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HOST RANGE AND TOTAL CELLULAR PROTEIN FINGERPRINT OF SOFT ROT *ERWINIA* ISOLATED FROM SOME VEGETABLES IN EGYPT

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Abstract

The aim of work was to isolate the soft rot pathogens from some vegetables, showing naturally soft rot symptoms, to study the differences in their host range and total cellular protein fingerprint. Seventeen of soft rot erwiniae isolates and identified according to pathological, morphological, cultural and biochemical characters as follows; fifteen of *Erwinia carotovora* subsp. *carotovora* isolates and two of *Erwinia chrysanthemi*. The soft rot erwiniae isolates differed in their pathogenicity and host range. The soft rot erwiniae also could utilize of carbon sources showing variable in acid and/or gas producing. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis of total cellular proteins of bacterial isolates revealed the different protein fingerprints .The obtained of protein fingerprints were distinguished into 15-17 discrete protein bands with molecular weight ranged from 253.287 to 14.594 KDa. The pair-wise similarity matrix based on DICE coefficient among protein fingerprints was in the range of 0.86 to 1.00 as well as the tree of dendrogram can be classified into two main groups includes many of subgroups.

Key words : Soft rot *Erwinia*, Host range, Protein fingerprints, Vegetables.

Introduction

Soft rot disease is one of the destructive diseases of vegetables and occurs worldwide as well as in Egypt. The soft rot *Erwinia viz*. *Erwinia carotovora* subsp. *carotovora* and *Erwinia chrysanthemi* were the common bacterial pathogen that infected freshly storage tissues of vegetables, than any other bacterial diseases. The soft rot *Erwinia* can be found on crops in the field, in transit, in storage and during marketing, where the bacteria enter the host tissue through injuries and by increase amounts of their pecteolytic enzymes which release resulted maceration of plant tissue (Bhat *et al*., 2010b; Opara and Asuquo, 2016). Soft rot *Erwinia* isolates from diseased vegetable samples of potato, tomato, carrot, chilies, and bell pepper using nutrient agar and identified as *Erwinia carotovora* subsp. *carotovora* through biochemical tests and their pathogenicity tests (Akbar *et al*., 2015). The soft rot *Erwinia* has a very wide host range of many vegetable species belonging to all families such as squash, eggplant, potato tubers, onion bulbs, garlic cloves, radish roots, carrot, sweet potato, rape, tomato, pepper, cauliflower, cabbage and cucumber (Bhat *et al*., 2010a; Ismail *et al*., 2012; Czajkowski *et al*., 2015; Himel *et al*., 2016). *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* was isolated from soften diseased potato tubers in Yugoslavia, where the isolated strains possess a wide host range besides on pathogenic characteristics (Obradovic and Arsenijevic, 1997).

The electophoretic of total bacterial cellular protein using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) is rapid method can be used to detect the differences among bacterial species (Laemmli, 1970; Euteneuer and Loos, 1985; Botha and Jooste, 1992; Millership, 1993). This method was applied for analysis and comparing *Xanthomanas campesteris* pv. *campestris* (Thaveechai and Schaad, 1986); *Erwinia chrysanthemi* strains (Uesugi *et al*., 1990b); phytopathogenic *Pseudomonas* and *Xanthomonas* species & pathovars (Van Zyl and Syteyn, 1990) and for

quick identification technique of *Pseudomonas* and *Erwinia* species (Lacroix *et al*., 1995). The protein fingerprints of *E. carotovora* subsp. *carotovora* and other *E. carotovora* strains had high similarity with one another, where the similarity ranged from 46 to 78% (Abd El-Khair, 2004). The proteins-SDS-PAGE analysis was applied to detect the differences between *E. carotovora* subsp. *carotovora* or *atroseptica* isolates (Khalil *et al*., 2014). The SDS-PAGE protein patterns of *E. carotovora* subsp. *carotovora* revealed that the 60.2 KDa peptide band was detected in all isolates (Uesugi *et al.*, 1990a) as well as the protein profiles had a unique peptide band with a molecular mass of approximately 28 KDa (Seo *et al*., 2004).

Therefore, this work is aimed to detect the common soft rot *Erwinia*, which infected some vegetables and study their differences in biochemical characters, host range and protein fingerprints of SDS-PAGE of total cellar proteins.

Materials and Methods

Sampling and isolation

Eight vegetables *viz*. potato tubers (*Solanum tuberosum* L.), sweet potato roots (*Ipomoea batatas* L.), cucumber fruits (*Cucumis sativus* L.), carrot roots (*Daucus carotovora*), eggplant fruits (*Solanum melongena* L.), chilli fruits (*Capsicum frutescens*), red sweet pepper fruits (*Capsicum annuum* L.) and tomato fruits (*Solanum lycopersicum* L.), showing natural soft rot symptoms, were collected from marketing and storage locations in Giza and Beni Suef Governorates, Egypt during 2014 season. All vegetable samples were kept in paper bags in ice box and then directly transported to the laboratory of Plant Pathology Department, National Research Centre. Isolation procedures of soft rot *Erwinia* were done according to standard bacteriological methods on nutrient glucose (2%) agar medium (3g beef extract, 5g peptone, 20g glucose and l liter distilled water, pH 7.2) (Ali *et al*., 2014).

Identification of soft rot *Erwinia* isolates

After performing Cokh's postulates, all pathogenic soft rot *Erwinia* isolates produced soft rot symptoms on plant species parts were selected to subsequent identification tests. The identification of *E. carotovora* subsp. *carotovora* isolates (Ecc_1-Ecc_{15}) as well as *E. chrysanthemi* isolates (Ech_1 & Ech_2) was done according to standard bacteriological methods of morphological, cultural and biochemical characters (Lelliott and Stead, 1987; Goszczynska *et al*., 2000).

Utilization of carbon sources by soft rot *Erwinia* isolates was carried out by basal synthetic medium (0.2g KCl; 0.2g $MgSO_4.7H_2O$; 1.0g $(NH_4)2HPO_4$ and 1 liter distilled water, adjusted the pH to 7.0). The carbon sources, *i.e*. arabnose, dextrose, fructose, lactose, maltose, mannitol, mannose, rhamnose, slaicin and xylose were incorporated as 1% in medium, except starch was added at 0.2 % of the liquid medium, together with bromothymol blue as indicator for acid production and Durham's fermentation tube indicator for gas production. Quadruplicated cultures were incubated at 30°C±2 for 7 days (Goszczynska *et al*., 2000).

Pathogenicity and host range of soft rot *Erwinia* isolates

The pathogenicity of 17 soft rot *Erwinia* isolates (fifteen *E. carotovora* subsp. *carotovora viz*. Ecc_1, Ecc_2, Ecc_3, Ecc_4, Ecc_5, Ecc_6, Ecc_7, Ecc_8, Ecc_9, Ecc_{10}, Ecc_{11}, Ecc_{12}, Ecc_{13}, Ecc_{14} & Ecc_{15} as well as two *E. chrysanthemi viz*. Ech_1 & Ech_2 was carried out on the same isolation host, while the host range was made using different isolation hosts. In all pathogenicity tests, re-isolation of bacterial pathogen was carried out.

Preparation of bacterial suspension

Each bacterial suspension, 17 soft rot *Erwinia* isolates was separately prepared by growing each bacterial isolate on Nutrient glucose (2%) agar medium in slant tubes. After incubation at 30°C±2 for 48h, the bacterial suspension for each bacterium was harvested by scraping the bacterial growth in 5ml of 0.2M sterile phosphate buffer (pH 7.2). Then, the bacterial inoculums was adjusted to a standard inoculums density at 10^9 colony forming unit (CFU)/ml by measuring the turbidity by using Unic Spectrophotometer UV-2000 at 610 nm and then the bacterial inoculums was kept at cool conditions until used (within 12h) (Bdliya and Dahiru, 2006).

Pathogenicity and host range procedures

The pathogenicty of isolated soft rot *Erwinia* was carried out on the same isolation host, while the host range was made using the different isolation hosts. Healthy potato tubers, sweet potato roots, cucumber fruits, carrot roots, eggplant fruits, chilli fruits, red sweet pepper fruits and tomato fruits was firstly surface sterilized by flaming. Then, each plant material was cut into slices (1 cm thick) by sterile knife under sterilized conditions. One slice of each plant material was separately put on sterilized filter paper in Petri dish containing 5ml of sterile distilled water. Each plant slice was separately inoculated with 0.1 ml of bacterial suspensions. For control, the plant slices were inoculated with phosphate buffer as well as distilled water. Five plant slices were used as replicates for each

treatment as well as the controls. All inoculated plant slices were incubated at 30±2°C. The soft rot symptoms were recorded after 3 days of incubation according to the disease scale described by Bartz (1999) as follows:- No rotting; + Arrested rot < 1cm; ++ Small active rot 1-< 2cm and +++ Large active rot > 2cm of soft rot diameter.

SDS-PAGE analysis

Total cellular protein preparation of *Erwinia* isolates

The total cellular proteins of *E. carotovora* subsp. *carotovora* (Ecc_1-Ecc_{15}) and *E. chrysanthemi* (Ech_1 & Ech_2) isolates were analyzed by SDS-PAGA (Laemmli 1970). Each bacterial isolate separately was grown in Luria broth medium (10g casein hydrolysate, 5g sodium chloride, 5g yeast extract, 1 litre of distilled water and adjusted to pH 7.0) for 24h at 30°C (Kado and Liu, 1981) for 48h at 30±2°C with shaking. The bacterial cells (about 10 ml of each bacterial culture) were harvested by centrifugation in cap tubes at 4500 rpm for 20 min at 4°C. By centrifugation at 13000 rpm for 2 min, the bacterial pellets were washed twice with sterile distilled water, and then the pellets were washed twice with 1000 µl of Tris -HCl - NaCl - Triton (50mM, 1M, 0.05% and pH7.5) buffer. The bacterial cell pellets were sonicated by Gallenkamp Ultrasonic Instrument at 24 amplitude microns, for about 2min (4x 30 sec.) (Bollag and Edelstein, 1992). The amount of total cellular protein in each bacterial suspension was determined spectrophotometerically at 550nm using bovine serum albumin as standard protein (Lowery *et al*., 1951). Soluble proteins of bacterial isolate were denatured by heating in presence of low molecular weight thio (2-mercaptoethanol) and sodium dodecyl sulphate. The soluble bacterial proteins was mixed with 150-200 µl of Laemmli buffer (Tris-HCl pH 6.8, 0.188 M; 2-mercaptoethanol .5% W/W; Glycerol, 10% W/W; SDS, 10% W/V and Bromophenol blue, 0.01 V/V of distilled water]. The bacterial suspensions with Laemmli buffer were incubated in water bath at 100°C for 2 min. Then, the suspension was quickly transferred to ice water and kept until loading in the gel.

Separating and stacking gel preparation

The gels were prepared form monomer solution of 30% acrylamide and 0.8% N-N-bis-methylene-acrylamide. One hundred ml of the denatured separating gel 15% (40.9 ml of acrylamide stock 30%; 25.0ml 1.5M Tris-HCL, pH 6.8; 100.0 µl TEMED; 500.0 µl SDS 10%; 1.0ml ammonium persulphate 10% and completed to 100ml with Distilled water was prepared and then the solution was immediately added to the gel unit and was left for at least one hour for polymerization. Fifty ml of the stacking gel 4% (6.5 ml of acrylamide solution 30%; 12.5 ml 0.5M Tris-HCl buffer, pH 6.8; 50.0 µl TEMED; 0.5 ml ammonium persulphate 10% completed to 50ml with distilled water) was prepared. The solution mixture was immediately added over resolving gel and the comb was placed at the same moment. The slab was left until polymerization of the staking gel was completed.

Loading of samples and running conditions

The comb was removed from polymerized gel. About 40 µl of denatured broken bacterial total cellular proteins as well as were put in each well using loading tip. The wide molecular weight protein marker (Sigma Co.) was loaded into the same gel. Then, the lid was placed on the unit and the electrophoresis run in the anode direction at 50 volt at 4ºC for 24 hours. After the completion of the run, the power supply was turned off. Then, the gel was in 100 ml of Coomassie Brilliant BlueR-250 solution in plastic overnight on a slow shaker. Then, the gel was rinsed in 100ml de-staining solution (140 ml methanol, 40ml glacial acetic acid and 520 ml distilled water) on shaker. Agitation was repeated three times with changing the de-staining solution until the protein bands became clear.

Then, data gels were photographed and the protein bands scanned using Gel-Pro Analyzer V.3 Package. The phonogram cluster among different isolates was determined by SPSS Programme. The pair-wise similarity matrix based on matching co-migrating band protein position between pairs of protein fingerprints of different soft rot isolates were calculated according to Dice (1945):

Similarity Dice (SD) =2a/ (2a-u) =2a/ (n1 + n2)

Where,

a = the number of common protein bands a pair of profiles.

u = the number of unmatched bands between each pair.

n_1 and n_2 = the total number of protein bands in the first and second profiles, respectively.

Results

Soft rot pathogens isolation

Isolation results showed that seventeen soft rot *Erwinia* isolates were isolated from vegetables showing naturally soft rot symptoms as follows: fifteen *E. carotovora* subsp. *carotovora* isolates *viz*. Ecc_1, Ecc_2, Ecc_3 & Ecc_4 (potato tubers); Ecc_5 (sweet potato roots); Ecc_6, Ecc_7 & Ecc_8 (cucumber fruits); Ecc_9, Ecc_{10} & Ecc_{11} (carrot roots); Ecc_{12} & Ecc_{13} (eggplant fruits) and

Table 1 : Pathological, cultural and biochemical characters *Erwinia carotovora* subsp. *carotovora* (Ecc_1 to Ecc_{15}) and *Erwinia chrysanthemi* (Ech_1 & Ech_2).

Tests	Reactions[1]	
	E. carotovora* subsp. *carotovra	***E. chrysanthemi***
Growth on NA medium	+	+
Soft rot on potato slice	+	+
Gram staining	G-	G-
Short rot shape	+	+
Yellow colonies on YDC	-	-
Fluorescent pigment on King's B	-	-
Orange colonies on M&M	-	-
Growth on H.S 40%	-	-
Pits formation on CVP	+	+
Anaerobic growth	+	+
Growth at NaCl solution 5% 7%	+ +	+ +
Growth at 37ºC	+	+
Sensitivity to Erythromycin	-	+
Gelatin lequification	+	+
Arginine dihydrolase	+	+
Levan production	-	-
Starch hydrolysis	+	+
H_2S production	+	+

[1] + positive reaction, – negative reaction.

Ecc_{14} & Ecc_{15} (chilli fruits) as well as two *E. chrysanthemi* isolates *viz*. Ech_1 (red sweet pepper fruits) and Ech_2 (tomato fruits). All soft rot *Erwinia* isolates were short rods, Gram-negative, motile, non-spore forming and non capsulated. The cultural characters of isolated bacteria colonies were circular, convex, entire, smooth, translucent, botryose and creamy white on nutrient glucose (2%) agar medium after 48h (table 1).

Biochemical and physiological characters of soft rot *Erwinia* isolates

All soft rot *Erwinia* isolates could grow on selective media producing specific characters as shown in table 1. The bacterial isolates grew under anaerobic conditions. The bacterial isolates were able to liquefy gelatin after three days of incubation, except *E. carotovora* subsp. *carotovora* (Ecc_8 & Ecc_{11}) and *E. chrysanthemi* (Ech_2) which liquefy gelatin after 14, 21 and 25 days of incubation, respectively. The soft rot *Erwinia* isolates showed different degrees of tolerance to NaCl solution. The most bacterial isolates could grow on NaCl solution at 5% concentration, while isolates *E. carotovora* subsp. *carotovora* (Ecc_7 & Ecc_{14}) and *E. chrysanthemi* (Ech_2) produced weakly grow and *E. carotovora* subsp. *carotovora* (Ecc_{15}) isolate couldn't grow. At 7% concentration, *E. chrysanthemi* (Ech_2) weakly grew, *E. carotovora* subsp. *carotovora* (Ecc_4, Ecc_7, Ecc_{12}, Ecc_{13}, Ecc_{14} & Ecc_{10}) couldn't grow, while other soft rot *Erwinia* isolates could grow well. Results revealed that negative reaction to erythromycin sensitivity was recorded with *E. carotovora* subsp. *carotovora* isolates, while the positive reaction was obtained with *E. chrysanthemi* isolates. Details of biochemical reactions of soft rot *Erwinia* isolates are listed in table 1.

The soft rot *Erwinia* isolates could utilize the carbon sources *viz*. arabnose, dextrose, fructose, lactose, maltose, mannitol, mannose, rhamnose, slaicin, starch, xylose, gylecrol and sucrose producing variable reaction for acid only and/or acid & gas production. Details of action carbon utilization by soft rot *Erwinia* isolates are listed in table 2. *E. carotovora* subsp. *carotovora* isolates showed different reaction for their utilization of carbon sources. *E. carotovora* subsp. *carotovora* (Ecc_1, Ecc_2, Ecc_3 & Ecc_8) and isolates (Ecc_3, Ecc_6, Ecc_8 & Ecc_{15}) could utilize both arabnose and dextrose producing acid only after 24h of incubation. *E. carotovora* subsp. *carotovora* (Ecc_1, Ecc_2, Ecc_3, Ecc_6, Ecc_8 & Ecc_{15}) and isolates Ecc_1, Ecc_2, Ecc_3, Ecc_8 & Ecc_{15} could utilize maltose mannitol produced acid only after 24h of incubation two *E. carotovora* subsp. *carotovora* (Ecc_6 & Ecc_{13}) and isolates (Ecc_3 & Ecc_8) could utilize mannose and rhamnose producing acid only after 24h. *E. carotovora* subsp. *carotovora* (Ecc_3, Ecc_8 & Ecc_{15}) and *E. chrysanthemi* (Ech_1) colud utilize slaicin producing acid only after 24h. *E. carotovora* subsp. *carotovora* (Ecc_3, Ecc_4, Ecc_8, Ecc_{13} and Ecc_{14} & Ecc_{15}) could utilize sucrose producing acid only after 24h.

Pathogenicity test of *Erwinia* isolates

Results showed that *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* isolates produce variable soft rot symptoms, when inoculated on surface of vegetable slices, after 72h of incubation (table 3). *E. carotovora* subsp. *carotovora* isolates produce soft rot symptoms as follows; on potato tuber slices, Ecc_4 isolate produce large active rot, while isolates Ecc_1, Ecc_2 & Ecc_3 gave small active rot. On sweet potato root slices, Ecc_5 produce large active rot. On cucumber fruit slices Ecc_7, Ecc_8 and Ecc_6 isolates produce large active rot, small active rot and arrested rot, respectively. On carrot root

Table 2 : Carbon utilization of *Erwinia carotovora* subsp. *carotovora* (Ecc_1 to Ecc_{15}) and *Erwinia chrysanthemi* (Ech_1 & Ech_2) isolates.

Soft rot *Erwinia* isolates	Carbon utilization*												
	Arabnose	Dextrose	Fructose	Lactose	Maltose	Mannitol	Mannose	Rhamnose	Salicin	Starch	Xylose	Gylecrol	Sucrose
Erwinia carotovora subsp. *carotovora*													
Ecc_1	A^+	-	$A^{[+]}$	$A^{[+]}$	$A^+G^{[+]}$	$A^+G^{\{+\}}$	$A^{[+]}$	-	-	-	$A^{[+]}$	-	-
Ecc_2	A^+	-	-	$A^{[+]}$	A^+	A^+	$A^{[+]}$	-	$A^{\{\pm\}}$	-	$A^{[+]}$	-	-
Ecc_3	A^+	$A^+G^{[+]}$	$A^{[+]}$	$A^{[+]}$	$A^+G^{[+]}$	$A^+G^{(\pm)}$	$A^{(+)}G^{[+]}$	A^+	$A^+G^{(+)}$	$A^{[+]}$	$A^{[+]}$	$A^{(+)}$	$A^+G^{(+)}$
Ecc_4	$A^{(+)}$	$A^{\{+\}}$	$A^{[+]}$	$A^{(+)}$	$A^{(+)}$	$A^{(+)}$	$A^{\{+\}}$	$A^{[+]}$	$A^{[+]}$	$A^{[\pm]}$	$A^{[+]}$	$A^{(+)}$	A^+
Ecc_5	$A^{[+]}G^{\{+\}}$	$A^{[+]}G^{\{+\}}$	$A^{[+]}$	$A^{[+]}$	$A^{[+]}$	$A^{\{\pm\}}$	$A^{(+)}G^{[+]}$	$A^{[+]}$	$A^{[+]}$	-	$A^{[+]}$	$A^{(+)}$	$A^{\{+\}}$
Ecc_6	$A^{(+)}$	$A^+G^{[+]}$	$A^{[+]}G^{[+]}$	A^+	A^+	$A^{(+)}$	$A^+G^{[\pm]}$	$A^{[+]}$	$A^{[+]}$	$A^{[+]}$	$A^{[+]}$	$A^{\{+\}}$	$A^{(+)}G^{[+]}$
Ecc_7	$A^{\{+\}}$	$A^{\{+\}}G^{\{+\}}$	-	$A^{[+]}$	$A^{[+]}$	$A^{\{+\}}$	$A^{(+)}$	$A^{[+]}$	$A^{\{+\}}$	-	-	-	-
Ecc_8	A^+	$A^+G^{(+)}$	$A^{[+]}$	$A^{(+)}$	$A^+G^{(+)}$	$A^+G^{(+)}$	$A^{(+)}G^{[\pm]}$	A^+	$A^+G^{(\pm)}$	$A^{\{\pm\}}$	$A^{[+]}$	$A^{\{+\}}$	$A^+G^{(+)}$
Ecc_9	$A^{\{+\}}$	$A^{(+)}G^{\{+\}}$	$A^{[+]}$	$A^{[+]}$	$A^{(+)}$	$A^{(\pm)}G^{\{\pm\}}$	$A^{(+)}G^{(+)}$	$A^{[+]}$	$A^{[+]}$	$A^{[\pm]}$	$A^{[+]}$	$A^{[+]}$	$A^{(+)}$
Ecc_{10}	$A^{[+]}$	$A^{[+]}G^{\{+\}}$	$A^{[+]}$	$A^{[+]}$	$A^{[+]}$	$A^{(\pm)}$	$A^{(+)}G^{[+]}$	$A^{[+]}$	$A^{[+]}$	$A^{[+]}$	$A^{[+]}$	$A^{(\pm)}$	$A^{(\pm)}$
Ecc_{11}	$A^{\{\pm\}}$	$A^{[+]}$	$A^{[+]}G^{[\pm]}$	$A^{(+)}$	$A^{[+]}G^{[\pm]}$	-	$A^{[+]}G^{\{+\}}$	$A^{[+]}$	$A^{\{+\}}$	$A^{[+]}$	$A^{[+]}$	$A^{\{+\}}$	$A^{(+)}G^{\{+\}}$
Ecc_{12}	$A^{\{\pm\}}$	$A^{[+]}G^{\{+\}}$	$A^{[+]}G^{[\pm]}$	$A^{[+]}$	$A^{(\pm)}$	-	$A^{(+)}G^{\{+\}}$	$A^{\{\pm\}}$	-	-	$A^{[+]}$	-	$A^{(+)}$
Ecc_{13}	$A^{\{\pm\}}$	$A^{[+]}G^{\{+\}}$	$A^{[+]}$	$A^{[+]}$	$A^{\{+\}}$	-	$A^+G^{\{+\}}$	-	-	-	$A^{[+]}$	-	$A^+G^{[+]}$
Ecc_{14}	$A^{\{\pm\}}$	$A^{\{+\}}$	$A^{[+]}$	$A^{\{+\}}$	$A^{[\pm]}$	$A^{\{+\}}$	$A^{[+]}G^{\{+\}}$	$A^{\{+\}}$	$A^{\{+\}}$	-	$A^{[+]}$	-	$A^+G^{[+]}$
Ecc_{15}	$A^{(+)}$	$A^+G^{(+)}$	$A^{[+]}$	$A^{\{+\}}$	A^+	$A^+G^{(+)}$	$A^{(+)}G^{\{+\}}$	$A^{[+]}$	A^+	$A^{\{+\}}$	$A^{[+]}$	$A^{(+)}$	$A^+G^{\{\pm\}}$
Erwinia chrysanthemi													
Ech_1	$A^{\{+\}}$	$A^{(+)}G^{\{+\}}$	$A^{[+]}G^{[\pm]}$	$A^{(+)}$	$A^{[+]}$	$A^{(+)}$	$A^{(+)}G^{\{+\}}$	$A^{[+]}$	A^+	$A^{[+]}$	$A^{[+]}$	$A^{(+)}$	
Ech_2	-	$A^{[+]}G^{\{+\}}$	-	-	-	-	$A^{\{+\}}$	$A^{[+]}$	$A^{[+]}$	-	-	-	-

*A^+ after 24h, (A^+) after 48h, $[A^+]$ after 72 -96h and $\{A^+\}$ after one week. G^+ after 24h, (G^+) after 48h, $[G^+]$ after 72 -96h and $\{G^+\}$ after one week.

slices, Ecc_{10} &Ecc_{11} isolates produce large active rot, while isolate Ecc_9 produce small active rot, respectively. On eggplant fruit slices, isolate Ecc_{12} produce large active, while Ecc_{13} produce small active rot, respectively. On chili fruit slices, both isolate Ecc_{14} & Ecc_{15} gave large active rot. *E. chrysanthemi* isolates produce soft rot symptoms as follows; isolates Ech_1 & Ech_2 produce large active rot on both red sweet pepper and tomato fruit slices (table 3).

Host range of soft rot *Erwinia* isolates

Results of host range of *E. carotovra* subsp. *carotovora* and *E. chrysanthemi* isolates revealed that different soft rot symptoms were recorded of inoculated vegetable slices *viz*. potato tubers, roots of sweet potato & carrot and fruits of cucumber, eggplant, red sweet pepper, chili & tomato, after 72h of incubation, Results revealed that soft rot *Erwinia* isolates differed in their pathogenicty on tested vegetable hosts according to recorded of soft rot symptoms (table 3). On potato tuber slices, *E. carotovra* subsp. *carotovora* isolates (Ecc_5, Ecc_6, Ecc_9, Ecc_{10}, Ecc_{11}, Ecc_{12} & Ecc_{13}) and *E. chrysanthemi* (Ech_1) produce large active rot. The small active rot was obtained with isolates *E. carotovra* subsp. *carotovora* (Ecc_7 & Ecc_8) and *E. chrysanthemi* (Ech_2), while the arrested rot was recorded with *E. carotovra* subsp. *carotovora* (Ecc_{14} & Ecc_{15}), respectively. On sweet potato root slices, *E. carotovra* subsp. *carotovora* (Ecc_9, Ecc_{10} & Ecc_{11}) produce large active rot, *E. carotovra* subsp. *carotovora* (Ecc_2, Ecc_3, Ecc_4, Ecc_6, Ecc_{12} & Ecc_{14}) and *E. chrysanthemi* (Ech_1&Ech_2) produce small active rot, while other *Erwinia* isolates produce arrested rot, respectively. On cucumber fruit slices with *E. carotovra* subsp. *carotovora* (Ecc_1, Ecc_2, Ecc_3, Ecc_4, Ecc_9, Ecc_{10} & Ecc_{11}) produce large active rot, while *E.*

Table 3 : Pathoginicity tests and host range of *Erwinia carotovora* subsp. *carotovora* (Ecc_1 to Ecc_{15}) and *Erwinia chrysanthemi* (Ech_1 & Ech_2) on the different plant host.

Soft rot *Erwinia* isolates	Isolated from	Soft rot on slices of							
		Potato tuber	Sweet potato roots	Cucumber fruits	Carrot roots	Eggplant fruits	Red sweet pepper fruits	Chili fruits	Tomato fruits
Erwinia carotovora subsp. *carotovora*									
Ecc_1	Potato	++	+	+++	++	++	+++	-	+++
Ecc_2		++	++	+++	++	+++	+++	-	+++
Ecc_3		++	++	+++	+++	+++	+++	+++	+++
Ecc_4		+++	++	+++	++	+++	+++	++	+++
Ecc_5	Sweet potato	+++	+++	++	+++	++	++	+++	+++
Ecc_6	Cucumber	+++	++	+	++	+	++	++	+++
Ecc_7		++	+	+++	-	+	+++	+++	+++
Ecc_8		++	+	++	+++	++	+++	+++	+++
Ecc_9	Carrot	+++	+++	+++	++	++	+++	+	+++
Ecc_{10}		+++	+++	+++	+++	+++	+++	+++	+++
Ecc_{11}		+++	+++	+++	+++	+++	+++	+++	++
Ecc_{12}	Eggplant	+++	++	++	++	+++	+++	+++	+++
Ecc_{13}		+++	+	++	+++	++	+++	+++	+++
Ecc_{14}	Chili	+	++	++	++	++	+++	+++	+++
Ecc_{15}		+	+	++	-	++	+++	+++	+++
Erwinia chrysanthemi									
Ech_1	Red sweet Pepper	+++	++	++	++	++	++	+++	+++
Ech_2	Tomato	++	++	++	-	++	+++	++	+++

*- No rotting ++ Small active rot + Arrested rot +++ Large active rot

carotovra subsp. *carotovora* (Ecc_5, Ecc_{12}, Ecc_{13}, Ecc_{14} & Ecc_{15}) and *E. chrysanthemi* (Ech_1 & Ech_2) gave small active rot, respectively. On carrot root slices, *E. carotovra* subsp. *carotovora* (Ecc_3, Ecc_5 and Ecc_8 & Ecc_{13}) produce large active rot. *E. carotovra* subsp. *carotovora* (Ecc_1, Ecc_2, Ecc_4, Ecc_6 and Ecc_{12} & Ecc_{14}) and *E. chrysanthemi* (Ech_1) produce small active rot, while no soft rot symptoms were recorded with *E. carotovra* subsp. *carotovora* (Ecc_7 & Ecc_{15}) and *E. chrysanthemi* (Ech_2), respectively (table 3).

On eggplant fruit slices, *E. carotovra* subsp. *carotovora* (Ecc_2, Ecc_3, Ecc_4 and Ecc_{10} & Eca_{11}) produce large active rot. The small active rot was obtained with *E. carotovra* subsp. *carotovora* (Ecc_1, Ecc_5, Ecc_8, Ecc_9 and Ecc_{14} & Ecc_{15}) and *E. chrysanthemi* isolates, while the arrested rot was recorded with other isolates. On red sweet pepper fruit slices, the most *E. carotovra* subsp. *carotovora* isolates as well as *E. chrysanthemi* (Ech_2) produce large active rot, while *E. carotovra* subsp. *carotovora* both Ecc_5 and Ecc_6 produce small active rot and arrested rot, respectively. On chili fruit slices, *E. carotovra* subsp. *carotovora* (Ecc_3, Ecc_5, Ecc_7, Ecc_8, Ecc_{10}, Ecc_{11}, Ecc_{12}, Ecc_{13}) and *E. chrysanthemi* (Ech_1) produce large active rot. *E. carotovra* subsp. *carotovora* (Ecc_4 & Ecc_6) and *E. chrysanthemi* (Ech_2) produce small active rot, while other *E. carotovra* subsp. *carotovora* (Ecc_9) and (Ecc_1 & Ecc_2) produce arrested rot and no soft rot symptoms, respectively. On tomato fruit slices, the soft rot *Erwinia* isolates produce large active rot, except *E. carotovra* subsp. *carotovora* (Ecc_{11}) produce small active rot, respectively (table 3).

Protein electrophoresis (protein fingerprints)

The discrete protein bands

The scan of SDS-PAGE protein fingerprints of total

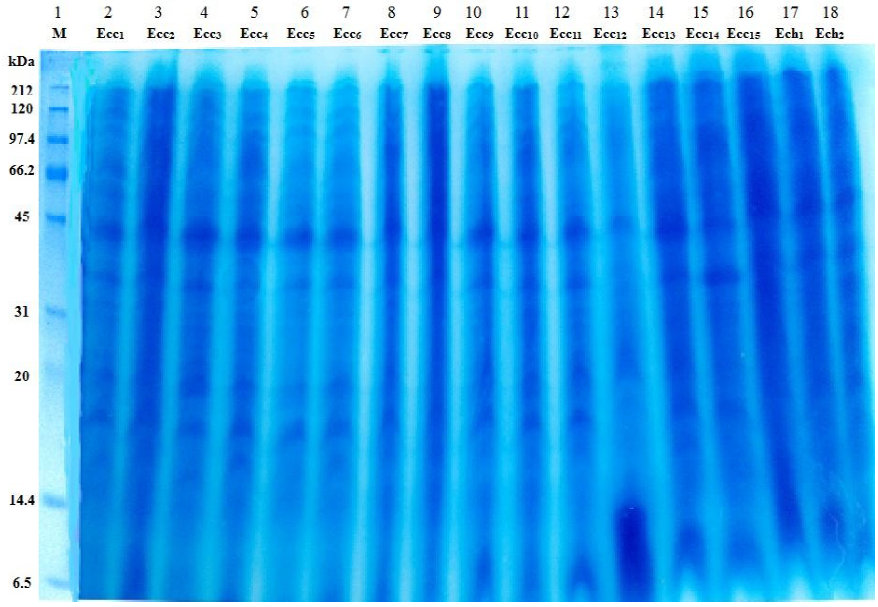

Fig. 1 : Protein fingerprints of total cellular protein of *Erwinia carotovora* subsp. *carotovra* lane1 (Protein marker); lane 2-Lane 16 (*Erwinia carotovora* subsp. *carotovra* isolates); lane 17 & Lane 18 (*Erwinia carotovora* subsp. *chrysanthemi* isolates).

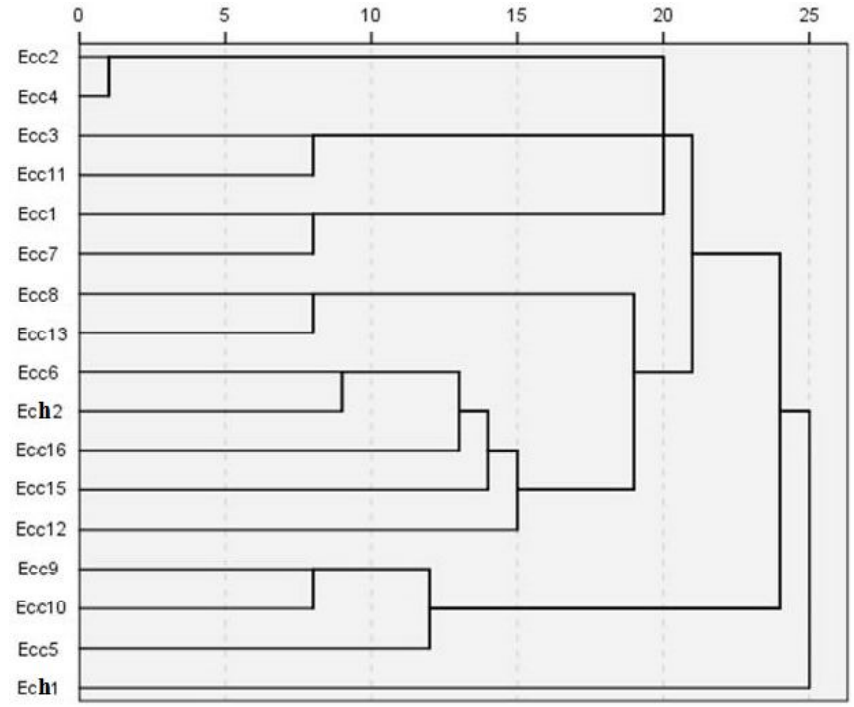

Fig. 2 : Phylogeny tree for seventeen of *Erwinia carotovora* subsp. *carotovora* (Ecc_1 to Ecc_{15}) and *Erwinia chrysanthemi* (Ech_1 & Ech_2) isolates of protein purification fractions separated by SDS-PAGE electrophoresis.

cellular proteins of soft rot *Erwinia* isolates distinguished into 15-17 discrete protein bands ,where the protein fingerprints of *E. carotovora* subsp. *carotovora viz*. Ecc_1, Ecc_2, Ecc_3, Ecc_4, Ecc_5, Ecc_6, Ecc_7, Ecc_8, Ecc_9, Ecc_{10}, Ecc_{11}, Ecc_{12}, Ecc_{13}, Ecc_{14} & Ecc_{15} contains 17, 17,17, 17, 17, 15, 16, 16, 17, 16, 16, 16, 17, 16 & 16 and the protein profiles of *E. chrysanthemi* Ech_1 & Ech_2 contains 17 and 16 bands, respectively (fig. 1 and table 4). The molecular weight of discrete protein bands ranged from 253.287 – 14.594 KDa. Comparison among protein fingerprints showed that protein profiles of soft rot *Erwinia* isolates were similar in some parts of the gel, where the discrete protein bands of 233.279, 193.489, 176.216, 155.173 and 131.626 KDa (high molecular weight); bands of 91.574, 68.402, 57.375, 49.773, 42.857 (moderate molecular weight) and bands of 35.546, 30.152,

26.851, 22.271 and 14.594 KDa (low molecular weight) were the common protein bands (table 4).

Electrophoresis data revealed that the protein fingerprints of *E. carotovora* subsp. *carotovra* isolates obtained from potato tubers were similar as well as the protein band of 20.512 KDa was common in all profiles of isolates , while the bands of 115.475 and 114.186 KDa was detected in profiles of Ecc_1 and Ecc_3, respectively . *E. carotovora* subsp. *carotovora* isolated from sweet potato roots only showed the occurrence of 20.743 KDa in their protein profile. The protein profiles of *E. carotovora* subsp. *carotovora* isolates which isolated from cucumber fruits were similar, except the bands of 250.461 and 115.475 KDa was detected in profiles of Ecc_8 and Ecc_7, respectively. The differences in protein profiles of *E. carotovora* subsp. *carotovora* isolates were obtained from carrot roots, in occurrence of protein bands 114.186; 107.956 and 17.794 KDa in protein profiles of Ecc_9; Ecc_{11} & Ecc_9 and Ecc_{10}, respectively. The protein profiles of *E. carotovora* subsp. *carotovora* (eggplant fruits) were similar, except the bands of 253.287 KDa (Ecc_{12}) and 243.988 & 105.166 KDa (Ecc_3), respectively. Results also showed that the protein profiles of *E. carotovora* subsp. *carotovora* isolated from chili fruits were similar, except the occurrence of 241.266 105.166 KDa in Ecc_{14} and 243.988 KDa in Ecc_{15}. The protein profiles of *E. chrysanthemi* were similar with occurrence the band of 103.993 and 102.832 KDa in profiles of Ech_1 and Ech_2, respectively (table 4).

Similarity matrix (DICE coefficient) and Dendrogram tree

The pair-wise similarity matrix (DICE coefficient) and dendrogram tree among soft rot *Erwinia* isolates, based on total cellular protein analysis using SDS-PAGE method are shown in table 5 and fig. 2. The similarity among protein profiles of bacterial isolates was ranged from 0.86-1.00. The highest similarity value (1.00) was recorded between the protein fingerprints of *E. carotovora* subsp. *carotovora* (Ecc_2 & Ecc_4) isolates. The lowest similarity value (0.86) was recorded between *E. carotovora* subsp. *carotovora* Ecc_5 and both of Ecc_1, Ecc_2, Ecc_3 and Ecc_4 isolates; between Ecc_9 and both of Ecc_1, Ecc_2, Ecc_3 and Ecc_4 isolates; between of Ecc_9 and both of Ecc_1, Ecc_2, Ecc_3 and Ecc_4 isolates; between Ecc_{13} and both of Ecc_1, Ecc_2, Ecc_3, Ecc_4 and Ecc_5 isolates, between Ecc_{14} and both Ecc_1, Ecc_2, Ecc_3 and Ecc_4, respectively. The similarity value (0.97) was recorded in protein fingerprints between Ecc_1 and Ecc_7, between Ecc_2 and Ech_1, between Ecc_3 and Ecc_{11}, between Ecc_5 and Ecc_{10}, between Ecc_6 and each of Ecc_7, Ecc_8, Ecc_{10}, Ecc_{11}, Ecc_{12}, Ecc_{15}, Ech_1 and Ech_2, between Ecc_7 and Ecc_{10}, between Ecc_8 and Ecc_{13}, between Ecc_9 and Ecc_{10}, respectively. Details of similarity values of 0.93 and 0.90 are shown in table 5.

The tree of dendrogram of protein profiles can be divided into two main groups, the first group include *E. chrysanthemi* Ech_1 isolate, while the second group include the other *E. carotovora* subsp. *carotovora* isolates *viz*. Ecc_1, Ecc_2, Ecc_3, Ecc_4, Ecc_5, Ecc_6, Ecc_7, Ecc_8, Ecc_9, Ecc_{10}, Ecc_{11}, Ecc_{12}, Ecc_{13}, Ecc_{14}, Ecc_{15} and *E. chrysanthemi* Ech_2 (fig. 2). The second group can be classified into four sub groups; the first sub-group includes the *E. carotovora* subsp. *carotovora* isolates Ecc_2, Ecc_4, Ecc_1 and Ecc_7, while the second sub-group includes the isolates *E. carotovora* subsp. *carotovora* Ecc_3 & Ecc_{11}. The third sub-group includes the isolates *E. carotovora* subsp. *carotovora* Ecc_8, Ecc_{13}, Ecc_6, Ecc_{15}, Ecc_{14} & Ecc_{12} and *E. chrysanthemi* Ech_2. The fourth sub-group includes the isolates *E. carotovora* subsp. *carotovora* Ecc_9, Ecc_{10} and Ecc_5 (fig. 2).

Discussion

Bacterial soft rot disease is a highly harmful disease for vegetables, whether during storage or marketing in Egypt. More studies indicated soft rot pathogens on one plant type, while this study is aimed to study the common soft rot bacteria in some vegetables such as potatoes, sweet potatoes, carrot, cucumbers, red sweet pepper, chili, eggplant and tomatoes. About 17 of soft rot *Erwinia* isolates different tested vegetables, where the different isolated were caused typical soft rot symptoms on the same host. Re-isolation procedures showed that the isolated bacteria were similar to the original bacterial culture. The bacteria were short rod and Gram negative with producing similar colonies type as those recorded by Abd El-Khair and Haggag (2007). According to pathological, cultural and biochemical characters, the soft rot *Erwinia* isolates (Ecc_1 to Ecc_{15}), which isolated from potato, sweet potato, cucumber, carrot, eggplant and chili can were identified as *Erwinia carotovora* subsp. *carotovora*, while soft rot *Erwinia* isolates (Ech_1 & Ech_2), which isolated from red sweet pepper and tomato were identified as *Erwinia chrysanthemi*. These results are agreement with those recorded by Perombelon and Kelman (1980) and Ali *et al*. (2114). They reported that the morphological and physio-chemical tests can be used to identify of *E. carotovora* subsp. *carotovora* and *E. chrysanthemi*. The pathological, cultural and biochemical characters also play an important role for identification of bacterial soft rot pathogen as recorded by Abd El-Khair (2004), Khalil *et al*. (2014). The bacterial pathogen

Table 4 : The discrete protein in protein fingerprints of total cellular proteins of *Erwinia carotovora* subsp. *carotovora* (Ecc_1 to Ecc_{15}) and *Erwinia chrysanthemi* (Ech_1 & Ech_2) isolates by SDS-PAGE analysis.

Band No.	MWKDa	Soft rot *Erwinia* isolates																
		Erwinia carotovora subsp. *carotovora*															*E.chrysanthemi*	
		Ecc_1	Ecc_2	Ecc_3	Ecc_4	Ecc_5	Ecc_6	Ecc_7	Ecc_8	Ecc_9	Ecc_{10}	Ecc_{11}	Ecc_{12}	Ecc_{13}	Ecc_{14}	Ecc_{15}	Ech_1	Ech_2
1	**253.287**	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
2	**250.461**	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-
3	**243.988**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
4	**241.266**	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
5	**233.279**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	**193.489**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	**176.216**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	**155.173**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	**131.626**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	**116.778**	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
11	**115.475**	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
12	**114.186**	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
13	**107.956**	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-
14	**105.166**	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
15	**103.993**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
16	**102.832**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
17	**91.574**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	**68.402**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	**57.375**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	**49.773**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21	**42.857**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
22	**35.546**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	**30.152**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	**26.851**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	**22.271**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26	**20.743**	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
27	**20.512**	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
28	**17.794**	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
29	**14.594**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(+) Refers to occurred of protein band, (-) Refers to not occurred of protein band.

Table 5 : Similarity matrix between pairs of protein fingerprints of *Erwinia carotovora* subsp. *carotovora* (Ecc_1 to Ecc_{15}) and *Erwinia chrysanthemi* (Ech_1 &Ech_2) isolates by matching co-migrating band position using DICE coefficient.

Soft rot *Erwinia* isolates	Soft rot *Erwinia* isolates																	
	Erwinia carotovora **subsp.** ***carotovora***															*E.chrysanthemi*		
	Ecc_1	Ecc_2	Ecc_3	Ecc_4	Ecc_5	Ecc_6	Ecc_7	Ecc_8	Ecc_9	Ecc_{10}	Ecc_{11}	Ecc_{12}	Ecc_{13}	Ecc_{14}	Ecc_{15}	Ech_1	Ech_2
Ecc_1	1.00																
Ecc_2	0.93	1.00															
Ecc_3	0.93	0.93	1.00														
Ecc_4	0.93	1.00	0.93	1.00													
Ecc_5	0.86	0.86	0.86	0.86	1.00												
Ecc_6	0.93	0.93	0.93	0.93	0.93	1.00											
Ecc_7	0.97	0.90	0.90	0.90	0.90	0.97	1.00										
Ecc_8	0.90	0.90	0.90	0.90	0.90	0.97	0.93	1.00									
Ecc_9	0.86	0.86	0.86	0.86	0.93	0.93	0.90	0.90	1.00								
Ecc_{10}	0.90	0.90	0.90	0.90	0.97	0.97	0.97	0.93	0.97	1.00							
Ecc_{11}	0.90	0.90	0.97	0.93	0.90	0.97	0.93	0.93	0.90	0.93	1.00						
Ecc_{12}	0.90	0.90	0.90	0.90	0.90	0.97	0.93	0.93	0.93	0.90	0.93	1.00					
Ecc_{13}	0.86	0.86	0.86	0.86	0.86	0.93	0.90	0.97	0.86	0.90	0.90	0.90	1.00				
Ecc_{14}	0.86	0.86	0.86	0.86	0.90	0.93	0.90	0.90	0.86	0.90	0.90	0.90	0.93	1.00			
Ecc_{15}	0.90	0.90	0.90	0.90	0.90	0.97	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.90	1.00		
Ech_1	0.90	0.97	0.90	0.90	0.90	0.97	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	1.00	
Ech_2	0.90	0.90	0.90	0.90	0.90	0.97	0.93	0.93	0.93	0.93	0.93	0.93	0.90	0.90	0.93	0.93	1.00

of tip-over disease (*E. carotovora* subsp. *carotovora* and *E. chrysanthemi*) was identified on the basis of morphological, cultural, physiological and biochemical characteristics and pathogenicity tests **(**Snehalatharani and Khan, 2010). On the basis of pathogenicity to potato, tomato, onion and cucumber as well as on the basis of the physiological and biochemical properties, the bacterial soft pathogen identified as *E. carotovora* subsp. *carotovora* (Karnjanarat *et al*., 1987).

Our study revealed that the soft rot *Erwinia* isolates differed in host range according to the soft rot symptoms resulting on plant hosts. It is clear the differences were recorded among soft *Erwinia* isolates in their disease capacity according to host. Similar results were recorded by many workers, where *Erwinia* causes soft rot for many vegetables in post-harvest such as tomato fruits (Lemma *et al*., 2014), potato tubers (Ali *et al*., 2014), sweet potato roots (Umunna and Anselem, 2014) and carrot roots. The soft rot *Erwinia* had wide host range of many important economically vegetables such as eggplants, squash, potato, onion bulbs, garlic cloves, reddish roots, carrot, sweet potato, tomato, pepper fruits, cabbage and cucumber (Hibark *et al*., 2007; Shrestha *et al*., 2009; Bhat *et al*., 2010b). The biochemical study revealed that soft rot *Ewinia* isolates were differs in their ability of fermentation of tested carbon sources producing acid only or acid and gas.

Some bacterial isolates showed the quickly fermentation of carbon sources, more compared to other isolates. It is possible to say that there is a similarity between the results of disease capacity and the biochemical characteristics of the causative agent, where the isolate more rapidly fermented carbon sources was the greater in the disease capacity. These results are agreement with similar results recorded by Abd El-Khair (2004) and Parthiban *et al*. (2012). They mentioned that *E. carotovora* isolates showing variable action with sugars until one week

of incubation. Our results showed that there is no clear relationship between the pathogenic ability of *E. carotovora* isolates and their ability to ferment the sugars. But, the isolates that characterized by their high disease ability as well as they characterized by their ability to ferment many sugars, especially dextrose, lactose and sucrose within 24 hours. On the other hand, the isolates that characterized by their moderate or weak disease capacity showed a weak reaction in their ability to ferment sugars. Therefore, the speed of *E. carotovora* in the sugars fermentation may play a role in the disease ability.

Comparison the protein fingerprints *E. carotovora* subsp. *carotovora* isolates (Ecc_1 to Ecc_{15}) and *E. chrysanthemi* (Ech_1 & Ech_2) revealed that molecular weight of proteins ranged of 253.287-14.594 KDa. A few of protein bands occurred in some protein fingerprints than other, for example the band of 20.512 KDa was noticed in protein profiles of potato isolates only. The comparison of similarities among soft rot *Erwinia* isolates, using DICE coefficient, showed the rates ranged from 0.86 to 1.00, where the similarity of 1.00 recorded between Ecc_1 & Ecc_4 isolates. The dendergram tree cleared that the protein profiles can be classified into two main clusters included some sub-clusters. These results are similar with those recorded by El-Sheikh (2010). He mentioned that on the basis of the results obtained from SDS-PAGE analysis of *E. carotovora* subsp. *carotovora* proteins, it could be detected that there are differences among isolates in protein profiles and molecular weights and application of these technique help to differentiate between the *E. carotovora* subsp. *carotovora* isolates. SDS-PAGE method is particularly useful to analyzing the complex profile created by a total soluble protein lysate. These results are agreement with those recorded by Avora *et al*. (2002) and Abd El-Khair (2004). They reported that differentiation among *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* using SDS-PAGE according to their molecular weight are very important. Such results suggested that the bacterial isolates were not genetically identical (Yon-Xiang and Geider, 1997).

This study confirms the differences between the soft rot bacteria in the disease or the biochemical differences, which were confirmed by the study of the protein test. Therefore, this study recommends taking precautions when developing resistance programs for this cause in vegetables.

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