato tubers by morphological, biochemical and pathogenicity tests in Chlef region in western Algeria. Sciences & Technologie C 26:61-67

Bukhalid RA, Chung SY, Loria R (1998). *Nec1*, a gene conferring a necrogenic phenotype, is conserved in plant pathogenic *Streptomyces* spp, and linked to a transposase pseudogene. Molecular Plant-Microbe Interactions 11(10):960-967.

Bukhalid RA, Takeuchi T, Labeda D, Loria R (2002). Horizontal transfer of the plant virulence gene, *necl*, and flanking sequences among genetically distinct *Streptomyces* strains in the diastatochromogenes cluster. Applied and Environmental Microbiology 68(2):738-744.

Gomez KA, Gomez AA (1984). Statistical procedures for agricultural research, 2nd ed. John Willey, New York.

Goyer C, Beaulieu C (1997). Host range of *Streptomycete* strain causing common scab. Plant Disease 81(8):901-904.

Goyer C, Otrysko B, Beaulieu C (1996). Taxonomic studies on streptomycetes causing potato common scab: a review. Canadian Journal of Plant Pathology 18(2):107-113.

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994). Bergey's manual of determinative bacteriology, 9th Ed Williams and Wilkins, Baltimore, MD.

Hosny M, Abo-Elyousr KAM, Asran MR, Saead FA (2014). Chemical control of potato common scab disease under field conditions. Archive of Phytopathology and Plant Protection 47(18):2194-2200.

Kers JA, Cameron KD, Joshi MV, Bukhalid RA, Morello JE, Wach MJ, Gibson DM, Loria R (2005). A large, mobile pathogenicity island confers plant pathogenicity on *Streptomyces* species. Molecular Microbiology 55(4):1025-1033.

Kochba J, Lavee S, Spiege-Roy P (1977). Difference in peroxidase activity and isozymes in embryogenic and non-embryogenic 'sharr outi' orange ovular callus lines. Plant and Cell Physiology 18(2):463-467.

Krieg NR, Holt JG (1984). Bergey's manual of systematic bacteriology, Vol 1. Williams and Wilkins Company, Baltimore MD, USA.

Kritzman G, Grinstein A (1991). Formalin application against soil-borne *Streptomyces*. Phytoparasitica 19:248.

Kyndt JA, Meyer TE, Cusanovich, MA Beeumen JJ (2002). Characterization of a bacterial tyrosine ammonia lyase, a biosynthetic enzyme for the photoactive yellow protein. FEBS Letters 512(1-3):240-244.

Lambert DH, Loria R (1989). *Streptomyces scabies* sp. nov., nom. rev. International Journal of Systematic and Evolutionary Microbiology 39(4):387-392.

Lisker N, Cohen L, Chaluz E, Fuchs Y (1983). Fungi infection suppress ethylene-induced phenylalanine ammonia-lyase activity in grape fruit. Physiological Plant Pathology 22(33):331-338.

Manome A, Kageyama A, Kurata S, Yakomaku T, Koyama O, Kanagawa T, Tamaki H, Tagawa M, KamagataY (2008). Quantification of potato common scab pathogen in soil by quantitative competitive PCR with fluorescent quenching-based probes. Plant Pathology 57(5):887-896.

Park DH, Yu YM, Kim JS, Cho JM, Hur JH, Lim CK (2003). Characterization of *Streptomycetes* causing potato common scab in Korea. Plant Diseases 87(11):1290-1296.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, NY.

Schaad NW (1988). Laboratory guide for identification of plant pathogenic bacteria, 2nd Ed APS Press.

Schaad NW (1992). Laboratory guide for identification of plant pathogen bacteria, 2nd ed. International Book Distributing Co, Lucknow pp 44-58.

Shihata ZA (1974). Studies on common scab disease of potato. MSc Thesis, Faculty of Agriculture, Assiut University.