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EFFICACY OF MOLECULAR DIAGNOSIS TO DETERMINE GENETIC DIVERSITY AMONG INDIGENOUS ISOLATES OF *Erwinia carotovora* UNDER EGYPTIAN CONDITION

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ABSTRACT: Thirty-Four isolates of *Erwinia carotovora* subspecies *carotovora* (Ecc) causing soft rot of potato, were collected from tubers of potato plants grown in North and East Egypt. The frequency of the isolated species of soft rot-associated bacteria, being 10, 10, 8 and 6 isolates from Governorates of Sinai, Sharqia, Gharbia and Beheira, respectively. The isolates were identified as Ecc by biochemical method and molecular identity then was confirmed by polymerase chain reaction (PCR) using specific primers *FEc1* and *REc1* which amplified the Eca-specific band of 690-733 bp. Pathogenicity test divided these 9 isolates into 3 aggressiveness groups, High infection (2 isolates), moderate infection (5 isolates) and low infection (2 isolates), receptively. These isolates were also characterized using the technique of Inter Simple Sequence Repeats polymerase chain reaction (ISSR-PCR) found that 61 amplified fragments, 56 were polymorphic (91.8%) and the percentage of polymorphism ranged from 100% (844_B) to 84.62% (HB_{12}); the fragment sizes were 224bp to 1.36 kb. The unweighted pair-group method with arithmetical average (UPGMA) cluster analysis based on pairwise genetic similarity coefficient revealed that the similarity between the selected 9 soft rot bacterial isolates ranged from 0.125 between Ec6 and Ec9 to 0.875 between Ec1, Ec3 and Ec4. The average of similarity among genotypes was 0.500 and divided the soft rot isolates into three well-defined clusters showing a great level of genetic diversity. However, these clusters were not specific to aggressiveness groups, origin or special potato variety. Isolates with different aggressiveness levels, originated from different potato varieties participate between the same clusters. This means that the isolates likely derived from the same source population and got scattered from one area to another through their hosts.

Key words: Potato (*Solanum tuberosum*) *Erwinia carotovora,* soft rot, genetic diversity, ISSR-PCR.

INTRODUCTION

In 2016, Egypt's potato (*Solanum tuberosum*) production - targeted within the River Delta within the North - has distended at a rate of over 5% a year. Between 1990 and 2016, annual output rose from 2.6 million tonnes to about 6.000.000 tonnes, creating Egypt Africa's Number one potato producer. Egypt conjointly ranks among the world's high potato exporters in 2016, exports destroyed over 380.000 tonnes of contemporary potatoes and 18.000 tonnes of frozen potato merchandise, destined principally for markets in Europe (**FAOSTAT, 2016**).

Soft rot area unit foremost diseases of potato that cause large losses to potato crop not solely within the field however additionally within the storage wherever the bacterium area unit transmitted from the pathologic tubers to the healthy ones. Multiple subsp. of *Erwinia*, together with (*Erwinia carotovora* subsp.

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atroseptica and *Erwinia carotovora* subsp. *carotovora* and *Erwinia carotovorum* subsp. *brasiliensis*). Ecc and Eca area unit the first eubacterium in control of soft decay of potato in temperate climates (**Pitman *et al.*, 2010**).

The genetic variability of Phyto-pathogenic bacteria are important to elucidate possible relationships between proven populations of the pathogen and the area from where they were originally isolated (**Scortichini, 2005**). Various techniques could be used to study genetic diversity present in a pathogen population. Inter Simple Sequence Repeats polymerase chain reaction (ISSR-PCR), using low strictness conditions and primers having short nucleotide sequences has been used efficiently to distinguish genetic diversity through some plant pathogenic bacteria (**Mello *et al.*, 2006)**.

The ISSR marker belongs to a class of multilocus, mostly dominant genetic markers that also include the amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) markers, and efficiency for studying diversity in pathogen populations **(Hsiang and Wu, 2000; Jawhar *et al.*, 2000)**.

Although the reproducibility of DNA fingerprints may be influenced by the reagents, thermocycler (**Tyler *et al.*, 1997**) and intensity of amplicons used to score the fingerprints (**Skroch and Nienhuis, 1995**), under well-established parameters the results can be very reproducible within a laboratory (**Toth *et al.*, 1999; Mello *et al.*, 2008**) evaluated a number of phenotypic and molecular typing techniques for determining diversity in *Erwinia carotovora* and concluded that among the molecular identified and ISSR analysis was the most discriminatory.

Erwinia carotovora is an important pathogen of potato in Egypt, especially the North and East part of the country. However, no research work has been done so far on the population structure of this important pathogen. Therefore, our long term goal has been to develop an effective disease management strategy and understanding the variation in the pathogen population is a must to achieve this goal. In this paper we report the pathogenic and genetic diversity found, using ISSR techniques, among the different isolates of *Erwinia carotovora* collected from different geographical areas of the North and East Egypt.

MATERIALS AND METHODS

Isolation and Purification of Bacteria Associated with Soft-rotted Tubers

Thirty-four samples of naturally infected potato tuber (*Solanum tuberosum* L.) were collected from four totally different farms situated in Sinai, Sharqia, Gharbia and Beheira Governorates. Infected tubers with soft rot bacteria *Erwinia carotovora* (Ec) were used (**Yuan *et al.*, 2004**).

Isolation and Pathogenicity Test

Potato tubers were surface disinfected by flaming, then cut exploitation with sterile knife into 1cm thick slices with virtually equal diameter. Every slice was placed on the surface of moistened sterilized paper into a Petri-dish. 0.5ml of bacterial inoculum of every isolate was used for artificial inoculation by pipetting at the middle of every slice. Three slices were used for every tested bacterial isolate likewise as management. All treatments were incubated at 30°±2°C for four days, then examined daily. Disease assessment of decayed tubers slices were expressed in keeping with 48 hours (**Lelliott and Stead, 1987**) as follow: (-): Negative infection, (+): Low positive infection, (++): Moderate infection, and (+++): High infection.

Bacterial DNA Extraction

To extract DNA, bacteria were grown overnight at 27± 2°C in 5ml LB broth in shaking incubator. Half ml of each isolate was poured in Eppendorf tubes, centrifuged at 7000 rpm at 4°C for 10 minutes, supernatants were discarded, and the pellets were resuspended in 100 µl 0.5N NaOH solution (**Wang *et al.*, 1993**). The suspension was centrifuged at 8000 rpm for 10 minutes, and supernatant was saved. For each isolate, 5µl of the supernatant were mixed with 45µl of 0.1 M TBE buffer and stored at 4°C until used. When needed, 3 µl of this mixture was directly added to the PCR tube.

Identification of the Selected Isolates

Identity of isolates was confirmed using biochemical tests (**Lelliott and Stead, 1987**) and molecular identified by subspecies-specific PCR primers (Table 1), was obtained from

Table 1. The sequences of forward and reverse used primers were as follows

Primer name	Sequences (5' to 3')	Target	Product size (bp)
FEc1	CGGCATCATAAAAACACG	*Erwinia carotovorum*	690-733
REc1	GCACACTTCATCCAGCGA		

Molecular Biology Lab., Agric. Research Park, Fac. Agric., Benha Univ., Qalubia, Egypt. The primers used in this study were synthesized by Thermo Fisher Scientific Inc. as described by **De Boer and Ward (1995)**.

DNA amplification was done according to the non-conventional method and the PCR reaction mix was in the final reaction volume of 25 µl contained; 5.0 µl 5X Taq buffer, 1.1 µl 25 mM $MgCl_2$, 0.5 µl 10 mM dNTPs, 0.13 µl 5 U/µl Taq polymerase, 1.25 µl 10 µM of each primer and 2.0 µl DNA template finalized to 25 µl by adding 13.77 dH_2O; PCR was performed in a thermal cycler T Professional (Biometra, Germany) using the following protocol and adjusted as needed. The reaction involved initial denaturation (94˚C, 5 min) followed by 35 cycles of denaturation (94˚C, 1 min), annealing (55˚C, 1 min), extension (72˚C, 1 min) with a final extension (72˚C, 7 min).

ISSR-PCR Technique

ISSR-PCR was carried out according to (**Welsh and McClelland, 1990; Williams *et al.*, 1990**) the primers used were 11 to 18 mer oligonucleotide; fifteen primers were evaluated. Only, five primers 814_A [$(CT)_8TG$)], 844_B [$(CT)_8$ GC],17898_A $(CA)_6AC$), HB_9 [$(GT)_6GG$] and HB_{12} [$(CAC)_3GC$] were selected for further molecular characterization of the (Ec) isolates because they amplified reproducible polymorphic bands for all the isolates.

PCR reactions were optimized and mixtures (25µl total volume) were composed of dNTPs (200 µM), $MgCl_2$ (1.5mM), 1x buffer, primer (0.2µM), DNA (50ng), Taq DNA polymerase (2 units). Amplification was carried out in a Biometra Cycler programmed for 94°C for 3 min (one cycle); followed by 94°C for 30sec,40°C for 45 sec and 72°C for 1 min (35 cycle), 72°C for 10 min (one cycle) then 4°C (infinitive).

Amplification products (25µl) were mixed with 3µl loading buffer and separated on 1.3% agarose gel and stained with 0.5 µg/ml ethidium bromide and visualized under ultraviolet light and photographed. DNA fragment sizes were determined by comparisons with the 1kb plus DNA ladder marker.

ISSR-PCR Analysis

Similarity coefficients were calculated according to dice matrix **(Nei and Li, 1979; Rohlf, 1993)**. Parents were grouped by cluster analysis with the similarity matrix and unweighted pair group method based on arithmetic mean (UPGMA).

RESULTS AND DISCUSSION

Isolation and Biochemical Identification of the Pathogenic Isolates

Biochemical assays for the isolated bacteria revealed that the 34 isolates are distributed among three species belonging to three genera grouped as *Erwinia* sp., *Bacillus* sp., *Pseudomonas* sp. At a frequency of 12, 13, and 9 isolates, respectively. Different governorates did not show much difference in the frequency of the isolated species of soft rot-associated bacteria, being 10, 10, 8 and 6 isolates at Sinai, Sharqia, Gharbia, and Beheira, respectively (Table 2).

Van derWolf and De Boer (2007) found that the main bacteria causing potato tuber soft rot are *Erwinia* sp., *P. carotovorum* subsp. *carotovorum*. *Pseudomonas syringae* van Hall found only two species of Gram-negative bacteria isolated from rotting potatoes collected from clamps in England in 1945-7 (**Jones and Dowson 1950**). The isolates of *Bacillus* spp. are thought to be saprophytic rather than pathogenic, as Bacilli are not previously reported to be the primary causal agents of soft rots. **Lund and Wyatt (1979)** highlighted that potato tubers may harbor pectolytic saprophytic bacteria (*Bacillus* spp., *Clostridium* spp., *Flavobacterium* spp. and *Pseudomonas* spp.) which, if given the chance, can also cause rotting (Table 3).

Table 2. Frequency of the isolated bacteria from potato tubers showing soft rot collected from four governorates

The isolated bacteria	Frequency of the isolated bacteria from				Total	Frequency (%)
	Sinia	Sharqia	Gharbia	Beheira		
***Erwinia* sp.**	4	3	3	2	12	35.3
***Bacillus* sp.**	3	4	3	3	13	38.2
***Pseudomonas* sp.**	3	3	2	1	9	26.5
Total	10	10	8	6	34	---

Table 3. Severity(*) of soft rot symptom on potato caused by confirmed isolates of *Erwinia carotovora*

Host species	Organ	Bacterial isolates								
		Ec1	Ec2	Ec3	Ec4	Ec5	Ec6	Ec7	Ec8	Ec9
Potato (*Solanum tuberosum)*	Tuber	+++	++	++	+	+++	+	++	++	++

*Severity of soft rot symptoms was assessed visually according to **Lelliott and Stead (1987)** as follows: (-): Negative infection, (+): Low infection, (++): Moderate infection, and (+++): High infection.

Pathogenicity Test

In the previous section, we showed that bacterial isolation trial from rotted potato tubers resulted in 9 bacterial isolates (Table 3). Results of pathogenicity assay showed that 9 of all the tested isolates were pathogenic on potato tubers. Out of them, only 2 isolates (Ec1 and Ec5) caused highly severe symptoms of soft rot on potato tubers, meanwhile, the remaining isolates (Ec2, Ec3, Ec7, Ec 8 and Ec9) showed moderate severity on potato tubers, while the Ec4 and Ec6 showed low infection.

Traditional identification of the 9 strains of the presumptive pathogen *Erwinia carotovorum* isolates resulted in the confirmation of all of them to be *Erwinia carotovorum* as they showed the following results: creamy, short rods, Gram negative, which did not have the ability to produce any pigment in Kings B medium. All the tested isolates were positive for motility, starch hydrolysis, growth at 37°C, growth in 5% NaCl, catalase activity, but negative for KOH 3%, pigment production. The strains also induced typical bacterial soft rot symptoms on tuber slices of cv. Spunta (Table 4).

Molecular Identification of the Pathogenic Isolates

Amplifications with the *FEc1* and *REc1* primers were positive in all strains (Ec1 to Ec9) and negative control B (-): *Bacillus* spp., P (-): *Pseudomonas* spp., respectively. Also, the *Fec1*and *Rec1* primers detected *Erwinia carotovorum* subsp. *carotovorum* DNA in the ten tested strains **(**Fig. 1). It found that PCR product was 690 – 733 bp long, which was the same size as the region on the *Erwinia carotovorum* subsp. *carotovorum* between the two primers. Results illustrated in (Figure 1) showed the sequence of this 690-733 bp PCR amplicon. These results were confirmed with those obtained by (**De Boer and Ward, 1995**) who proved that for definitive identification of *Erwinia carotovorum* strains.

Genetic Diversity of *Erwinia carotovorum* Strains

Inter simple sequence repeats (ISSR) polymorphism of *Erwinia carotovorum* isolates varied in their virulence on potato tubers

Amplification of soft rot (*Erwinia carotovora*) DNA from 9 bacterial strains were done with

Table 4. Biochemical characters of isolated bacteria (*Erwinia carotovorum*)

Identification test	Ec1	Ec2	Ec3	Ec4	Ec5	Ec6	Ec7	Ec8	Ec9
KOH 3%	-	-	-	-	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+	+	+
Tolerance NaCl (5%)	+	+	+	+	+	+	+	+	+
Catalase activity	+	+	+	+	+	+	+	+	+
Pigment production	-	-	-	-	-	-	-	-	-
Pectate degradation	+	+	+	+	+	+	+	+	+
Sucrose reduction	-	-	-	-	-	-	-	-	-
Acid prod. Gas glucose	+	+	+	+	+	+	+	+	+

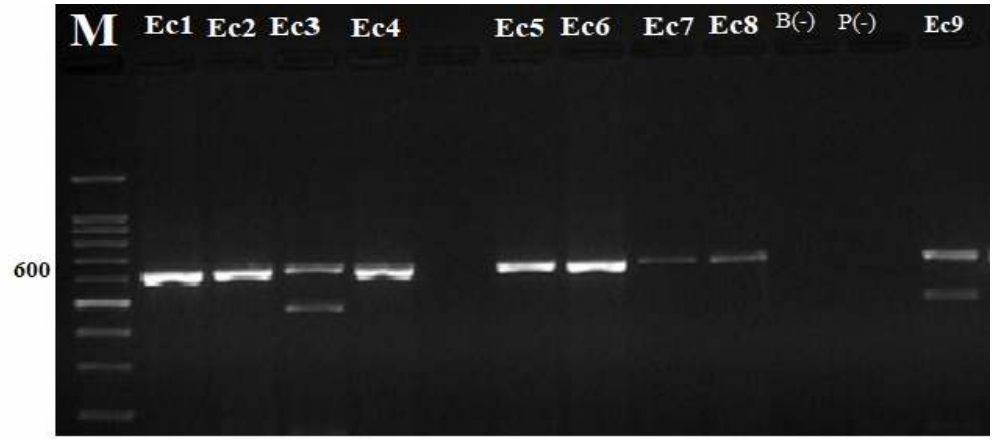

Fig. 1.Agarose gel electrophoresis for PCR amplified DNA of *Erwinia carotovorum* subsp. *carotovorum* isolates using *Fec1* and *Rec1* primers with the expected amplified product of 690 to 733 bp. Lane M represents Ladder 1.5 Kb. Lanes 1-4; isolates Ec1, Ec2, Ec3, Ec4, Lanes 6-9; Ec5, Ec6, Ec7, Ec8 and Lanes 12; Ec9, and negative control: E (-) *Erwinia carotovorum* subsp. *carotovorum,* B (-): *Bacillus* spp. and P (-): *Pseudomonas* spp., respectively.

five ISSR primers of 61 amplified fragments, 56 were polymorphic (91.8%) and the percentage of polymorphism ranged from 100% (844_B) to 84.62% (HB_{12}); the fragment sizes were 224 bp to 1.36 kb (Table 5 and Fig. 2).

The number of bands which obtained by each primer was 9 with 814_A primer, 16 with the 844_B primer, 12 with 17898A primer, 11 with both HB_9 primer and 13 with HB_{12} primer. The 844_B primer had the higher polymorphism (100%) but HB_{12} primer gave the lowest polymorphism (84.62%). Finally, 61 bands loci were amplified by ISSR primers which 56 loci of them were polymorphic and 5 loci were monomorphic.

Jaccard's Similarity Coefficient:

The UPGMA cluster analysis based on pairwise genetic similarity coefficient revealed that the similarity between the 9-soft rot bacterial isolates ranged from 0.125 between Ec6 and Ec9 to 0.875 between Ec1, Ec3 and Ec4; the average of similarity among genotypes was 0.500 (Table 6 and Fig. 3).

The dendrogram constructed from ISSR analysis of *Erwinia carotovora* isolates collected from geographically diverse zones of the North and East Egypt separated the 9 isolates into three major ISSR groups (Fig. 3).

Table 5. Levels of polymorphism, total number of bands, monomorphic bands, polymorphic bands and percentage of polymorphism as revealed by ISSR markers among the 9 *Erwinia carotovorum* Isolates

Code	ISSR primer	Nucleotide sequence 5' to 3'	Total number of bands	Monomorphic Bands	Polymorphic bands	Polymorphism (%)
ISSR1	814A	$(CT)_8TG$	9.0	1.0	8.0	88.89
ISSR2	844B	$(CT)_8GC$	16.0	0.0	16.0	100.0
ISSR3	17898A	$(CA)_6AC$	12.0	1.0	11.0	91.67
ISSR4	HB9	$(GT)_6GG$	11.0	1.0	10.0	90.90
ISSR5	HB12	$(CAC)_3GC$	13.0	2.0	11.0	84.61
Total			61.0	5.0	56.0	91.8

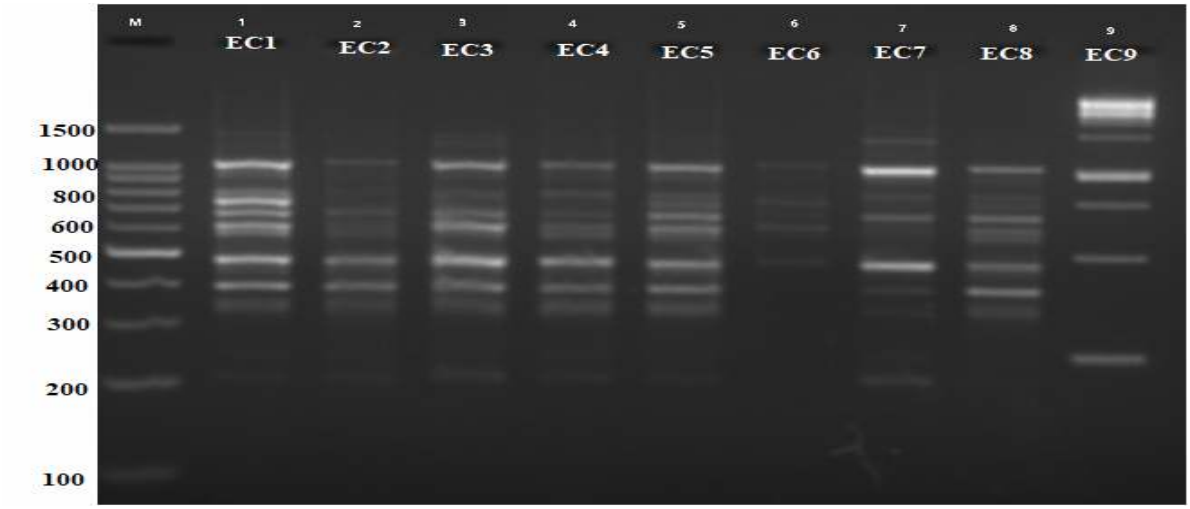

Fig. 2. Amplification profiles of 9 *Erwinia carotovorum* subsp. *carotovorum* isolates with primer 844_B. Lane 1-9 = isolates Ec1 to Ec9. M = size marker represents Ladder 1.5 Kb.

Table 6.Genetic distances among the nine bacterial Isolates as estimated using ISSR-PCR data

Isolate	Ec1	Ec2	Ec3	Ec4	Ec5	Ec6	Ec7	Ec8	Ec9
Ec1	1	0.556	0.875	0.778	0.417	0.500	0.250	0.231	0.083
Ec2		1	0.625	0.556	0.250	0.250	0.182	0.273	0.222
Ec3			1	0.875	0.455	0.375	0.273	0.250	0.091
Ec4				1	0.545	0.333	0.364	0.333	0.083
Ec5					1	0.083	0.455	0.545	0.077
Ec6						1	0.0	0.0	0.125
Ec7							1	0.500	0.200
Ec8								1	0.182
Ec9									1

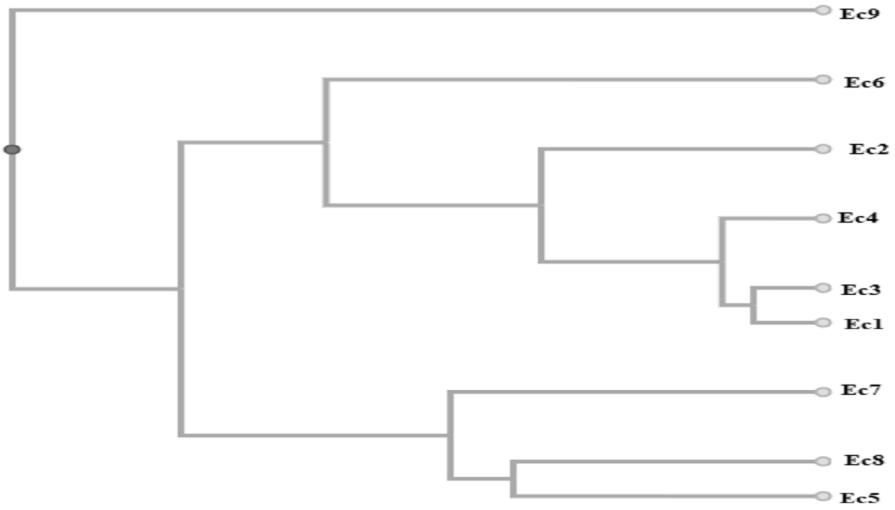

Fig. 3. Dendrogram showing genetic similarities among 9 Ec (*Erwinia carotovorum* subsp. *carotovorum)* isolates using the unweighted pair-group method with arithmetical average (UPGMA) of Genetyx software, (version 7.0)

The first group was formed by Ec 5, 8 and 7; Only Ec 7 and 8 belongs to "Moderate infection". The second group consisted of five *Erwinia carotovora* isolates, two isolates (Ec4 and Ec6) of which belongs to "low positive infection" and Ec2 and Ec3 strains to "Moderate infection"; Only one isolate (Ec1) of this group belong to "High infection". Finally, the isolate Ec9 formed the third group and only Ec1 strain of this group belong to "Moderate infection". Found the different within groups, the various bacterial strains diverged slight from each other. Never close relationship was observed among the ISSR and infection groups or between ISSR groups and geographic origin and conformable results were reported by **Uddin *et al.* (2013)**.

The actuality that various *Erwinia carotovora* isolates can cause different amounts of soft rot under similar experimental conditions propose that the population of the pathogen is not homogeneous and there is pathogenic variation between the isolates examined.

The less virulent isolates might have evolved from extremely virulent ones just by losing virulence gene clusters as is that the case of *P. wasabiae* isolates (**Kim *et al.*, 2009; Pitman *et al.*, 2010**). instead, the weakly virulent isolates might be due to the existence of lower virulent phenotypes in natural environments along with the aggressive strains, as a part of population diversity (**Yap *et al.*, 2004**). It can't be dominated out that these weakly virulent strains might possess a role in the survival and institution of the population underneath specific environmental conditions (**Yishay *et al.*, 2008**). **Yahiaoui-Zaidi *et al.* (2010**) found that *Erwinia carotovora* isolates were a lot of aggressive on their origin plant of than on alternative potato varieties. Our information showed that there was variation within the pathogenicity of *Erwinia carotovora*.

Similar results were reported by other researchers (**Arabi and Jawhar, 2007; Jawhar and Arabi, 2009**) studying different plant pathogens. This can be due to genotype-isolate interactions where different virulence genes are operating in the pathosystem (**Van der plank, 1984**).

ISSR analysis potentially provides information across the entire genome as it uses non-specific primers which bind randomly to regions over the whole genome. ISSR analysis would even detect smaller changes caused by point mutations, thus offering a higher degree of sensitivity as compared to that obtained by other methods. As compared to other related *Erwinia* sp. is relatively homogeneous (**Parent *et al*., 1996**). Such relatively low levels of genetic diversity may be due to a subspecies having more recent origins, limited geographical

distribution and limited host range. **Avrova *et al*., (2002)** however, found quite a high level of genetic diversity among 9 strains of *Erwinia carotovora* using amplified fragment length polymorphism. Other researchers studying genetic differences among isolates of enterobacteria on potatoes also demonstrated a greater diversity of pectolytic soft rot infecting potatoes than previously thought (**Oliveira *et al*., 2003; Yahiaoui-Zaidi *et al*., 2003; Yap *et al*., 2004**). Phylogentic trees of **Kim *et al*. (2009)** suggest that *Erwinia carotovora* subsp. *carotovora* is as variable as *Erwinia carotovora* subsp. *atroseptica*, if the branch lengths of the phylogenetic trees are considered as surrogates for diversity among strains.

REFERENCES

Arabi, M.I.E. and M. Jawhar (2007). Molecular and pathogenic variation identified among isolates of *Cochliobolus sativus*. Aust. Pl. Pathol., 36: 17-21.

Avrova, A.O., L.J. Hyman, R.L. Toth and I.K. Toth (2002). Application of amplified fragment length polymorphism fingerprinting for taxonomy and identification of the soft rot bacteria *Erwinia carotovora* and *Erwinia chrysanthemi*. Appld. and Envir. Microbiol., 68 (4): 1499-1508.

De-Boer, S.H. and L.J Ward (1995). PCR detection of *Erwinia carotovora* subsp. *atroseptica* associated with potato tissue. Phytopathol., 85: 854-858.

FAOSTAT (2016): International Year of the Potato. Food and Agriculture Organization of the United Nations (FAO). http:// www. fao.org/potato-2008/en/world/africa.html.

Hsiang, T. and C. Wu (2000). Genetic relationships of pathogenic *Typhula* species assessed by RAPD, ITS-RFLP and ITS sequencing. Mycol. Res., 104: 16-22.

Jawhar, M. and M.I.E. Arabi (2009). Heterogeneity in the internal transcribed spacers of the ribosomal DNA in *Cochliobolus sativus* as revealed by IRAP. J. Pl. Pathol., 91(1): 123-127.

Jawhar, M., R.S. Sangawn and M.I.E. Arabi. (2000). Identification of *Drechslera graminea* isolates by cultural characters and RAPD analysis. Cer. Res. Commun., 28: 89-93.

Jones, D.R. and W.J. Dowson (1950). On the bacteria responsible for soft rot in stored potatoes, and the reaction of the tuber to invasion by *Bacterium carotovorum* (Jones) Lehmann and Neumann." Ann. Appl. Biol., 37 (4): 563-569.

Kim, H.S., B. Ma, N.T. Perna and A.O. Charkowski (2009). Phylogeny and virulence of naturally occurring type III secretion system-deficient Pectobacterium strains. Appl. and Envir. Microbiol., 75: 4539-4549.

Lelliott, R.A. and D.E. Stead. (1987). Methods for the Diagnosis of Bacterial Diseases of Plants. Oxford, UK Blackwell Scientific Publications.

Lund, B.M. and G. Wyatt (1979). A method of testing the effect of antibacterial compounds on bacterial soft rot of potatoes, and results for preparations of dichlorophen and sodium hypochlorite. Potato Res., 22 (3):191-202.

Mello, A.F.S.,R.K. Yokomi, U. Melcher, J. Chen and J. Fletcher (2006). Assessment of genetic diversity in *Spiroplasma citri* by RAPD, rep-PCR and bacteriophage primers. (Abstr.) Phytopath. 96 (suppl.): S77.

Mello, A.F.S., R.K. Yokomi, U. Melcher, J.C. Chen, A.C. Wayadande and J. Fletcher. (2008). Genetic diversity of *Spiroplasma citri* strains from different regions, hosts, and isolation dates. Phytopath., 98: 960-968.

Nei, M. and W.H. Li (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci., 76: 5269-5273.

Oliveira, A.M.R., V. Duarte, J.R.P. Silveira and M.G. Moraes (2003). Incidence of pectolytic erwinias associated with blackleg of potato in Rio Grande do Sul. Fitopatol. Bras., 28: 49-53.

Parent, J.G., M. Lacroix, D. Page and L. Vezina (1996). Identification of *Erwinia carotovora* from soft rot diseased plants by random amplified polymorphic DNA (RAPD) analysis. Pl. Dis., 80: 494-499.

Pitman, A.R., A.H. Sally and B.V. Sandra (2010). Genetic characterization of Pectobacterium wasabiae causing soft rot disease of potato in New Zealand. Eur. J. Pl. Pathol., 126: 423 - 435.

Rohlf, F.J. (1993). NTSYS-pc numerical taxonomy and multivariate analysis system, version 1.70. Exeter Software, Setauket, New York.

Scortichini, M. (2005). The population structure of some plant pathogenic bacteria: an ecological and adaptive perspective. J. Pl. Pathol., 87: 5-12.

Skroch, P. and J. Nienhuis (1995). Impact of scoring error and reproducibility of RAPD data on RAPD based estimates of genetic distance. Theor. Appld. Genet., 91: 1086-1091.

Toth, I.K., Y. Bertheau, L.J. Hyman, L. Laplaze, M.M. Lopez , J. Mc. Nicol, F. Niepold, P. Persson, G.P.C. Salmond, A. Sletten, J.M. Vander-Wolf and M.C.M. Perombelon. (1999). Evaluation of phenotypic and molecular typing techniques for determining diversity in *Erwinia carotovora* subsp. *atroseptica*. Appld. Microbiol., 87: 770-781.

Tyler, K.D., G. Wang, S.D. Tyler and W.M. Johnson (1997). Factors affecting reliability and reproducibility of amplification-based DNA fingerprinting of representative bacterial pathogens. J. Clin. Microbiol., 35: 339-346.

Uddin, M., N. Saifullah, M. Ahmad, I. Ali, A. Iqbal and N.U. Khan (2013). Genetic characterization of *Verticillium chlamydosporium* isolated from Pakistan using random amplified polymorphic DNA (RAPD) primers. Pak. J. Bot., 45 (2): 467-472.

Van derWolf, J. and S. De Boer (2007). Bacterial pathogens of potato. In: Vreugdenhil D, Bradshaw J, Gebhardt C et al. (eds) Potato biology and biotechnology: advances and perspectives. Elsevier, Oxford, UK, 595–619.

Van-der plank, J.E. (1984). Disease Resistance in Plants. 2nd Ed. New York, USA, Acad. Press.

Wang, H., M. Qi and A.J. Cutler (1993). A simple method of preparing plant samples for PCR. Nuc. Acids Res., 21: 4153-4154.

Welsh, J. and M. McClelland (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res., 18: 7213-7218.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531- 6535.

Yahiaoui-Zaidi, R., B. Uan and D. Divon (2003). Biochemical and molecular diversity among Erwinia isolates from potato in Algeria. Pl. Pathol., 52: 28-40.

Yahiaoui-Zaidi, R., R. Ladjouzi and S. Benallaoua (2010). Pathogenic variability within biochemical groups of *Pectobacterium carotovorum* isolated in Algeria from seed potato tubers. Int. J. Biotech. and Mol. Biol. Res., 1 (1): 001-009.

Yap, M.N., J.D. Barak and A.O. Charkowski (2004). Genomic diversity of *Erwinia carotovora* subsp. *carotovora* and its correlation with virulence. Appl. Environ. Microbiol., 70: 3013-3023.

Yishay, M., B. Saul, V. Angel, L. Tal, O. Ron and Y. Iris (2008). Differential pathogenicity and genetic diversity among *Pectobacterium carotovorum* ssp. *carotovorum* isolates from monocot and dicot hosts support early genomic divergence within this taxon. Environ. Microbiol., 10 (10): 2746–2759.

Yuan, F., S. Qu, C. Cui, M. Cao and M.C. Qing (2004). A New Strain of *Erwinia carotovora* subsp. *carotovora* Isolated from Soft-rotted Chinese Cabbage. Acta Microbiologica Sinica, 44 (2): 136-140.

كفاءة التشخيص الجزيئي لتحديد التنوع الجيني بين عزلات جنس أرونيا كاروتفورا تحت الظروف المصرية

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تم جمع أربعة وثلاثين عزلة من فصائل جنس أرونيا كاروتفورا التى تتسبب في العفن الطرى فى البطاطس، من درنات نباتات البطاطس التي تزرع في شمال وشرق مصر و معدل تكرارالأنواع المعزولة من البكتيريا المسببة للتعفن الطرى فى البطاطس، وهي ١٠ و ١٠ و ٨ و ٦ عزلات في محافظات سيناء والشرقية والغربية والبحيرة على التوالي، تم تعريف وتوصيف العزلات، ٩ عزلات على أنها من جنس أرونيا كاروتفورا بواسطة الطريقة البيوكيميائية والتعريف الجزيئية ثم تم تأكيدها من خلال تفاعل البلمرة المتسلسل (PCR) باستخدام بادئات محددة FEc1 و REc1 التي تضخم النطاق الخاص بـ جنس أرونيا كاروتفورا من ٦٩٠-٧٣٣ بايت وقسمت الأختبارات القدرة المرضية لهذه العزلات التسع إلى ثلاث مجموعات مرضية ، عدوى عالية (٢ عزلة) ، عدوى معتدلة (٥ عزلات) وعدوى منخفضة (٢ عزلة) ، بشكل متوالى، وقد تم تمييز التنوع الوراثى بين هذه العزلات التسعة المنتخبة بأستخدام تقنية تفاعل البلمرة بين التكررات البسيطة المتسلسل (ISSR) حيث نتج عدد ٦١ شظية مضخمة منها ٥٦ شظية متعددة الأشكال بنسبة (٩١,٨%) ونسبة تعدد الأشكال تراوحت من ١٠٠% مع البادى (844B) إلى ٨٤,٦٢% مع البادى (HB12) وكانت أحجام الشظايا الوراثية المكبرة تتراوح من ٢٢٤بايت إلى ١,٣٦ كيلو بايت و قد أظهر تحليل العنقودى UPGMA على أساس معامل التشابه الوراثي للأزواج أن التشابه و القرابة بين التسع عزلات من بكتيرية التعفن الطري فى البطاطس المختارة تراوح بين ٠,١٦ بين Ec6 و Ec9 إلى ٠,٨٧٥ بين Ec1 و Ec3 وEc4 ؛ كان متوسط التشابه بين الأنماط الوراثية هو ٠,٥٠٠ وقسمت عزلات التعفن الطرى فى البطاطس إلى ثلاث مجموعات محددة جيدا تظهر مستوى عالى من التنوع الجيني، ومع ذلك، فإن هذه المجموعات لم تكن محددة لمجموعات القدرة المرضية، أو الأصل أو أنواع البطاطس الخاصة وتتشارك العزلات ذات مستويات القدرة المرضية المختلفة من أصناف بطاطس مختلفة تشارك بين نفس العناقيد، وهذا يعني أن العزلات من المحتمل أن تكون مستمدة من نفس العشيرة وتتأثر من منطقة إلى أخرى عبر العوائل المرضية المختلفة.

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