

# Disease severity and diversity among *Actinomyces* isolated from diseased potato

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**Abstract**— *Actinomyces* are soil habitant which among them *Streptomyces* are plant pathogens. They were isolated from potato scab lesion complex collected from crop growing area. Pathogenicity on mini-tuber and plants were done and they were tested for: the presence of *nec1* gene, diversity and differences in disease severity induction. Results indicated that most of the strains harbor *nec1* and they were belong to five groups similar to *S. scabies*, *S. acidiscabies*, *S. turgidiscabies*, *S. caviscabies* and *Streptomyces sp.* Severity disease induction trial showed significant differences with scale ranged from zero to 10. They induced raised or pitted lesion in three levels: strong, mild and weak. Diversity and disease severity spectrum could be due to introduction of mother potato tuber from different area and courtiers. Continuing of this strategy result emergence of new pathogenic strains as pathogenicity genes reside on a large mobile pathogenicity island in some potato pathogenic *Streptomyces* spp.

**Keywords**— *nec1*, Pathogenicity island, Raised symptoms, *Streptomyces*

## I. INTRODUCTION

**A**CTINOMYCETES are Gram positive bacteria which are abundant in soil, but only a small numbers belong to the genus *Streptomyces* including *S. scabies*, *S. turgidiscabies* and *S. acidiscabies* are the main causal agents of scab disease on potato, carrot, radish, beet, parsnip and turnip. They induce raised, netted, russet and deep or shallow-pitted lesions on abovementioned and some other plants and cause yield losses and diminish product marketability [4, 15 & 16]. Plant pathogenic *streptomyces* have been classified into a number of species based on DNA sequence relatedness, 16s ribosomal DNA sequence, and biochemical characteristics [1, 12 & 17]. The most widely described common scab pathogen is *Streptomyces scabies* which occurs worldwide. *S. scabies*, *S. europaescabiei* and *S. stelliscabiei*; have been isolated in France, the eastern United States and Canada, other species causing common scab include *S. acidiscabies*, from eastern North America, Japan, and Korea *S. turgidiscabies* from Japan, Korea, Finland, and Sweden *S. aureofaciens* from Finland and three new species from Korea [1, 2, 12, 15 & 17]. In *S. scabies* a single 425-kb restriction endonuclease

fragment of pathogenicity island (PAI) contains genes for biosynthesis of thaxtomin, tomatinase and a necrosis-inducing protein [2, 5, 6 & 11]. Grouping within the genus *Streptomyces* according to numerical using physiological tests classification revealed 15 major and 34 minor clusters as well as 40 single member clusters, have been defined at the 81.5% similarity level [3]. The present study was undertaken to identify the diversity and disease severity difference among the Iranian scab inducing *Streptomyces* strains in order to apply in disease management strategy in future.

## II. MATERIALS AND METHODS

Isolation and pathogenicity test of *Streptomyces* strains: Potato tubers that apparently showed symptoms of scab diseases were collected from the main potato growing areas in Iran. Affected potato samples were disinfected and tissue below a lesion was aseptically removed and transferred to water agar [1.2%] containing 50 mg of nystatin, 5 mg of polymixin B, and 50 mg of cycloheximide per liter and kept at 28°C for 8 days. Pathogenicity tests on potato mini tubers were done according to Loria *et al.* [11] and under green house condition according to Goyer and Beaulieu [4]. Physiological feature determination and PCR test For DNA extraction YEME medium containing 20% sucrose were used. *Nec1 F* [5'-AAGATGAGCGCGAACGGAAGCC -3'] and *Nec1 R* [5'-CCTACTTTCTCGTTATCCATATA -3'] primers was used for amplification of *nec1* gene [2 & 7].

Diversity and disease severity determination To investigate of diversity, phenotypic features of the *Streptomyces* strains were determined based on standard bacteriological methods and presence and pattern of linear plasmid by pulse field electrophoresis. For disease severity scaling potato tuber variety Agria dressed with bacterial suspension [ $10^7$  CFU] for 30 minutes and dried in room temperature for 12 h. Tubers were put in pasteurized soil and watering was done normally basically according to Goyer and Beaulieu and Domkarova *et al.*, [7 & 14]. Scoring was done based on the severity of induced symptoms. The experiment was done in a randomized design with three replicates and data analyzed by SAS software. Pathogenicity tests on potato whole and sliced mini tubers were done according to Loria *et al.* [11].

## III. RESULTS

Results of PCR experiments showed a DNA amplified band with nearly 700 bp which is absent in some strains [Fig 1.].

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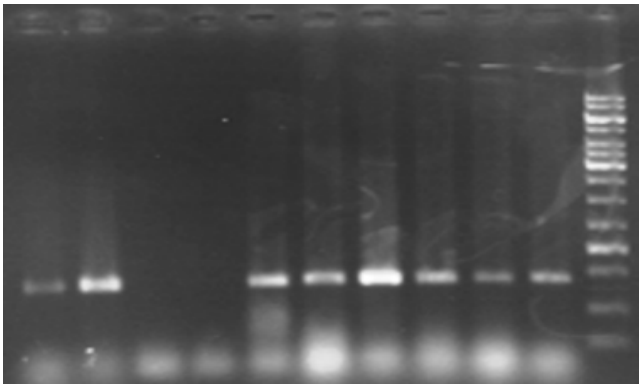


Fig. 1. A 700 bp PCR amplified band correspond to *nec 1* gene using Necf/NecR primers in *Streptomyces* pathogenic on potato.

Phenotypic features determination results summarized in table1.

TABLE I

SOME PHENOTYPIC FEATURES OF *STREPTOMYCES* STRAINS CAUSED AND ASSOCIATED WITH POTATO SCAB DISEASE.

Characteristics	Group 1	Group 2	Group 3	Group 4	Group 5
Spore Chain	Spiral	Flexuous	Flexuous	Straight	Straight
Colony color on YME	Gray	Yellow	Gray	white	Pale red
Tyrosinase	+	+	-	-	-
Utilization of:					
L-Arabinose	+	-	+	-	-
D-Fructose	+	+	+	-	+
D-Glucose	+	+	+	+	+
D-Mannitol	+	+	+	-	+
raffinose	+	+	-	+	-
rhanmose	+	-	-	-	-
sucrose	+	+	+	+	+
D-Xylose	+	-	-	-	-
i-Inositol	+	-	+	-	-
Growth pH	5	5	4.5	5	5.5
Growth in:					
5% NaCl	+	-	-	+	+
6% NaCl	+	-	-	-	+
7% NaCl	-	-	-	-	-
Thallium acetate 10 µg/ml	-	-	+	+	-
Thallium acetate 100 µg/ml	-	-	-	-	-
Crystal violet 0.5 µg/ml	-	-	+	+	+
Phenol 0.1%	-	-	+	+	+
Penicillin 10 µg/ml.	-	-	+	+	+
Streptomycin 20 µg/ml.	-	-	+	+	+

\*+ =positive reaction; - =negative reaction

Characterization of *Streptomyces* strains revealed five groups similar to *S. scabies*, *S. acidiscabies*, *S. turgidiscabies*, *S. caviscabies* and *Streptomyces* sp. respectively *S. scabies* is characterized by spiral gray spores, production of melanin and utilization of all tested sugars. Most pathogenic strains isolated from potato lesions belong to *S. scabies* (table I).

Disease severity induction showed significant differences among the tested strains (figures 2 & 3).



Fig.2. Severity of symptoms induced by *Streptomyces* strains. First column on the left side show healthy slice, mini-tuber and plants respectively

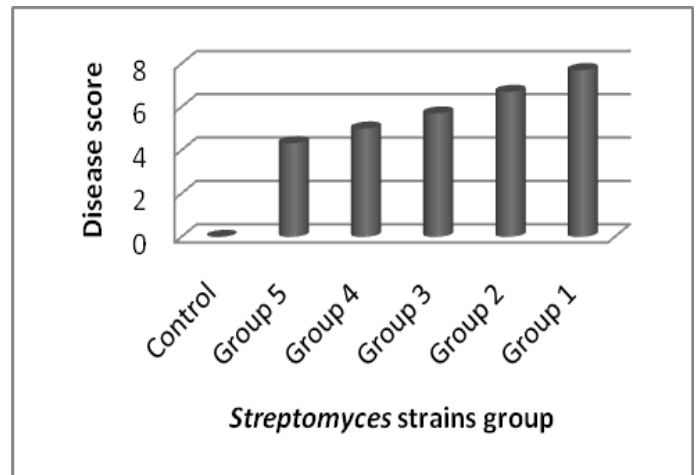


Fig. 3. Mean disease severity at harvest following inoculation of pathogenic *Streptomyces* strains group

Under natural and greenhouse condition potato pathogenic *Streptomyces* spp. induce different symptoms on potato tubers including raised netted russet and deep or shallow-pitted lesions.

#### IV. DISCUSSION

Until now more than 10 *Streptomyces* spp. have been described as the causal agents of potato scab complex disease from different geographical region worldwide. In the present study *Streptomyces* strains associated with potato scab lesion were isolated which they were five groups similar to *S. scabies*, *S. acidiscabies*, *S. turgidiscabies*, *S. caviscabies* and *Streptomyces* sp. Most of the common scab-causing strains were belong to *S. scabies* as found in other studies [13 &17] .

Present study and other studies [1 & 12] suggests that there may be differences in the population profiles of *Streptomyces* species causing common scab in different parts of the world. PCR experiment showed that most of the tested strains carry sequences related to *necl* gene although this gene was absent in some pathogenic strains as described by other studies [2 & 16]. In this study *Streptomyces* isolated from scabby potato plants, were diverse in morphology, *necl* related sequence and virulence. Strains of potato scab lesion associated *Streptomyces* differed in above-mentioned features were reported by other researchers [[22. Kinkel, L. L., Bowers, J. H., Shimizu, K., Neeno-Eckwall, E. C., and Schottel, J. L.1998. Quantitative relationships among thaxtomin A production, potato scab severity, and fatty acid composition in *Streptomyces*. Can. J. Microbiol. 44:768-776.. Leiner et al. [26] [26. Leiner, R. H., Fry, B. A., Carling, D. E., and Loria, R. 1996. Probable involvement of thaxtomin A in pathogenicity of *Streptomyces scabies* on seedlings. Phytopathology 86:709-713.] These finding suggested that pathogenicity factors or determinants in addition to thaxtomin may be involved in disease etiology caused by *Streptomyces*. Kinkel *et al.* noted that 60% of the variation in disease severity on tubers was attributable to thaxtomin levels, leaving 40% to be accounted for by other factors [8, 9 & 10]. Leiner *et al.* [10] showed data indicating that *Streptomyces* cultures produce more symptoms on seedlings than culture supernatants, suggesting that additional virulence or phytotoxicity components could be present. Tested *Streptomyces* strains induced a spectrum disease severity in green-house trial and showed significant differences. They induced typical lesions which were raised, or pitted by different levels. Introduction of new mother potato tuber from different geographical area and courtiers during the time maybe the reason for this diversity which encounter the disease management with notable problem.

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