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Serological Characteristics of *Erwinia carotovora* Isolated from Potato Fields in Egypt

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Abstract: Six isolates of *Erwinia carotovora* were isolated from naturally infected potato tubers in different localities in Egypt. The isolates were tested pathologically, morphologically and serologically. All *E. carotovora* isolates exhibited the same results in the morphological and pathological tests and identified as *E. carotovora*. The isolates showed variation in their virulence. The serological tests such as Crossed Immunoelectrophoresis (CIE) and Double Gel Diffusion (DGD) were used to study serological differences between *E. carotovora* isolates. The serological differences between *E. carotovora* isolates using CIE were clearer than in DGD, since the numbers of precipitin peaks detected in the homologous and heterologous reaction were almost twice than the numbers of precipitin bands in DGD. The obtained results using CIE with *E. carotovora* isolates were emphasized by reciprocal analysis procedure. The common and specific antigens for each isolate were clearly detected. At least five antigens were found to be common among *E. carotovora* isolates. One and two antigens were found to be specific to *E. carotovora* isolates No. 1, 2 and 4, 5, respectively, while four antigens were found to be specific to *E. carotovora* isolate No. 6. No antigens were found specific to *E. carotovora* isolate No. 3.

Key words: *Erwinia carotovora*, crossed immunoelectrophoresis, double gel diffusion, soft rot disease

INTRODUCTION

The production of potatoes is accompanied by severe diseases caused by bacterial phytopathogens leading to enormous losses in yield and quality worldwide (Oerke *et al*., 1994; Rasche *et al*., 2006). One of the most important diseases of potatoes is bacterial soft rot, caused by *Erwinia carotovora*. Losses due to infection of tubers by bacterial soft rot during storage varied from 31.3- 36.8% (Rasul *et al*., 1999).

Erwinia carotovora is facultative anaerobe, nonspore-forming, Gram-negative enterobacteria that causes disease in a wide range of plants including many economically important crops (Perombelon and Kelman, 1980; Schuerger and Batzer, 1993; Agrios, 2005). The extent of losses varies from country to country and is affected by the climate as well as the conditions of plant growth and storage. Infections by *E. carotovora* ssp. *carotovora* can occur worldwide on the field or in storage after harvesting. All the strains of *E. carotovora* ssp. *carotovora* caused creamy white or light brown soft rot in potato slices (Obradovic and Arsenijevic, 1997). These symptoms appeared about 10 h after inoculation and the slices degraded completely in the course of the next 48 h. The infection of seed tubers by *E. carotovora* ssp. *atroseptica* can lead to the development of different symptoms during vegetative growth stage, including non-emergence of plants, chlorosis, wilting, haulm desiccation and typical blackleg (Helias *et al*., 2000). Additionally, they found that the method of inoculation and inoculum concentration provided major factors for the subsequent development of symptom.

Several workers used different methods in preparing the bacterial antigen for immunization and serological tests. For example, the pathogenic isolates of *P. syringae* were divided into 10 distinct serotypes based on the reaction of their heat-stable antigens in gel-diffusion tests (Otta and Harley, 1970). Composite antigens containing sonicated cells of seven of the major *P. syringae* serotypes were used to obtain composite antisera containing antibodies against heat-stable antigens of each serotype. Rough and smooth isolates possessed

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serologically identical heat-stable antigens, were not consistently different pathologically and did not agglutinate in up to 10% saline solutions. Isolates of *P. aptata* and *P. morsprunorum* were similar enough to *P. syringae* in all tests and could be considered synonymous. Many investigators used Double Gel Diffusion (DGD) and Crossed Immunoelectrophoresis (CIE) as successful tests for differentiation between bacterial strains including either the same or different species. Also, these techniques were used to distinguish between virulent and avirulent strains of bacteria. A serologically specific strain of *E. carotovora* var. *carotovora* (aroideae type) caused black leg disease in potato plant and was distinguishable from the soft rot organism *E. carotovora* var. *carotovora* (Tanii and Akai, 1975). Tohamy (1992) carried out a comparison between *E. carotovora* ssp. *carotovora* and *E. carotovora* sub sp. *atroseptica* using CIE techniques. Double diffusion agar test and immunoelectrophoresis techniques were used to investigate serological differences and common antigens between virulent and its mutant of *P. solanacearum* (Abd El-Rehim *et al*., 1995). They obtained mutants by treating wild type virulent strain K60 with N-methyl-N-nitro-N-nitrose-guanidine. Auxotrophic and antibiotic resistant mutants were used as markers for successful mutation. Pathogenicity test on tomato and potato indicated that leu, leu, +thr and ampr mutant were virulent, while penr and strr were avirulent. They indicated that the virulent mutants have common antigens that are not shared by the avirulent ones and there are no differences between individual mutants.

Serological detection techniques and also nucleic acid based techniques could not differentiate between virulent and avirulent isolates *P. solanacearum*. Vruggink and Geesteranus (1975) mentioned that in sap of infected potato stems and rotted tubers the bacterium (*E. carotovora* var *atroseptica)* could be distinguished serologically from *E. carotovora* var *carotovora* by double gel diffusion tests. In latent infections, tubers were treated to increase the number of bacteria which were then detected serologically either in the rotted tissues or more reliable on agar. The serological detection method appears more reliable than bacteriological techniques.

So, the objectives of this study were the isolation and identification of soft rot *E. carotovora*, differentiation between *E. carotovora* isolates, early detection of cultivar resistance to the disease and serodiagnosis of *E. carotovora* isolates in oozes of naturally infected potato tubers.

MATERIALS AND METHODS

Isolation and identification of *E. carotovora*: Isolation of soft rot bacteria was carried out from rotted potato tubers collected from four locations (Kafr El-sheikh, El-Gharbeya, El-Beheira and El-Monefeya) (Table 1). Diseased materials were washed thoroughly with tap water. Small portions of the inner tissues were surface sterilized. Rotted tubers were macerated in small amount of sterile water in test tube and full loop of the resulting suspension was streaked over the surface of plates containing nutrient agar media (pH 7.0). Plates were incubated for 48 h at 28°C. Bacteria were purified through single colony isolation technique (single colonies were picked up, streaked on slants of the same media and incubated at 28°C for 48 h). Identification was carried out according to Bergey's Manuals of Systematic Bacteriology (Bergey *et al*., 1984).

Table 1: Studied *E. carotovora* isolates, host plant and localities

E. carotovora isolates	Host plant	Locality (Governorate)	Degree of virulence	Cultivars
1	Potato	Kafr El-Sheikh	++	Kara
2	Potato	El-Gharbeya	++	Flora
3	Potato	El-Behira	+	Kara
4	Potato	El-Menofeya	++	Harmony
5	Potato	El-Gharbeya	+++	Flora
6	Potato	El-Behira	+++	Harmony

+++: Highly virulent, ++: Moderately virulent, +: Weakly virulent

Pathogenicity tests: For testing the rotting ability of the isolates, standard tubers (60 g in weight) of Kara (susceptible cultivar) were used for the determination of the rotting ability of *E. carotovora* isolates. A bacterial suspension O.D. (0.4) prepared from 48 h old culture at 28 °C. Tubers of the kara potato cultivar were thoroughly washed and sterilized by flaming. A hole was made in each tuber by a sterilized cork borer and 0.5 mL of the standard bacterial suspension (10^7 CFU mL^{-1}) was pipetted in each hole. The hole was then closed with the removed cylinder. Inoculated tubers were kept in sterilized polyethylene bags for 4 days at 28°C. Control tubers were prepared by the same way by using sterile tap water instead of the bacterial suspension. Inoculated tubers as well as the control were cut at transverse direction and the diameters of rotted area were estimated in centimeters according to Hollis and Goss (1950). The mean amount of rot produced by each isolate on potato tubers was considered a good indication for the rotting ability of isolates.

Serological studies

Preparation of bacterial antigens: The bacterial cells of each studied isolate were harvested after 3-5 days growing on nutrient broth medium with shaking at 30°C, then centrifuged at 8000 rpm for 20 min, washed in distilled water and recentrifuged twice before taking up in normal saline 0.85%. Cell suspensions were sonicated with ultrasonicator for 2 min. The protein constituents of

the bacterial sonicated suspensions were determined according to Lowry *et al.* (1951) method and adjusted to 20 mg mL^{-1}.

Preparation of plant antigens: Plant leaves were mixed with saline solution (1:1 w/v), homogenized and centrifuged at 8000 rpm for 20 min. The supernatant was purified in dialysis bags and used as antigens (El-Kady *et al.*, 1989).

Immunization with *E. carotovora* isolates: The experiment was carried out according to El-Kady *et al.* (1986) and Sidaros *et al.* (2006). All the tools used for antigens and antisera preparation were sterilized. The bacterial antigen of each of the six isolates (No. 1, 2, 3, 4, 5 and 6) and its mutants of *E. carotovora* were injected subcutaneously then intramuscular in Boscat rabbits (3-3.5 kg) which were previously bled to obtain normal serum. Each Boscat rabbit was received a course of ten injections (two injections per week) with increasing doses of prepared antigen (0.5, 0.75, 1, 1.25, 1.50, 1.75, 2, 2.25, 2.50, 2.75 mL). Fruend's complete adjuvant was used only with the first injection, while the incomplete adjuvant used with the following injections. The rabbits were bled by cutting the lateral veins of the other ear at 7 days after the last injection. The blood was received in a sterilize test tube and left to clot at 37°C for 2 h. The clot was loosen from the wall of the tube with a fine glass rod, then the blood tube was kept at 4°C overnight to allow the clot to retract and express the straw coloured serum. The antisera were obtained and centrifuged at 8000 rpm for 20 min to remove any precipitates and kept at 0°C. Sodium azide (0.02%) was added to prevent any contamination.

Serological tests

Double gel diffusion test (DGD): Agar double gel diffusion plate method (Ouchterlony, 1949) was carried out by using difico agar 1.2% (w/v) incorporated in physiological solution with sodium azide 0.2% (w/v). The gel was poured in Petri dishes to a depth of about 2 mm. Holes were cut in a convenient pattern using a cork borer 8 mm in diameter. A pattern consists of a central hole with four circumferential holes to that of the peripheral wells were filled with antiserum dilution. To study the serological relationship between the antiserum and its homologous and heterologous antigens, the antiserum was placed in the central well, while the antigens were placed in the peripheral ones. Plates were stored at room temperature (25-28°C) for 10 days. The plates were observed daily for lines of precipitate between the antiserum and antigen wells. The titer was determined by the dilution of antiserum after which no bands were detected.

Crossed immunoelectrophoresis (CIE) preparations

Preparation of the glass plates and gels for immunoelectrophoresis: The 80×60×1 mm glass plates were cleaned by detergent followed by repeated washing by distilled water and a final washing by ethanol, then dried. Glass plates were coated with thin film of agarose in order to get a better contact between the gel and the glass by spreading a few milliliter agarose on the plates with a tampon or by gently moving the edge of another glass plate at 45°C angle along the surface of the electrophoric plate. The agarose was dried to a fine film in hot air from a hair drier for a few minutes. The coated plates were marked with an X on the opposite side to signify that the coating has been carried out. The coated plates were stored for weeks at room temperature (Axelsen *et al.*, 1975).

Coating of glass plates was made in the second dimension of the crossed-immunoelectrophoresis. Making a uniform gel was obtained by coating the gel on a glass plate as the following: the clean and dry glass plate, coated and preheated (if necessary), was laid on a horizontal table (leveled before the pouring of agarose). The tubes containing the agarose solution were incubated in a water bath (45-55°C) near the table. The tubes with agarose solution were taken out of the thermostated water bath and immediately poured onto the middle of the glass plate and left a few minutes for solidification.

Isolation of gama immunoglobulins: Isolation of immunoglobulin was carried out according to the methods of Axelsen *et al.* (1975) and Harboe and Inglid (1973). The isolation of IgG+IgA from rabbit antiserum was left for about 20 h at room temperature. By centrifugation at 4000 rpm for 3 min, 98% of the antibody activity precipitated. The supernatant was discarded. The precipitate was washed with approximately 5 mL 1.75 M $(NH_4)_2SO_4$, centrifuged and the supernatant was again discarded. The washing procedure was repeated twice. By adding a small amount of water the antibody containing precipitate was transferred to 10 mL dialysis bag. The dialysis was performed at 4°C for 2×12 h against distilled water, 1×24 h against 0.050 M Na-acetate 0.021 M HAC pH 5.0, 2×12 h against distilled water and 1×24 h against the acetate buffer. During the dialysis, a precipitate from lipoproteins appeared which was removed by centrifugation. The supernatant was transferred to a column containing 25 mL DEAE-sephadex A50 equilibrated with the acetate buffer pH 5.0. The immunoglobulins appeared in the elute. The column was eluted with approximately 25 mL acetate buffer. By this procedure rabbit IgG and bacterial IgA were recovered almost quantitatively. The IgM was lost.

Finally, immunoglobulins were dialysed against 0.1 M NaCl, 15 mM NaN_3 and stored in this solution in the refrigerator (4°C).

Crossed immunoelectrophoresis techniques (CIE): Crossed immunoelectrophoresis (CIE) and CIE with intermediated gel were performed according to published procedures of Axelsen *et al.* (1975) with some modifications. Electrophoresis in first dimension was performed on 80×60×1 mm glass plates. The pre warmed plates (50°C) were covered with 8 mL of 1% agarose gel (Barbital buffer, pH 8.6, 1 = 0.02) to give an agarose volume to surface area ratio of 0.15 mL cm^{-2}. After cooling at room temperature, six antigen wells (4 mm in diameter) were punched out 3.5 cm from the cathodic end and 1 cm apart in the vertical axis of the glass plate. Antigen samples (15 mL/well) which contained 10 mg protein/mL were added to each well and electrophoresis was performed for 70 min in a water cooled electrophoresis chamber at 10 V cm^{-1}; 0.01% bromophenol blue was mixed with one sample as an indicator for migration of the samples. After the first dimensional electrophoresis, agarose strips (2×6 cm) containing the antigens were cut and transferred on one side of glass plate (6×8 cm) for the second dimension electrophoresis. The remaining space on the plate was then covered with agarose containing immunoglobulins "Ig" (5 mL lagarose +0.4 mL Ig). For CIE with an intermediated gel, a brass barrier was placed 2 cm from the first dimension antigen containing strip and space filled with agarose containing Ig (2.1 mL agarose + 0.15 mL Ig). After getting the gel, the intermediate was trimmed to 2 cm width and the remaining space was covered with agarose containing Ig for other samples (3.75 mL agarose +0.25 Ig). Electrophoresis in the second dimension was performed at 2 V cm^{-1} for 16 and 20 h. After immunoelectrophoresis, gels were stained with coomassie brilliant blue R-250 according to method of Axelsen *et al.* (1975). The glass plates were stained according to Laurell (1972).

RESULTS

Isolation, identification and pathogenicity of *E. carotovora* isolates: Six isolates of *Erwinia carotovora* were isolated from naturally infected potato tubers in different governorates in Egypt (Table 1). *Eruinia carotovora* isolates were varied in the degree of their virulence, since isolates No. 5 and 6 were highly virulent, while isolates No. 1, 2 and 4 were moderate but isolate No. 3 showed weak virulence (Table 1).

Table 2: Number of precipitin bands detected in the heterologous and homologous antigen-antibody reactions of *E. carotovora* isolates using double gel diffusion test (DGD)

E. carotovora isolates	Representative antigens							
	1	2	3	4	5	6	Control	Specific antigens
1	5*	4	4	4	3	4	0	1
2	4	6*	5	4	4	5	0	1
3	4	5	5*	2	5	5	0	0
4	4	4	5	6*	3	5	0	1
5	3	4	2	3	6*	5	0	1
6	4	6	5	5	4	8*	0	2

*Homologous reactions

Table 3: Number of precipitin peaks detected in the heterologous and homologous antigen-antibody reactions of *E. carotovora* isolates using crossed immunoelectrophoresis (CIE)

E. carotovora isolates	Representative antigens							
	1	2	3	4	5	6	Control	Specific antigens
1	9*	6	8	8	6	6	0	1
2	6	10*	8	8	9	7	0	1
3	8	8	8*	5	8	8	0	0
4	8	8	5	10*	8	6	0	2
5	6	9	8	8	11*	8	0	2
6	6	7	8	6	8	12*	0	4

*Homologous reaction

Serological differences between *E. carotovora* isolates using

Double gel diffusion test (DGD): Data presented in Table 2 and Fig. 1 showed that antibodies of *E. carotovora* isolate No. 1 gave 5, 4, 4, 4, 3 and 4 precipitin bands when allowed to react with antigens of isolates No. 1, 2, 3, 4, 5 and 6, respectively (Fig. 1). Antibodies of *E. carotovora* isolate No. 2 showed 4, 6, 5, 4, 4 and 5 precipitin bands when reacted with antigen of *E. carotovora* isolates No. 1, 2, 3, 4, 5 and 6, respectively (Fig. 1c, d). Also, antibodies against *E. carotovora* isolate No. 3 formed 4, 5, 5, 2, 5 and 5 precipitin bands when reacted with antigens of *E. carotovora* isolates No. 1, 2, 3, 4, 5 and 6, respectively (Fig. 1e, f). Antibodies against *E. carotovora* isolate No. 4 gave 4, 4, 5, 6, 3 and 5 precipitin bands when reacted with antigens of *E. carotovora* isolates no. 1, 2, 3, 4, 5 and 6, respectively (Fig. 1g, h). Antibodies against *E. carotovora* isolates No. 5 detected 3, 4, 2, 3, 6 and 5 precipitin bands when reacted with antigens of *E. carotovora* isolates No. 1, 2, 3, 4, 5 and 6, respectively (Fig. 1i, j). Finally, antibodies against *E. carotovora* isolate No. 6 gave 4, 6, 5, 5, 4 and 8 precipitin bands when reacted with antigens of *E. carotovora* isolates No. 1, 2, 3, 4, 5 and 6, respectively (Fig. 1k, l). The results clearly showed that at least two antigens were found common among *E. carotovora* isolates, while one antigen was found specific to each isolate of *E. carotovora* (No. 1, 2, 4 and 5). Two antigens were found specific to *E. carotovora* isolate No. 6, while no antigens were found specific to isolate No. 3.

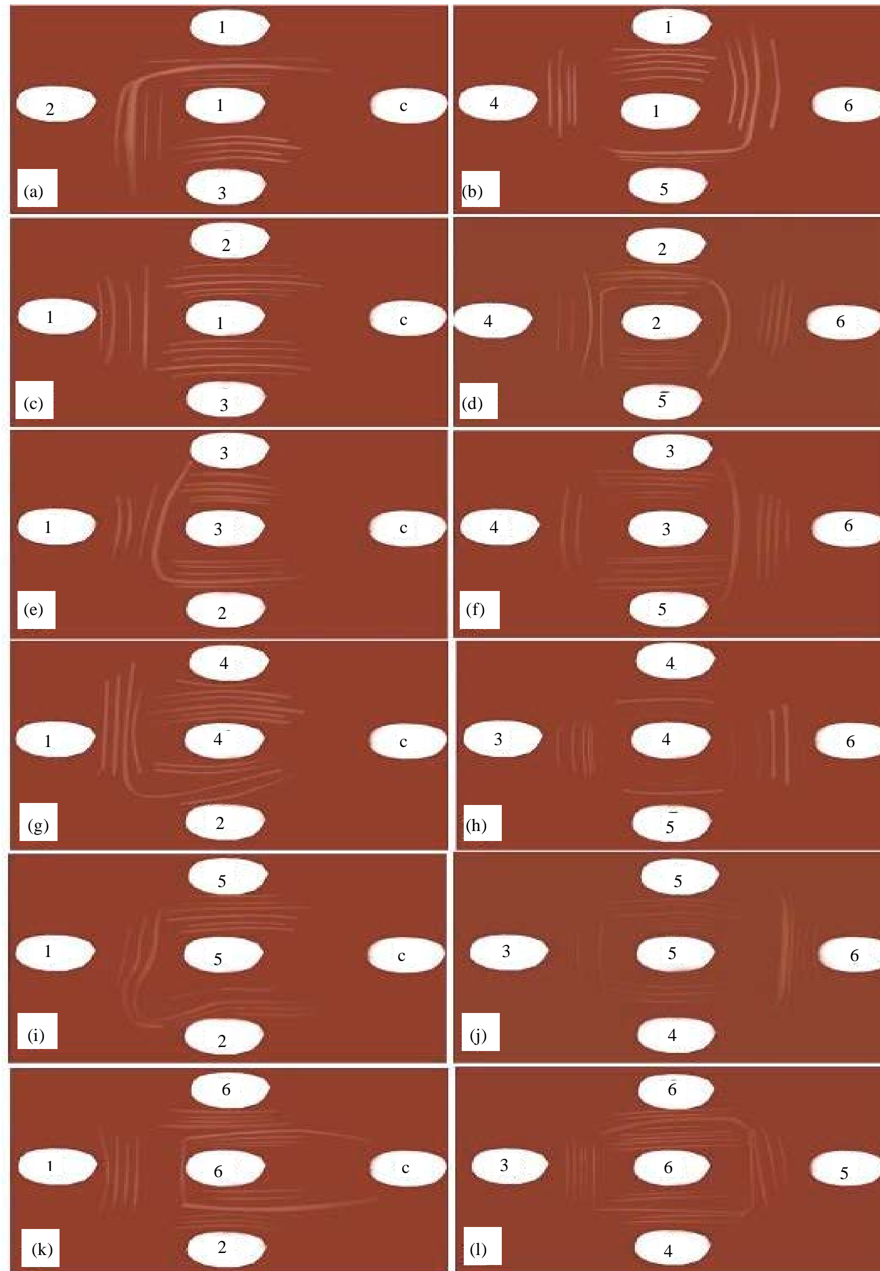

Fig. 1(a-l): Double gel diffusion test (DGD) for the serological reactions between antigens and antibodies of *E. carotovora* isolates. Antibodies of isolate No. (a, b) 1 , (c, d) 2, (e, f) 3, (g, h) 4, (i, j) 5 and (k, l) 6 in the central holes, while the isolates antigens in the outer holes, C: Saline solution (control)

Crossed immunoelectrophoresis (CIE): The CIE and CIE with an intermediate gel were used as available and sensitive techniques for distinguishing between *E. carotovora* isolates. Data presented in Table 3 and Fig. 2 and 3 showed the following results.

Antibodies against *E. carotovora* isolate No. 1 gave 9, 6, 8, 8, 6 and 6 precipitin peaks when electrophorized with antigens of *E. carotovora* isolates No. 1, 2, 3, 4, 5 and 6, respectively. Also, antibodies against *E. carotovora* isolate No. 2 showed 6, 10, 8, 8, 9 and 7 precipitin peaks

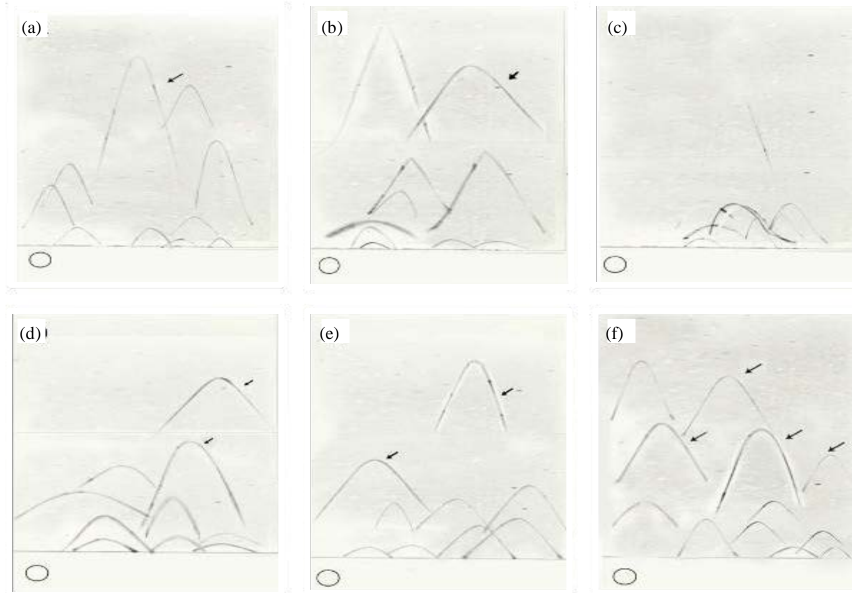

Fig. 2(a-f): Crossed immunoelectrophoresis (CIE) for the homologous serological reaction between antigens and antibodies of *E. carotovora* isolates No. (a) 1, (b) 2, (c) 3, (d) 4, (e) 5 and (f) 6, respectively. Anode was put on the right and the top. Arrows refer to the specific antigens of isolate

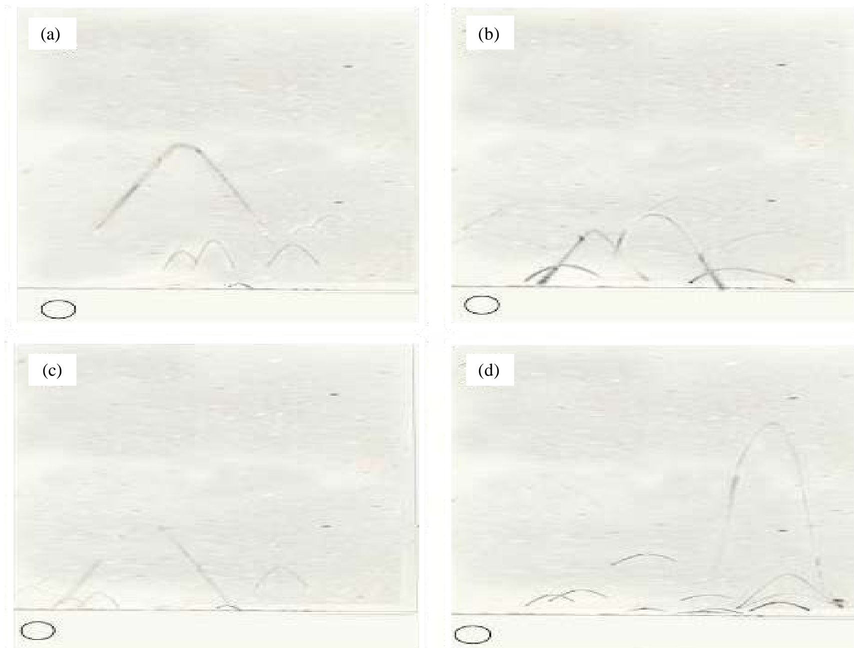

Fig. 3(a-d): Crossed immunoelectrophoresis (CIE) for the heterologous serological reactions between antigens and antibodies of *E. carotovora* isolates. Anode was put on the right and the top. (a) Antigens of isolate No. 1 electrophorized with antibodies of isolate No. 2, (b) Antigens of isolate No. 2 electrophorized with antibodies of isolate No. 3, (c) Antigens of isolate No. 3 electrophorized with antibodies of isolate No. 4 and (d) Antigens of isolate No. 4 electrophorized with antibodies of isolate No. 5 antibodies

Table 4: Number of precipitin peaks detected in cross absorption experiments between *Erwinia carotovora* isolates using crossed immunoelectrophoresis (CIE)

Absorbed antibodies of *E. carotovora* isolates	Antigens used for absorption	Antigen used for testing after absorption							
		1	2	3	4	5	6	Control	Specific antigens
1	1	0	0	0	0	0	0	0	1
	2	3	0	2	0	0	0	0	
	3	1	0	0	0	0	0	0	
	4	1	0	0	0	0	0	0	
	5	3	0	2	0	0	0	0	
	6	3	0	2	0	0	0	0	
2	1	0	4	2	2	3	1	0	1
	2	0	0	0	0	0	0	0	
	3	0	2	0	0	1	0	0	
	4	0	2	0	0	1	0	0	
	5	0	1	0	0	1	0	0	
	6	0	2	1	1	2	0	0	
3	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	
	3	0	0	3	0	0	0	0	
	4	3	3	0	0	3	3	0	
	5	0	0	0	0	0	0	0	
	6	0	0	0	0	0	0	0	
4	1	0	0	0	2	0	0	0	2
	2	0	0	0	2	0	0	0	
	3	3	3	0	5	3	1	0	
	4	0	0	0	0	0	0	0	
	5	0	0	0	2	0	0	0	
	6	2	2	0	4	2	0	0	
5	1	0	3	2	2	5	2	0	2
	2	0	0	0	0	2	0	0	
	3	0	1	0	0	3	0	0	
	4	0	1	0	0	3	0	0	
	5	0	0	0	0	0	0	0	
	6	0	1	0	0	3	0	0	
6	1	0	1	2	0	2	6	0	4
	2	0	0	1	0	1	5	0	
	3	0	0	0	0	0	4	0	
	4	0	1	2	0	2	6	0	
	5	0	0	0	0	0	0	0	
	6	0	0	0	0	0	0	0	

when electrophorized with antigens of *E. carotovora* isolates No. 1, 2, 3, 4, 5 and 6, respectively. Antibodies against *E. carotovora* isolate No. 3 formed 8, 8, 8, 5, 8 and 8 precipitin peaks when electrophorized with antigens of *E. carotovora* isolates No. 1, 2, 3, 4, 5 and 6, respectively. Antibodies against *E. carotovora* isolate No. 4 showed 8, 8, 5, 10, 8 and 6 precipitin peaks when electrophorized with antigens of *E. carotovora* isolates No. 1, 2, 3, 4, 5 and 6, respectively. Also antibodies against *E. carotovora* isolate No. 5 detected 6, 9, 8, 8, 11 and 8 precipitin peaks when electrophorized with antigens of *E. carotovora* isolates No. 1, 2, 3, 4, 5 and 6, respectively. Antibodies against *E. carotovora* isolate No. 6 formed 6, 7, 8, 6, 8 and 12 precipitin peaks when electrophorized with antigens of *E. carotovora* isolates No. 1, 2, 3, 4, 5 and 6, respectively.

The previous results using CIE clearly showed that at least five antigens were found to be common among *E. carotovora* isolates, while one antigen was found to be specific for each *E. carotovora* isolates (No. 1, 2). Two precipitin peaks (antigens) were found to be specific to *E. carotovora* isolates No. 4 and 5. Four antigens were found to be specific to *E. carotovora* isolate No. 6. On the other hand, no antigens were found to be specific to *E. carotovora* isolate No. 3.

Reciprocal analysis procedure: According to data obtained from the homologous and heterologous reactions using CIE and CIE with an intermediate gel (Table 2 and Fig. 2-4) the common and specific antigens of *E. carotovora* isolates were recorded in Table 4. One antigen was found to be specific for each *E. carotovora* isolates (No. 1 and 2), while two antigens were found to be specific for each *E. carotovora* isolates (No. 4 and 5). Four antigens were found to be specific to *E. carotovora* isolate No. 6. In CIE with an intermediate gel, the specific antigens were formed in the upper gel, while the common antigens were formed in the intermediate gel (Fig. 4). The Fig. 4 showed that one precipitin peak (antigen) was detected when the antigens of *E. carotovora* isolate No. 1 were electrophorized and intermediate gel contained antibodies of isolate No. 4,

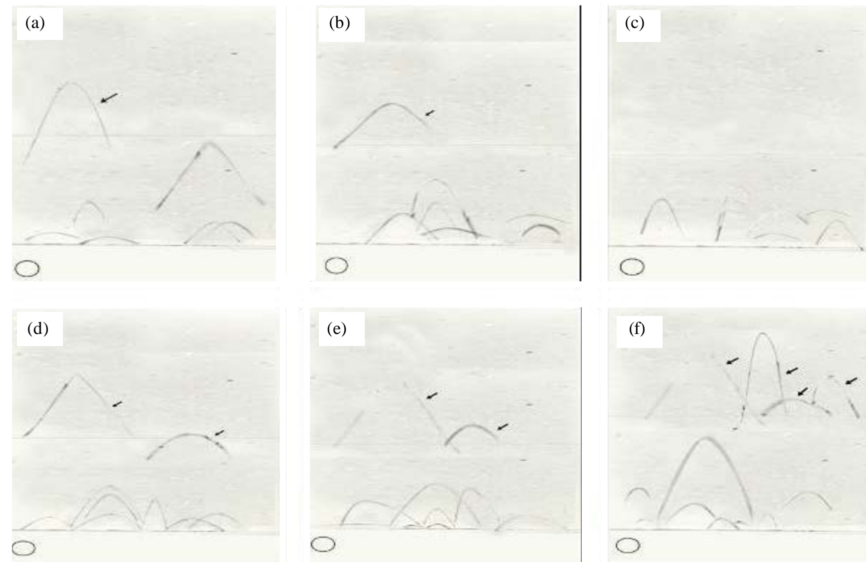

Fig. 4(a-f): Crossed immunoelectrophoresis (CIE) with an intermediate gel of *E. carotovora* isolates. Anode was put on the top and the right. Upper gel contains antibodies against the electrophorized antigens. Arrows refer to the specific antigens of isolate. (a) Antigens of isolate No. 1 electrophorized, intermediate gel contains antibodies of isolate No. 3, (b) Antigens of isolate No. 2 electrophorized, intermediate gel contains antibodies of isolate No. 3, (c) Antigens of isolate No. 3 electrophorized, intermediate gel contains antibodies of isolate No. 5, (d) Antigens of isolate No. 4 electrophorized, intermediate gel contains antibodies of isolate No. 2, (e) Antigens of isolate No. 5 electrophorized, intermediate gel contains antibodies of isolate No. 2 and (f) Antigens of isolate No. 6 electrophorized, intermediate gel contains antibodies of isolate No. 3

while the upper gel contained antibodies of isolate No. 1 (Fig. 4a). Also, another antigen was detected when antigens of isolate No. 2 were electrophorized, while the intermediate gel contained antibodies of isolate No. 5 (Fig. 4b). On the other hand, No specific antigens were observed with isolate No. 3, sine no precipitin peaks were detected in the upper gel (Fig. 4c). Two precipitin peaks (antigens) were formed in the upper gel when isolate No. 4 and 5 antigens were electrophorized against antibodies of isolate No. 2 in the intermediate gel (Fig. 4d, e). Four precipitin peaks (antigens) were detected in the upper gel when antigens of isolate No. 6 were electrophorized and intermediate gel contained antibodies of isolate No. 3 (Fig. 4f).

Common antigens between *E. carotovora* isolates and host plant using CIE: Antibodies against *E. carotovora* isolates No. 1 (moderately virulent), 3 (weakly virulent) and 6 (highly virulent) were electophorized with antigens of potato, tomato, squash and carrot plants. When the potato antigens were electrophorized with antibodies against *E. carotovora* isolates No. 1, 3 and 6, two, one and three precipitins peaks antigens were detected, respectively. The same results were obtained when squash antigens were electrophorized with antibodies against *E. carotovora* isolates. On the other hand, no antigens were detected between the antibodies against these isolates and carrot and tomato antigens (Table 5 and Fig. 5). The results showed that the number of common antigens between the pathogen and host may be associated to the degree of susceptibility of the host plant.

Table 5: Number of precipitin peaks detected between antibodies of *E. carotovora* isolates No. 1, 3 and 6 and antigens of potato, tomato, squash and carrot plants using Crossed immunoelectrophoresis (CIE)

Antibodies of *E. carotovora* isolates	Representative antigens				
	Potato	Tomato	Squash	Carrot	Control
1**	2	0	2	0	0
3*	1	0	1	0	0
6***	3	0	3	0	0

**Moderately virulent, *Weakly virulent, ***Highly virulent

Comparison between crossed immunoelectrophoresis (CIE) and double gel diffusion test (DGD): According to the data presented in Table 3 and 4 and showed the

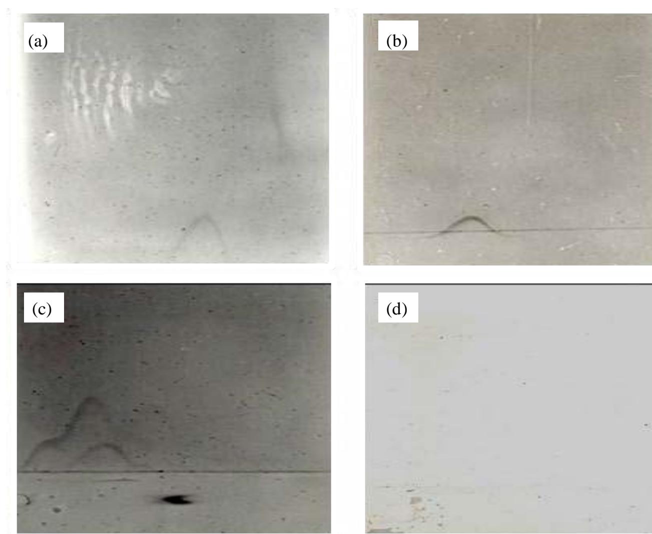

Fig. 5(a-d): Crossed immunoelectrophoresis (CIE) for the serological reactions between antibodies of *E. carotovora* isolates and antigens o f host plant. Anode was put on the top and the right. Arrows refer to the host-pathogen common antigens. (a) Antigens of potato leaves were electrophorized against antibodies of isolate No. 1, (b) Antigens of potato leaves were electrophorized against antibodies of isolate No. 3, (c) Antigens of potato leaves were electrophorized against isolate No. 6 and (d) Antigens of tomato leaves were electrophorized against antibodies of isolate No. 6

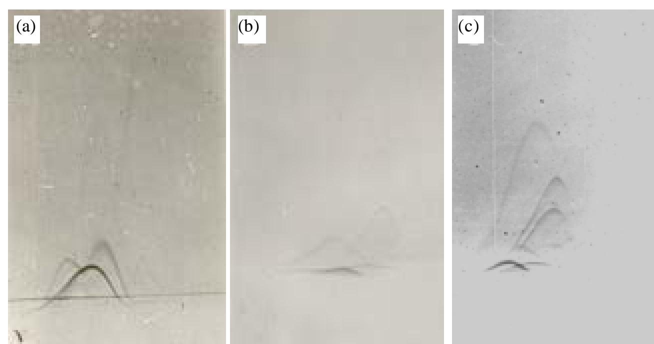

Fig. 6(a-c): Serodiagnosis of *E. carotovora* in naturally infected potato tubers using CIE. Antigens of infected potato tubers (oozes) were electrophorized with antibodies against *E. carotovora* isolates No. (a) 1, (b) 3 and (c) 6. Anode was put on the right and the top

comparison between CIE and DGD revealed that the numbers of precipitin peaks in CIE were almost twice the numbers of precipitin bands detected in DGD for the same reactions indicating that CIE is more sensitive, valuable and successful than DGD. Both CIE and DGD proved that the serological relationships between *E. carotovra* isolates No. 2, 5 and 6 were more closely than that found among the other tested isolates, also both the two techniques proved that each isolate of *E. carotovora* had specific antigens (except isolate No. 3).

Serodiagnosis of *Erwinia carotovora* in naturally infected potato tubers using CIE: Naturally infected potato tubers by *E. carotovora* were serologically tested using CIE. The oozes produced by the water-soft rot tissues were used as antigens and electrophorized with antibodies against *E. carotovora* isolates No. 1, 3 and 6, the number of detected precipitin peaks were 4, 3 and 5 peaks, respectively. No precipitin peaks were detected when the tissue extracts of healthy potato tubers were tested with antibodies against *E. carotovora* isolates (Fig. 6).

DISCUSSION

No doubt in the importance of potato as economically essential crop in the world. Infection by *E. carotovora* can occur worldwide in the field or in storage after harvesting. The preparations of antigens and immunization procedure for *R. solanacearum* were studied by many authors. Quinon *et al*. (1964), prepared antisera from rabbits immunized with tomato, ginger and bird of paradise strains of *P. solanacearum* and normal serum prepared from uninjected rabbit were tested against 3 strains employing the tube agglutination procedures. A two fold dilution series from 1:4 through 1:4096 was tested. Normal serum did not agglutinate ginger, tomato and bird of paradise strains of *P. solanacearum.* Agglutination test with antisera and strains indicated relationship among the 3 strains. In the current study, six isolates of *E. carotovora* were isolated from naturally infected potato tubers in different localities in Egypt. The isolates were tested morphologically, pathologically and serologically. The isolates showed similar results in the morphological and pathological tests and identified as *E. carotovora* according to the Garrity *et al*. (2005), while the isolates showed variation in their virulence. Serological studies using crossed immunoelectrophoresis (CIE) and Double Gel Diffusion (DGD) tests were done.

No one can deny the essential role of the serology in the field of plant pathology especially using sensitive, available and successful techniques such as double gel diffusion test (DGD) and crossed immunoelectrophoresis (CIE). Recently, Omar *et al.* (2006) found genetic diversity among isolates of *Spiroplasma citri* based on the crossed immunoelectrophoresis (CIE). The serological differences between *E. carotovora* isolates were demonstrated using DGD test. In the homologous reactions, 5, 6, 5, 6, 6 and 8 precipitin bands were detected with antigens of *E. carotovora* isolates no. 1, 2, 3, 4, 5 and 6, respectively. In the heterologous reactions, at least two antigens were found to be common among *E. carotovora* isolates. Some antigens were found to be specific for each isolate (except *E. carotovora* isolate No. 3). One antigen was found to be specific for *E. carotovora* isolates (No. 1, 2, 4 and 5), while two antigens were found specific to *E. carotovora* isolate No. 6. In the present study, CIE differentiated between *E. carotovora* isolates and detected certain specific antigens for each isolate (except isolate No. 3). Both of CIE and DGD showed serological differences between *E. carotovora* isolates. But the serological differences between *E. carotovora* isolates using CIE were clearer than in DGD. The obtained results by using CIE with *E. carotovora* isolates were emphasized by using reciprocal analysis procedure. The common and specific antigens for each isolate were clearly detected. Other investigators distinguished serologically between *E. carotovora* isolates and other *Erwinia* species (Tanii and Akai, 1975; Vruggink and Geesteranus, 1975; Mehiar *et al*., 1979). Also, in the other bacterial species the serological differences were demonstrated as in; *A. tumefaciens* (El-Kady and Sule, 1982), *Pseudomonas* spp. (El-Kady *et al*., 1986) and *S. scabies* (Omar *et al*., 1989). The present study found relationship between the host plant and the pathogen according to the number of common antigens detected. High number of antigens was found common between *E. carotovora* isolate No. 6 (highly virulent) and potato (or squash) plants, while no antigens were found common with tomato and carrot plants. This finding may explain the theory of "gene for gene" for flora. So, we can understand early the best cultivars for disease resistance (or at least less susceptible to disease) according to the number of common antigens detected. Also, the present study could detect the pathogen in the oozes of infected tubers serologically, since four, three and five antigens were detected between the oozes (as antigens) and antibodies against *E. carotovora* isolates No. 1, 3 and 6, respectively. Finally, more extensive specific serological studies needed to explain the mechanism of disease resistance in host-pathogen interactions.

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