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RAPD-PCR Technique and Protein (SDS-PAGE) Analysis for Differentiation of *Erwinia carotovora* subsp. *carotovora* and Relation to Virulence

Mohamed A. El-Sheikh

Dept. of Plant Pathology, Fac. Agric. Damanhour Univ..

Abstract:

Three isolates out of 18 isolates of *Erwinia carotovora* subsp. *carotovora* were isolated from diseased potato tubers showing soft rot symptoms, and chosen to assess their virulence and to differentiate them on the basis of RAPD-PCR technique and protein SDS-PAGE analysis. Nine cultivars of potato differed in their susceptibility to the three tested isolates of Ecc, which varied in their virulence on potato tubers of different cultivars where isolate A was the most virulent followed by isolate B whereas, isolate C was the least virulent one. Five primers i.e: Bic 1, A_9B_4, A_9A_{10} , BAQ and BAR were chosen based on cost consideration and their capacity to discriminate between the bacterial isolates. All the five primers used showed different levels of polymorphism and revealed clear differences among the three isolates of Ecc. Distinguish differences were detected in the number and molecular weights (M.W) of the extracted protein bands among more virulent and less virulent isolates of Ecc. On the basis of RAPD-PCR technique and SDS-PAGE analysis, our results indicated that there are two main groups. Group 1 include one sample represent isolate C, however, group 2 include two subgroups represent isolate A and B, that are similar at the DNA level.

Key words: RAPD-PCR, protein (SDS-PAGE), *Erwinia carotovora* subsp. *carotovora*, identification, virulence, potato.

Introduction:

Erwinia carotovora subsp. *carotovora* is the causal agent of bacterial soft rot, a severe and devastating disease. Many economically important food crops as potatoes, tomatoes and chinese cabbages can be affected by this disease. Soft rot Erwinias are facultative anaerobes, nonsporeforming, gram-negative enterobacteria that cause disease in a wide range of plants (Zhao *et al*., 2000, Lopez *et al*., 2001, Perombelon, 2002, and Agrios, 2005). *Erwinia carotovora* subsp. *carotovora* strains have a wide distribution in both temperate and tropical zones, showing wider host ranges than these of the other sub species (Perombelon and Kelman, 1980, Wells and Moline, 1991). The plant pathogenic species *Erwinia carotovora* is a complex toxin

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consisting of strains with a range of different phenotypic, biochemical, host range, and genetic characteristics. This species is well suited for studying the ecology, specialization, and pathogenicity of enterobacterial pathogens since it is wide spread in the environment, it can infect numerous plant species, and many of its virulence genes have been identified, including genes encoding degradative enzymes, diverse regulatory systems (Toth *et al*., 2003). Pectolytic soft rot bacteria are usually identified by biochemical tests. These tests are lenthy and costly. DNA analysis techniques are now widely used and could replace biochemical tests. Specific detections of *E. carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica* were obtained with DNA probes (Ward and De Boer, 1990 and 1994), but are also limited by time and cost consideration. Recently, the random amplified polymorphic DNA (RAPD) technique was developed for the genetic analysis of DNA (Wilsh and McClelland, 1990; Williams *et al*., 1990). The technique is based on the enzymatic amplification of non selected DNA fragments, initiated by arbitrarily chosen DNA primers. With this technique, individual genomes can be grouped and classified by screening primers and selecting those that generate adequate levels of DNA polymorphism. The RAPD technique is simple, fast, and inexpensive, and hence could be an alternative to biochemical identification of soft rot bacteria. Based on assay of protein profiles of *Bacillus anthracis* isolates by sodium-dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique, Poerwadikata (1998) found that, the molecular weights of the strains from different areas differed and ranged from 18 to 86 Kda. For intraspecific grouping of *Ralstonia solanacearum,* outer membrane protein for 23 strains isolated from Brazil were analysed by SDS-PAGE and the strains had proteins bands of approximately 30 and 42 Kda and these strains showed various polypeptide patterns associating with both original hosts and biovar (Melo and Furuya, 1998). In addition, El-Ariqi (2001) and El-Sheikh (2004) found that the visible differences in protein profiles of *R. solanacearum* were correlated with differences in virulence, but not in colony morphology. The objectives of this study were the identification of *Erwinia carotovora* subsp. *carotovora* from soft rot diseased plants by random amplified polymorphic DNA (RAPD) analysis and the correlation of the genomic diversity of the isolates and their virulence.

Materials and Methods

Isolation and Identification

Isolation trials were carried out from infected samples of potato tubers collected from fields in El-Behera and El-Sharkia governorates during 2008-2009 growing seasons. Diseased materials were washed thoroughly

with tap water, small portions of the inner tissues of rotted tubers were macerated in small amount of sterile water in test tube, loop-full of the resulting suspension was streaked on the surface of plates containing the nutrient agar medium and incubated for 48 h at 28°C. Bacteria were purified through single colony isolation technique. Identification was carried out according to Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984 and Garrity *et al.*, 2005). Cultural, morphological, physiological and biochemical characteristics of the bacteria were performed as recommended by Klement *et al.* 1990 and sensitivity, to erythromycin and indole production, sucrose reduction, acid production from methyl glucoside and growth at 36°C (Dickey and Kelman, 1988).

Pathogenicity tests:

Bacterial suspensions of the tested *E.c.* subsp. *carotovora* were prepared from 48 h old culture and adjusted to 10^8 cfu / ml as determined by turbidemeter at 560 nm Tubers (60 g in weight) of cvs. Nicola, Sponta, Draga, Valor, Anabell, Diamont, Hermis, Lady rosetta, and Korag were thoroughly washed and sterilized by flaming. Five tubers four each cultivar were taken and a hole was made in each tuber by a sterilized cork-borer (0.5 cm in diameter), and 0.5 ml of the standard bacterial suspension (10^8cfu/ml) was pipetted in. The hole was then covered with the removed cylinder and inoculated tubers were kept in sterilized polyethylene bags for 5 days at 28°C. Control tubers were prepared by using sterile water instead of the bacterial suspension. At the termination of the 5th day after inoculation, inoculated tubers as well as the control were cut at transverse direction and the diameters of rotted area were estimated in millimeters according to Hollis and Goss 1950. The mean diameter of rotted tissue produced by each isolate was considered a good indication for the rotting ability of isolates. Statistical analysis was made using SAS program (SAS Inc., 2000). Least significant differences (LSD) were used to separate mean differences and to rank isolates.

Extraction of genomic DNA from *Erwinia carotovora* subsp. *carotovora* isolates:

The bacterial genomic DNA was extracted using wizard Genomic DNA purification kit QIAGENE DNA purification kit (Germany). 1 ml of an overnight culture was added to 1.5 ml microcentrifuge tube. Centrifugation at 16,000 x g for 2 minutes was carried out to pellet the cells and the supernatant was removed. A 600µl of Nuclei Lysis Solution was added to the cell pellets and gently pipette until the cells are completely suspended. Incubate at 80°C for 5 minutes was carried out to lyse the cells: then was cooled at room temperature. 3 µl of RNase solution was added to the cell lysate then the tube was inverted

2-5 times to mix. Sample was incubated at 37°C for 15-60 minutes, then it was cooled to room temperature. 200 µl of Protein Precipitation Solution was added to the RNase-treated cell lysate. Then it vortex vigorously at high speed for 20 seconds to mix the protein. Precipitation Solution with the cell lysate. The sample was incubated on ice for 5 minutes. Sample was centrifuge at 13,000-16,000 x g for 3 minutes. Transfer the supernatant containing the DNA to a clean 1.5 ml microcentrifuge tube containing 600 µl of room temperature isopropanol. The supernatant containing the DNA was Transfered to a clean 1.5 ml microcentrifuge tube containing 600 µl of room temperature isopropanol. Gently sample was mixed by inversion until the thread-like stands of DNA form a visible mass. Sample was centrifuged at 13,000-16,000 x g for 2 minutes. Carefully the supernatant was pour off and the tube was drain on clean absorbent paper. Also 600 µl of 70 % ethanol was added on room temperature and gently the tube was inverted several times to wash the DNA pellet. Centrifuge at 13,000-16,000 x g for 2 minutes. Carefully ethanol was aspirate. Drain the tube on clean absorbent paper was carried out to dry the pellet on air for 15 minutes. 100 µl of DNA Rehydration Solution was added to the tube and DNA was rehydrate by incubating at 65°C for 1 hour the solution was periodically mixed by gently tapping the tube. Alternatively, DNA was rehydrate by incubating the solution overnight at room temperature or at 4 ^{o}C. DNA was stored at 2-8°C.

Random Amplified Polymorphic DNA (RAPD):

Five random primers, each consist of 20 bases were used to differentiate and finger print the isolates of *Erwinia carotovora* subsp. *carotovora* under study using genomic DNA sequences of all primers are illustrated in Table (1).

Table 1: List of primers name and their nucleotide sequences employed in the RAPD -PCR analysis.

NUMBER	NAME	SEQUENCE
a	**Bic 1**	**CAG CCC CCT CCA GCA CCC AC**
b	**A9B4**	**GGT GAC GCA GGG GTA ACG CC**
c	**A9A10**	**GGA CTG GAG GTG GAT CGC AG**
d	**BAQ**	**GGT CTT GAA GTC GAG CGC AG**
e	**BAR**	**CCA GGC AAT TTC ATC AAG CC**

For RAPD analysis, PCR amplification was carried out in total volume 25 µl containing 2.5 µl 10 x buffer, 2 µl 25 µl mM $MgCl_2$, 2 µl 2.5 µl mM dNTPs, 1 µl pmol primer, 1 µl 50 ng of bacterial genomic DNA and 0.2 µl (5 units/ µl) Taq DNA polymerase (Promega Germany). The following PCR programme was applied : initial denaturation at 95°C for 5 min, 40 cycles of

95ºC for 1 min annealing at 30ºC for 1 min. Finally, an exta final extension step at 72°C for 10 min (Istock *et al*., 2001). Two µl of loading dye was added prior to loading of 10 µl per gel solt. Electrophoresis was performed at 100 volt with 0.5 x TBE as running buffer in 1.5 % agarose / 0.5x TBE gels and then gel was stained in 0.5 µg/cm^3 (w/v) ethidium bromide solution and destained in deionized water. Finally the gell was visualized and photographed using gel documentation system. Presence and absence of RAPD bands produced from the use of five primers were scored visually from the resulting photographs. .

Dendogram construction base on the RAPD-PCR band patterns:-

Data were scored for computer analysis on the basis of the presence and absence of the amplified productes for each primer. A product present in a bacterial isolate was designated (1) and when absence it was designated (0) after excluding common bands. Pairwise comparison of bacterial isolates based on the presence or absence of unique and shared polymorphic products was used to generate similarity coefficients according to Jaccard (1980). were used to construct a dendogram by UPGMA (Unweighted pair-Group Method with the similarity coefficients Arithmatical Averages) using statistical program.

Protein (SDS-PAGE) analysis:

Preparation of sample for SDS-PAGE:

Growth of bacterial cells and protein extraction procedure:-

Cultures of three isolates of *E. c.* subsp. *carotovora* were grown for 48 h at 28°C on a rotary shaker (150 rpm) in 250 ml Erlenmeyor flasks containing 100 ml of fresh glycerol nutrient agar (GNA) medium broth. Cells were collected by centrifugation and suspended in ice-cold phosphate buffer saline. Cells were concentrated 10-fold by centrifugation, then transferred to a new appendorf tube and stored at 20ºC until used (El-Moflehi, 2001).

Electrophoresis stock solutions:

The stock solutions used for protein electrophoresis were as follows:

A. 30 % Acrylamide stock solution (kept in dark at 4 °C):

Acrylamide 29.0 g, Bis-acrylamide 1.0 g. Deionized distilled water up to 100 ml. Any insoluble materials were removed by filtration through Whatman filter paper No. 1.

B. Sodium dodecylsulphate (10 % w/v SDS):

Ten grams of sodium dodecylsulphate were dissolved in 100 ml deionized distilled water.

C. Ammonium persulphate solution (10 % w/v):

One gram of ammonium persulphate was dissolved in 10 ml deionized distilled water and kept at 4ºC. The solution unstable and must be made just before use.

D. Buffers :

I) Separating gel buffer (1.5 M Tris-HCl, pH 8.8, kept in dark at 4ºC) :

Tris-base (18.15 g) was dissolved in 50 ml deionized distilled water and adjusted to pH 8.8 using concentrated HCl. The final volume was made up to 100 ml.

II- Staking gel buffer (0.5 M tris-HCl, pH 6.8, kept in dark at 4ºC) :

Tris-base (6.05 g) was dissolved in 50 ml deionized distilled water and adjusted to pH 6.8 using concentrated HCl. The final volume was made up to 100 ml. **III- Electrophoresis buffer (pH 8.3 – 8.5) :** The tank buffer consists of 3 g Tris-base, 14.4 g glycine and 1 g sodium dodecylsulphate dissolved in 1000 ml deionized distilled water.

Separating and stacking gel preparation:

Vertical slab (16 x 18 cm) gel electrophoresis apparatus (Bio-Rad, USA) was used. All glass plates were washed with deionized distilled water, then surface sterilized with ethanol. Spacers of 1.5 mm were used. Gels were prepared according to the protocol of Laemmli (1970). **a) 12 % separating gel (30 ml solution):** 30 % Acrylamide stock solution 12 ml, 1.5 M Tris-HCl (pH 8.8) 7.5 ml, 10 % (w/v) SDS 0.3 ml, 10 % Ammonium persulphate solution 0.3 ml, Deionized distilled water 9.9 ml, TEMED (added last) 12 µl, Separation gel solution was instantly swirled, then poured simultaneously to a height of 1.5 cm below the bottom of the comb and left to polymerize for at least 30 min. Separating gels were overlaid with 1 ml of water which removed before the stacking gel solution was poured. **b) 5 % Stacking gel (10 ml solution):** 30 % Acrylamide stock solution 1.7 ml, 0.5 M Tris-HCl (pH 8.8) 1.25 ml, 10 % (w/v) SDS 0.1 ml, 10 % Ammonium persulphate solution 0.1 ml, Deionized distilled water 6.8 ml, TEMED (added last) 10 µl, Stacking gels solution was quickly poured over the separating gel, and combs were used. Gels were left to polymerize for 30 min, before running. .

Protein sample preparation:

Sodium dodecyle sulphate (SDS) was add to the sample at a rate of 4 mg SDS/l mg protection, mixed with 50 µl 2-mercaptoethanol, then boiled at 100ºC in water bath for 3-5 min.

Loading and running of the samples:

Twenty microlitters of each protein sample were loaded in the wells of the staking gel. The samples were recovered with electrode buffer. Few drops of bromophenol blue (4 mg/100 ml deionized water) were added to the electrode (tracking dye). Protein maker with molecular weight ranged from 14.4 to 116.6 KDa was used a standard. Electrophoresis was carried out at constant voltage of 100 V for 3 h in electrophoresis running buffer (pH 8.3-8.5).

Staining and distaining of the gel:

The gel was stained in 50 ml of staining solution (0.125 % coomassie blue R-250, 50 % methanol and 10 % acetic acid) and then the gel was distained in a destaining solution (20 % methanol, 10 % acetic acid and 70 % H_2O). The gel was placed between two sheets of cellophane membrane and dried on gel drier for 2 hr, and photographed by digital camera.

Results:

Isolation trials from potato tubers showing soft rot symptoms during the 2008-2009 growing summer seasons from different localities in Egypt yielded eighteen isolates of *E. carotovora* subsp. *carotovora*. Three isolates out of 18 isolates differed in their virulence were chosen in this study. Data in table (2) show the morphological and biochemical of the three isolates which were similar to these of *E. c.* subsp. *carotovora*. (Klement *et al*., 1990, and Dickey and Kelman, 1988).

Table 2: Morphological, and biochemical characteristics of the three isolates of*Erwinia carotovora c.* subsp. . *carotovora* obtained from rotted potato tubers.

Characteristics		Result
Gram staining		**-**
Shape		**Short rods**
Sporulation		**-**
Motility		+
Colony type on (GNA)		**Round, convex, mucoid with entire margins, grayish white**
An aerobic growth		+
Growth at 36 C^o		+
Maceration of potato slices		+
Catalase activity		+
Starch hydrolysis		**-**
O_2 requirements		**Facultative an aerobic**
Growth in 6 % NaCl		+
Sensitivity to erythromycin (15 µg/disk)		**-**
Gelatin liquefaction		+
Production of acid from		
	Arabinose	+
	Lactose	+
	Mannose	+
	Maltose	**-**
	Adonitol	**-**
	Sorbitol	+
	dextrin	**-**

Reaction of potato cultivars against the three isolates of *E. c.* subsp. *carotovora*:-

It is clear from data in table (3) that potato cultivars differed in their susceptibility to the tested isolates of *E. c.* subsp. *carotovora*. Diamont was less susceptible to infection as compared with other cultivars followed by Nicola, Anabell and Lady rossetta where the rotted area recorded were 8.2, 9.06, 9.2 and 9.4 mm in diameter respectively. On the contrary, Sponta was the most susceptible cultivar to the tested isolates of *E. c.* subsp. *carotovora*, followed by Draga and Hermis where the rotted area measured were 26.6, 22.5 and 18.2 mm in diameter respectively. In case of Korag and Valor, the rotted area was 11.8 and 14.4 mm in diameter. Consequently, the cv.s Diamont, Nicola, Anabell and Lady rossetta could be considered as relative resistant, while Sponta, Draga and Hermis are regarded as highly susceptible. Meantime, Korag and Valor were moderately in their concern.

Table 3: Reaction of tubers of nine potato cultivars artificially inoculated with three isolates of *Erwinia. carotovora.* subsp. *carotovora.*

Cultivars	Diameter of rotted are a (m.m)			
	Bacterial isolates			
	A	B	C	Mean
Nicola	12.5	11.2	3.5	9.06[e]
Sponta	32.0	29.6	18.2	26.6[a]
Draga	29.0	25.0	13.5	22.5[b]
Valor	24.03	15.2	4.0	14.4[d]
Anabell	13.0	11.0	3.6	9.2[e]
Diamont	12.0	10.0	2.6	8.2[e]
Hermis	27.0	22.0	5.6	18.2[c]
Lady rossetta	13.2	11.6	3.4	9.4[e]
Korag	17.5	13.5	4.4	11.8[de]
Mean	20.02[a]	16.56[a]	6.53[a]	14.37

- Data are average of 3 replicates, $LSD_{0.05}$ for bacterial isolates = 3.52, $LSD_{0.05}$ for cultivars = 2.03, $LSD_{0.05}$ for interaction = not significant, Diameter of the rotted area was measure, 5 days after inoculation
- Means followed by the same letter in the same column o row are not significantly different.

In addition, data presented in table (3) showed that the three isolates of *E. c.* subsp. *carotovora* differed in their virulence against potato cultivars where the isolate A was the most virulent followed by isolate B whereas the isolate C was the least virulent in this respect.

Differentiation of *E. c.* subsp. *carotovora* isolates using Random Amplified Polymorphic DNA (RAPD):-

Results revealed that all primers used showed different levels of polymorphism. Using of five primers in RAPD-PCR showed clear differences among the three isolates of *E. c.* subsp. *carotovora* (Table 4). On the basis of amplified product, band patterns observed with each primer (Fig 1) a, b, c, d and e. Primer Bic 1 showed 14 polymorphic bands between the three isolates of *E. c.* subsp. *carotovora*, expect 3 bands were found in all isolates at 1000 bp, 850 bp and 110 bp. In case of primer A9B4, polymorphic profiles were detected among all isolates and the lowest number of bands was found in isolate B where one band was detected at 900 bp. There are 13 polymorphic bands between the three isolates except two bands were found in all isolates at 150 bp and 500 bp in case of primer A9A10. In addition, primer BAQ exhibited polymorphic patterns among all isolates except one band at 900 bp was observed in all isolates. However, it could be observed that the primer BAR amplified the lowest number of bands compared with other primers. The phylogenetic trees generated from RAPD-PCR of three samples represent three isolates of *E. c.* subsp. *carotovora* indicated that there are two mains group. Group 1 include one sample represent isolate c, however, group 2 include two subgroups represent isolate A and B of Ecc. Fig (2). Results obtained revealed that the primer A_9B_4, BAQ, andA_9A_{10} were more efficient to detect the degree of polymorphism and differentiate between the isolates of Ecc.

Protein (SDS-PAGE) analysis of *E. c.* subsp. *carotovora* isolates:

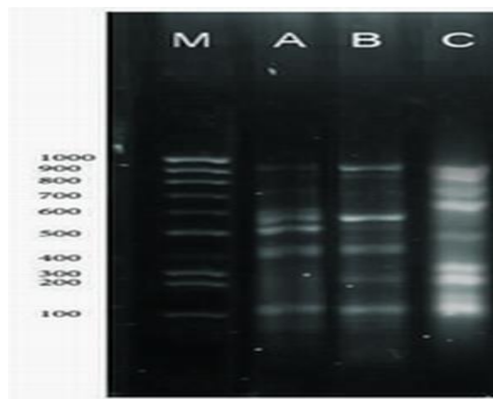
This study showed the differences in buffer soluble protein extracted from the three isolates of *E. c.* subsp. *carotovora* (Fig 3). Results showed distinguish differences in the number and size of extracted protein bands among the three isolates. The obtained data cleared that there are ten polymorphic soluble protein bands of M.W (120, 100, 70, 60, 50, 45, 40, 24, 20 and 19 KDa), however, four monomorphic bands were found in all three isolates at M.W (10, 15, 17 and 25 KDa). The phylogenetic tree generated from (SDS-PAGE) of the three isolates of *E. c.* subsp. *carotovora* revealed that there are two main group, group 1 include one isolate C and the group two include two subgroup represent isolate A and B. The similarity between isolate A and B was over 71 %, however, the similarity between the isolate C and the two other isolates was only 43 % with isolate A and 36 % with isolate B. consequently, these results indicate that isolate A and B are similar at the DNA level. (Table 5).

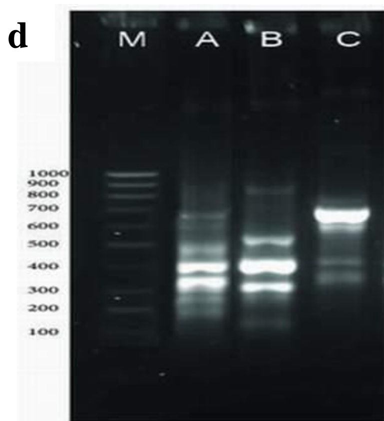
Table (4): Degree of polymorphism between the isolates of E.c subsp. carotovora for each primer.

Primers	No. of monomorphic bands	No. of polymorphic bands	Total bands	Percent
BIC 1	3	14	17	82.35
A_9B_4	-	17	17	100
A_9B_{10}	2	13	15	86.7
BAQ	1	15	16	93.8
BAR	4	1	5	20

Table (5): Percent of similarity between *E. c*. subsp. *carotovora* isolates by (SDS - Page) analysis.

Isolates	**A**	**B**	**C**
A	**100**	**071**	**043**
B		**100**	**036**
C			**100**





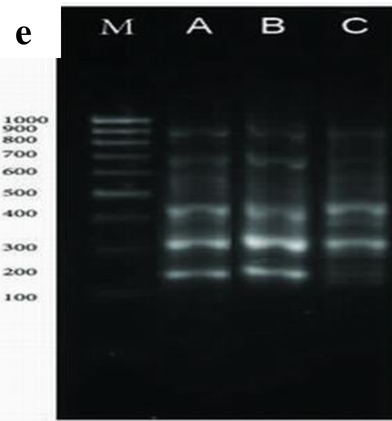


Figure (1): RAPD-PCR using primers (a: Bic_1, b: A_9B_4, c: A_9A_{10}, d: BAQ, e: BAR) M: DNA marker. Lanes A, B and C are *E. c*. sub sp *carotovora* isolates.

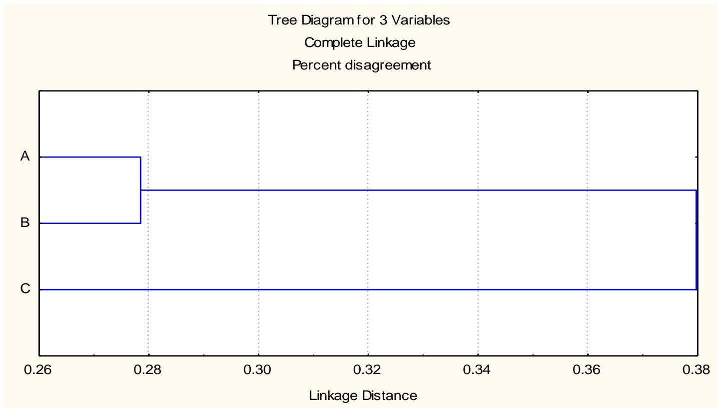


Figure (2) : Dendogram obtained by clustering (UPGMA method) based on the band pattern obtained by the RAPD-PCR analysis of three isolates of *E. c.* sub sp *carotovora*

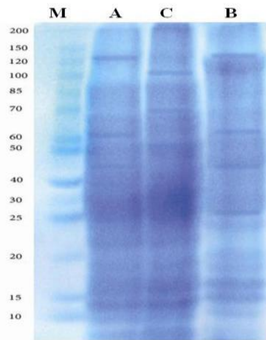


Figure (3): Protein (SDS-PAGE) analysis of the three isolates of *E. c*. sub sp *carotovora*. M: Protein marker. Lanes A, C and B are *E. c. c.* isolates.

Discussion:

On the basis of the results obtained from the pathogenicity tests, it could be concluded that Diamont, Nicola, Anabell, and Lady rossetta may be considered as relative resistance, while Sponta, Draga and Hermis are highly susceptible. On the other hand, Korag and Valor are moderately in this concern. This results agree with those obtained by Gomah and Mahmoud (2007). Data presented also showed that the three isolates of *Erwinia carotovora*. sub sp. *carotovora* differed in their virulence on potato tubers of different cultivars, where isolate A was the most virulent followed by isolate B whereas the isolate C was least virulent in this respect. Concerning the RAPD-PCR technique, all primers used showed different levels of polymorphism and revealed clear differences among the three isolates of *E. c*. subsp. *carotovora* that differed in their virulence. Our results are in harmony with the findings of Parent

et al., (1990) who distinguished between *Erwinia carotovora* and pectinolytic pseudomonas by RAPD-PCR and with Valkama and Karjalainen, (1994) who found that RAPD markers is a convenient method for identification of *E. c.* subsp. *carotovora* at species levels and differentiated between the bacterial strains in virulence on the same host. Our results also, in agreement with Coplin *et al*., (2002) who found that the RAPD-PCR analysis cleared the diversity between *E .c.* subsp. *carotovora* and the high virulent isolates had more bands than the low virulent ones. Data obtained from pathogenicity tests were in harmony with those observed from RAPD-PCR and (SDS-PAGE) analysis where indicated that there are two main groups. Group 1 represent isolate C, the least virulent, however, group 2 represent isolate A and B, which were more virulent. In addition, our results are in harmony with Gabriel *et al*., (1998) who showed that sensitivity of RAPD markers could reveal the genetic variation among the pathogenic *E. c.* subsp. *carotovora* and degree of polymorphism among the isolates could be a good tool to differentiate the degree of virulence in Ecc strains. Additionally, we are in accordance with (Valkama and Karjalainen 1994) where they found extensive genetic diversity within the Ecc isolates, and with (S. T. Seo *et al*., 2002), where they found the genetic analysis revealed striking differences among strains of Ecc from different areas and similarities between isolates from the same area. We have evaluated pathogenic and genetic diversity of Ecc strains from different localities in Egypt as an initial step in understanding the structure of pathogen population, where the isolates A and B isolated from the same area, however the isolate C recovered from another one. We don't know the reason for this difference, but these results may provide a clue about the route of distribution of Ecc strains. Our results also are in harmony with those obtained by (Mee-Ngan *et al*., 2004) that revealed considerable genomic diversity among the strains of Ecc isolated from the same host in the same season. Sensitivity of RAPD markers to reveal genetic variation among Ecc strains suggests its suitability for epidemiological analysis in this group. On the basis of the results obtained from protein (SDS-PAGE) analysis, it could be detected that there are differences in protein profiles and molecular weights (M.W) among the three isolates of Ecc. It is evident from such results that ten polymorphic soluble protein bands of different molecular weights were detected, however four monomorphic bands were found in the all three isolates of Ecc. The results obtained from RAPD-PCR were in accordance with that revealed by protein (SDS-PAGE) analysis. Consequently, the application of protein (SDS- PAGE) analysis and RAPD PCR techniques may aid

to detect and differentiate between the Ecc isolates and introducing the best procedures for evaluating the spreading of the pathogen. This research represents an important step towards understanding the evolution and differentiation of Ecc isolates. However, more extensive collection of isolates will be needed to study the pattern of genetic polymorphism of the Ecc group.

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استخدام تقنية RAPD-PCR وفصل البروتينات الذائبة بطريقة (SDS-PAGE) analysis للتفرقة بين عزلات البكتيرة ايروينيا كاروتوفورا كاروتوفورا وعلاقتها بالقدرة الامراضية

محمد احمد الشيخ
قسم امراض النبات – كلية الزراعة بدمنهور – جامعة دمنهور

تم عزل ثماني عشرة عزلة من البكتيرة ايروينيا كاروتوفورا كاروتوفورا من مناطق مختلفة، تم اختيار ثلاث عزلات منها تختلف فى قدرتها الامراضية على درنات اصناف مختلفة من البطاطس وهى نيقولا، سبونتا، دراجا، فالور، انابل، دايمونت، هيرمس، ليدى روزيتا، كوراج وذلك لدراسة إمكانية التفرقة بين عزلات البكتيرة ايروينيا كاروتوفورا عن طريق فصل البروتينات الذائبة باستخدام طريقة (SDS-PAGE) analysis وكذلك تقنية RAPD-PCR وعلاقة ذلك بالقدرة الامراضية للعزلات البكتيرية. واوضحت النتائج ان الثلاث عزلات تختلف فى هذا الصدد حيث ان العزلة A هى اقوى العزلات يليها العزلة B ثم العزلة C وان اصناف دايمونت، نيقولا، انابل، ليدى روزيتا كانت اقل قابلية للاصابة على التوالي وان اصناف سبونتا، دراجا، هرمس هى الاكثر قابلية للاصابة على التوالى بينما كانت الاصناف كوراج، فالور متوسطة القابلية للاصابة .

وقد اثبتت النتائج المتحصل عليها وجود فروق بين العزلات الثلاث فى عدد الاشرطة البروتينية (الحزم البروتينية) ووزنها الجزيئى حيث وجد 10 حزم بروتينية polymorphic ذات اوزان جزيئية 120، 100، 70، 60، 50، 45، 40، 24، 20، 19 KDa بينما وجدت اربع اشرطة بروتينية monomorphic متماثلة فى العزلات الثلاث ذات اوزان جزيئية 10 ، 15 ، 17 ، 25 KDa. كما اظهرت الدراسة وجود اختلافات فى عدد الاشرطة البروتينية الناتجة وكذا اوزانها الجزيئية بين الثلاث عزلات عند استخدام تقنية RAPD-PCR حيث وجد ثلاث عشرة حزمة بروتينية مميزة بين الثلاث عزلات. كما انه بدراسة شجرة النسب الوراثية للثلاث عزلات وجد ان درجة التماثل بين العزلتين A ، B كانت 71% بينما العزلة C كانت درجة التماثل بينها وبين كل من العزلتين A ، B 43% ، 36% على التوالي . كما ان دراسة شجرة النسب الوراثية وضعت الثلاث عزلات فى عنقودين اساسيين هما A ، B فى عنقود والعزلة C فى عنقود آخر مما يشير الى وجود قرابة وتشابة بين العزلتين A ، B على مستوى الـ DNA ويشير الى علاقة ذلك بالقدرة الامراضية.