Identification and Pathogenicity of Phytopathogenic Bacteria associated with soft rot disease on some Potato Cultivars

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Received on: 23/10/2016 *Accepted: 28/12/2016*

ABSTRACT

Pathogenicity of potato soft rot and blackleg caused by *Pectobacterium*, *Dickeya* and *Enterobacter* pathogens were investigated in this study. Twenty three soft rot bacterial isolates were obtained from different locations of North West of Egypt. Pathogenicity assessment was resulted in rotting on potato slices with tested isolates. Also pathogenicity was performed on seedling (Cara, Spunta and Ladybalfor cvs), showed tubers soft rot followed by leaves chlorosis and wilting as well as blackleg. Identification based on morphological, differential media, physiological and biochemical characteristics were proved that tested isolates belonging to *Pectobacterium* (Pcc), *Dickeya* and *Enterobacter*. Disease severity was estimated as percentage to express disease index of rotted tissue weight of tubers (cv.Cara, Agria, Bern, Diamont, Ladybalfor and Valor). Results indicated that Pcc isolates AS1 and AC5 were the most aggressive isolates and with highest percentage 26.21%, 23.34% respectively of infection, while Pcc KM15 isolate exhibited weak infection (18.17%).Whereas, no significant differences were found among isolates of *Dickeya* sp. in disease index. Potato cultivar Agria was the most sensitive versus Cara cv. was the most resistant to *Enterobacter* (KM23) isolate. Overall, Cara cultivar was more resistant to the infection by all tested isolates than other tested cultivars. On the other hand Valor and Ladypalfor cvs. were more susceptible of tested soft rot bacterial isolates.

Key words: *Pectobacterium*, *Dickeya*, *Enterobacter*, Soft rot, Blackleg, Disease severity, Disease index, Potato Cultivars.

INTRODUCTION

Potato (*Solanum tuberosum*, L.) is one of the most important vegetable crops in Egypt. Potato production of approximately 4,800,000 tons, produced from approximately 178,000 hectares, making Egypt Africa's biggest potato producer (FAO STAT, 2013). The soft rot *Enterobacteriaceae* (SRE) *Pectobacterium* and *Dickeya* species cause soft rot diseases on potato and other horticultural crops (Gardan *et al*., 2003). They affect the growing potato causing blackleg and they are responsible for tuber soft rot in storages thereby reducing yield and quality.

Pectobacterium, *Dickeya* and *Enterobacter* species are among the best characterized potato pathogens. They cause foliage diseases known as blackleg, aerial stem rot and stem wet rot as well as soft rot in tubers (van der Wolf and De Boer, 2007; Ashmawy *et al*, 2015). Blackleg disease caused by *P. atrosepticum*, *P.carotovorum* subsp. *brasiliensis*, *P. wasabiae* and *Dickeya* species appears as a slimy, wet and black rot lesion that spreads from the rotting mother tuber to the stems under humid conditions (Duarte *et al*., 2004; Pitman *et al*., 2010).

Identification of the soft rot erwinias, especially for *Dickeya* (=*E.chrysanthemi*) and *Pectobacterium* (=*E. carotovora*) had been performed for many years mainly based on biochemical tests, physiological assays and host range. The biochemical tests were used as a standard method for the erwinia identification (Dye, 1968 and Dye, 1969). Until now, the methods have still been widely performed by many scientists to differentiate *Pectobacterium* and *Dickeya* species from other bacteria (Ma *et al*., 2007).

The objectives of this study were to isolate soft rot bacteria from potato samples in cultivation areas in North West of Egypt; to identify and differentiate among soft rot bacterial isolates by biochemical tests and physiological assays; to determinate the disease severity of different potato cultivars.

MATERIALS AND METHODS

Isolation of soft rot bacteria

Naturally infected potato tuber and stem samples showing typical symptoms of soft rot or blackleg disease were collected from fields and stores from different locations of North West of Egypt. Diseased tubers were first washed with tap water then surface sterilized with 1% sodium hypochlorite solution (NaOCl) for 3 min then rinsed thoroughly 3 times with sterilized distilled water; the rotted tissues of tuber were put into sterilized mortar and homogenized then left to stand for 20 min then a loopful of the resulting suspension was streaked into plates containing Glycerol nutrient agar (GNA) medium according to Abo-El-Dahab and El-Goorani. (1969).

Pathogenicity test

Potato slices

All the bacterial isolates were tested for pectinolytic activity on potato tuber slices (Diamont

cv.). Development of rot on the slices was examined 24 h. after incubation at 25ºC (Ngadze, *et al*., 2012). Tubers were treated with sterile distilled water as control.

Potato planted cultivars in the greenhouse

Soft rot bacterial isolates *Pectobacterium* (AS1) isolate and *Dickeya* spp. (MS21) isolate were tested for pathogenicity on potato cultivars Cara, Ladybalfor and Spunta. Surfaces of the aforementioned cultivar potato tubers were sterilized with 1% sodium hypochlorite for five minutes washed with sterile water and planted in plastic pots 15 cm diameter filled with sterile peat moss and clay (one tuber per pot). When plants reached 15-20 cm in length, stems were inoculated by forcing a sterilized needle through to a depth of 0.25 ml and inject a drop of *Dickeya* suspension ca.10^9 CFU/mL (A_{600}, 0.5) into the stem at a height of 5 cm above the soil level (Prior and Steva, 1990), while injection with ca.10^8 CFU/mL (A_{600}, 0.4) of Pcc isolate into tuber directly inoculated Plants were placed in a greenhouse at 25 $\pm$ 2ºC. Three replicates were used and plants injected with sterile distilled water served as control.

Classical identification methods of soft rot bacteria

With morphological, physiological and biochemical tests, single colonies of each isolate of total 23 isolates were identified according to Dye, (1969); Cowan, (1974); Klement *et al*, (1990); Staly *et al*, (2005).

Differential media were used for identification of bacteria isolates. Logan's medium was used for *Dickeya* spp. (Ds) and *P. cartovorum* subsp. *cartovorum* (Pcc) (Fahy and Hayward, 1983), King's B medium for *Pseudomonas* (Saettler *et al*., 1989; Kelman and Dickey, 1988; Lelliott and Stead, 1987), Levure Peptone Glucose Agar medium (LPGA) for Pcc (Kettani-Halabi *et al*., 2013; Amdan *et al*., 2015) and Nutrient agar-Glycerol-Manganese chloride(NGM) for *Dickeya* spp. (Lee and Yu, 2005).

Disease severity

Disease severity was estimated according to Yaganza *et al*., (2004), as percentage of rotted tissue weight according to the change of tuber weight before and after treatment divided on weight of tuber before treatment as following formula:

PDI = (W1-W2)/W1 ×100

Whereas: PDI = percentage of disease severity index, W1= weight of whole tuber before treatment and W2= weight of tuber after removal of the rotten tissue.

The soft rot bacterial isolates were determined by inoculating intact unblemished healthy potato tubers of six cultivars (Cara, Agria, Bern, Diamont, Ladybalfor and Valor). This was done by surfaced-sterilized potato tuber for 10 min with 1% (v/v) sodium hypochlorite solution, rinsed thoroughly, and allowed to air dry. For each isolate, 3 tubers were cutting in half and a hole was made in half tuber center approximately 1 cm deep and 1 cm wide with a sterilized cork borer (1 cm in diameter), and 100 µL of ca. 10^8 CFU/mL bacterial suspensions prepared from 24 h and then placed into the wound (Marquez-Villavicencio *et al*., 2011). Sterile distilled water was used to inoculate negative controls. Potato tubers were placed randomized in plastic trays supplemented with sterilized moist cotton to maintain high humidity, and incubated for 48 h at 25+2°C after inoculation. Statistical analysis was made using SAS program v. 9.2. Least significant differences (LSD) were used to separate mean differences and to rank isolates (SAS Inc., 2009).

RESULTS

Isolation and pathogenicity assessment of soft rot bacteria

Twenty one isolates of soft rot bacteria were isolated from naturally infected potato cultivars tubers collected from fields and cold storage houses and 2 isolates were obtained from stems showed soft rotted and blackleg symptoms collected from fields. The locations included; Alexandria (8 isolates), Kafr El-Sheikh (10 isolates) and Matrouh Governorates (5 isolates) demonstrated in (Fig.1, Table 1).

All tested isolates observed as rotting of the entire thickness of potato slices within 24h. Results in Fig. (2) showed the artificially inoculation of potato cultivars (Cara, Spunta and Ladybalfor) with AS1 and MS21 isolates in the greenhouse.The symptoms appear as plant wilting and tuber soft rot after 4 days of inoculation with AS1 isolate, while recorded a stem blackleg and rotted potato tuber after one week by MS21 isolate. Whereas, Cara cv. was more resistant for the infection by PS1 isolate than MS21 isolate, the leaves wilt after one week post inoculation by AS1, while stem blackleg and plant wilt observed after 10 days with MS21 isolate.

Identification of soft rot bacteria based on classical methods

Results of morphological, physiological and biochemical characteristics of the tested pectinolytic bacterial isolates are presented in Table (2). Out of 23 bacterial isolates, 19 were identified as *Pectobacterium cartovorum*, in addition 3 isolates as *Dickeya* sp. and one isolate of *Enterobacter* sp..

Characteristic features of colonies on differential media used for identifying *P. carotovorum* subsp. *carotovorum* on LPGA medium, showed smooth and light-yellow colonies, while on Logan's medium showed small colonies with a light red centre. Colonies of *Dickeya* sp. isolates were dark red colonies on Logan's medium. Colonies of *Dickeya* sp. on NGM medium produced blue brownish pigment called indigoidine, which differentiate it from *Pectobacterium* spp. colonies that lack of indigoidine production.

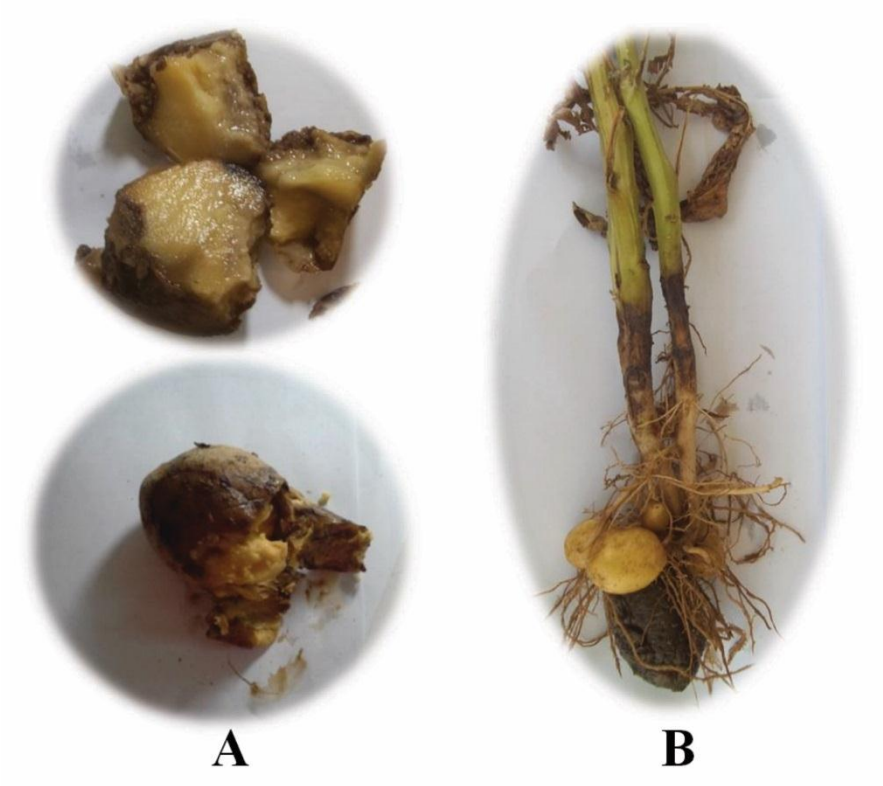

Fig.1: Natural infection of potato by phytopathogenic bacteria: Potato tubers soft rot symptoms (A) and aerial stem rot and blackleg symptoms (B).

Table 1: Source of bacterial isolates used in this study

Isolates Code	Potato part	Cultivar	Location
AS1	tuber	Spunta	Alexandria
AS2	"	"	"
AK3	"	Klose	"
AK4	"	"	"
AC5	"	Cara	"
AC6	"	"	"
AH7	"	Hermes	"
AH8	"	"	"
KM9	"	Mondial	Kafr El-Sheikh
KM10	"	"	"
KM11	"	"	"
KM12	"	"	"
KM13	"	"	"
KM14	"	"	"
KM15	"	"	"
KM16	"	"	"
KM17	"	"	"
MS18	"	Spunta	Matrouh
MS19	"	"	"
MS20	Stem	"	"
MS21	"	"	"
MS22	tuber	"	"
KM23	"	Mondial	Kafr El-sheikh

Table 2: Morphological traits, physiological and biochemical reactions of soft rot bacterial isolates

Bacteria	Isolates code	Characteristic																
		Shape (rods)	Gram staining	Motility	aerobic growth	Potato soft rot	Growth at 37°C	Gelatin liquefaction	Production of indole	Sensitivity to erythromycin	Growth in 5% NaCl	Glucose	Fractose	Maltose	Lactose	Soucrose	Sorbitol	α-methyle glucoside
Pectobacterium carotovorum	AS1	+	-	+	+	+	+	+	-	-	+	A/G	A	-	A	A	-	-
	AS2	+	-	+	+	+	+	+	-	-	+	A	A/G	-	A	A	-	-
	AK3	+	-	+	+	+	+	+	-	-	-	A	A/G	-	A	A	-	-
	AK4	+	-	+	+	+	+	+	-	-	-	A	A	-	A	A	-	-
	AC5	+	-	+	+	+	+	+	-	-	+	A/G	A	A	A	A	-	A
	AC6	+	-	+	+	+	+	+	-	-	+	-	-	A	A	A	-	A
	AH7	+	-	+	+	+	+	+	-	-	+	A	A	-	A	A	-	-
	AH8	+	-	+	+	+	+	+	-	-	+	A	A/G	A	A	A	-	A
	KM9	+	-	+	+	+	+	+	-	-	+	-	-	-	A	A	-	-
	KM10	+	-	+	+	+	+	+	-	-	+	A/G	-	-	A	A	-	-
	KM11	+	-	+	+	+	+	+	-	-	+	A	A	-	A	A	-	-
	KM12	+	-	+	+	+	+	+	-	-	+	A/G	A/G	-	A	A	-	A
	KM13	+	-	+	+	+	+	+	-	-	+	A	A	-	A	A	-	-
	KM14	+	-	+	+	+	+	+	-	-	+	A/G	A/G	-	A	A	-	-
	KM15	+	-	+	+	+	+	+	-	-	+	A/G	A/G	-	A	A	-	-
	KM16	+	-	+	+	+	+	+	-	-	+	A	A/G	-	A	A	-	-
	KM17	+	-	+	+	+	+	+	-	-	+	A/G	A/G	-	A	A	-	-
	MS18	+	-	+	+	+	+	+	-	-	+	-	-	A	A	A	-	A
	MS19	+	-	+	+	+	+	+	-	-	+	A/G	A	A	A	A	-	-
Ds	MS20	+	-	+	+	+	+	+	+	+	+	-	-	A	A	A	-	-
	MS21	+	-	+	+	+	+	+	+	+	+	A/G	A	A	A	A	-	-
	MS22	+	-	+	+	+	+	+	+	+	+	A	-	A	A	A	-	-
En	KM23	+	-	+	+	+	+	+	-	-	+	A/G	A	A	A	A	-	A

+ = More than 80% of isolates gave positive reaction; - = Less than 20% of isolates gave negative reaction; A=acid and G=gas

Disease Severity

Results in Figure (3) showed differential severity of soft rot symptoms among tested isolates on potato cultivars (Cara, Agria, Bern, Diamont, Ladybalfor and Valor)**.**

Presented data in Table (3) showed the mean effect of disease severity of 19 *P. carotovorum* isolates. The isolate AS1 gave the highest disease index (26.21%) followed by isolate AC5 (23.34%) and Alexandria isolate AS2 (22.84%). Meanwhile isolates KM15 and KM9 observed the least disease index, 18.17 and 18.29% respectively with no significant difference between them. Whereas the mean effect of potato cultivars showed different levels of sensitivity to infection by Pcc isolates. Potato cultivars Valor and Ladybalfor were affected more than Cara cultivar. In addition, results indicated that Valor and Ladybalfor cultivars were more susceptible to infection by isolates of Pcc (AH7, AS1 and AS2) than the other cultivars. While, the cultivar Cara was highly resistant to infection by isolates Pcc (AK3 and KM15). In this respect, Agria, Diamont and Bern cultivars were moderately susceptible for infection by the Pcc isolates.

No significant differences were found between *Dickeya* sp. isolates in disease index as shown in Table (4), while data of cultivars mean effect showed different degrees in susceptibility of potato cultivars to *Dickeya* sp. isolates. On the other hand, Cara cultivar exhibited resistance than Ladybalfor and Diamont cultivars of infection, whereas, Agria,Valor and Bern cultivars were moderately susceptible. The interaction between 6 potato cultivars and 3 *Dickeya* sp. isolates showed that MS21 isolate had the highest disease index (33.66%) with Ladybalfor cv. and least disease index (7.83%) with Cara cv.

Data present in Table (5) indicated that significant differences between potato cultivars and *Enterobacter* isolate. Agria cultivar was highly susceptible and Cara cv. was the highly resistant to KM23 isolate.

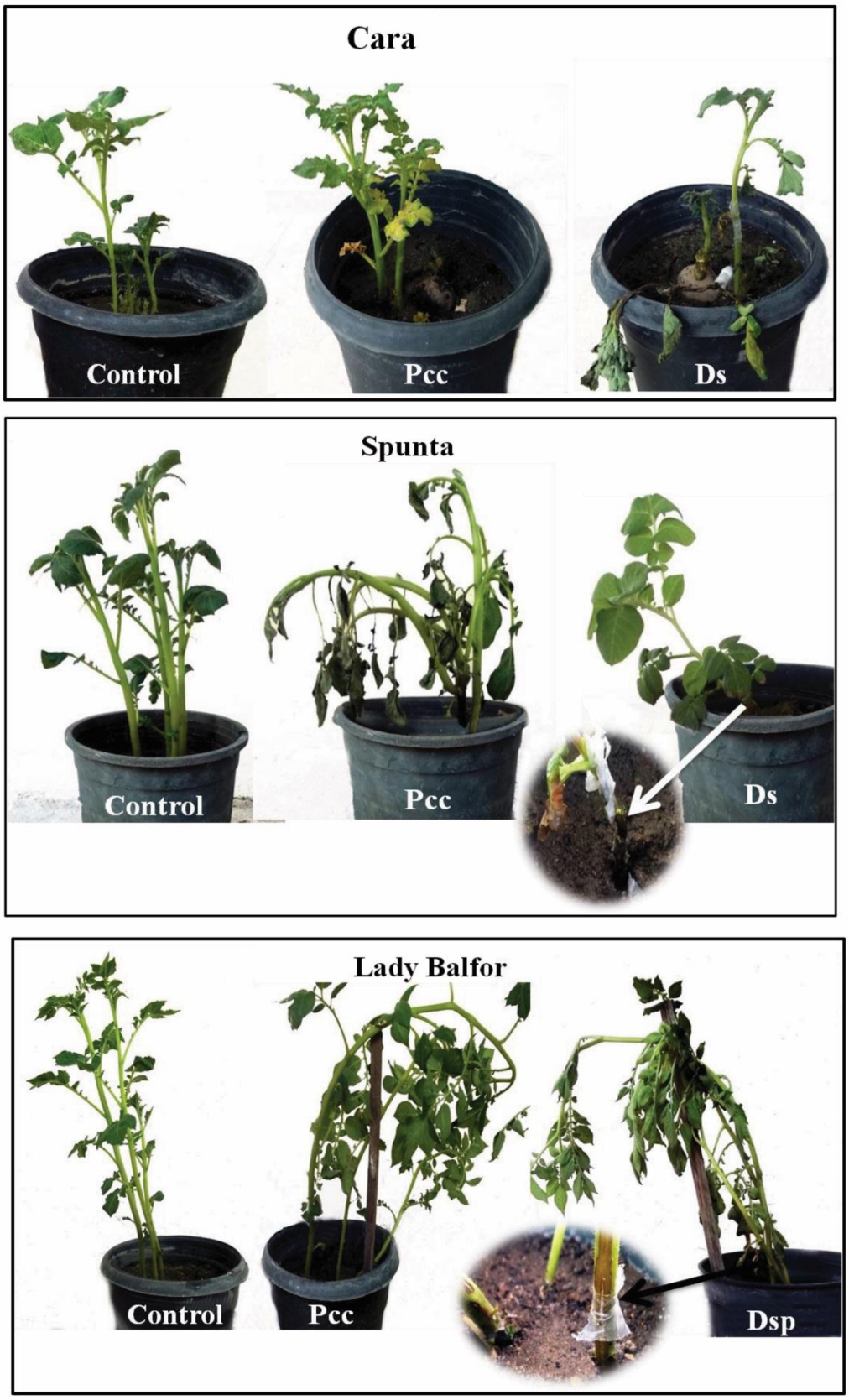

Fig. 2: Artificially inoculated potato tuber and aerial stems of Cara, Spunta and Ladybalfor cultivars with Pcc AS1 and Ds MS21 isolates

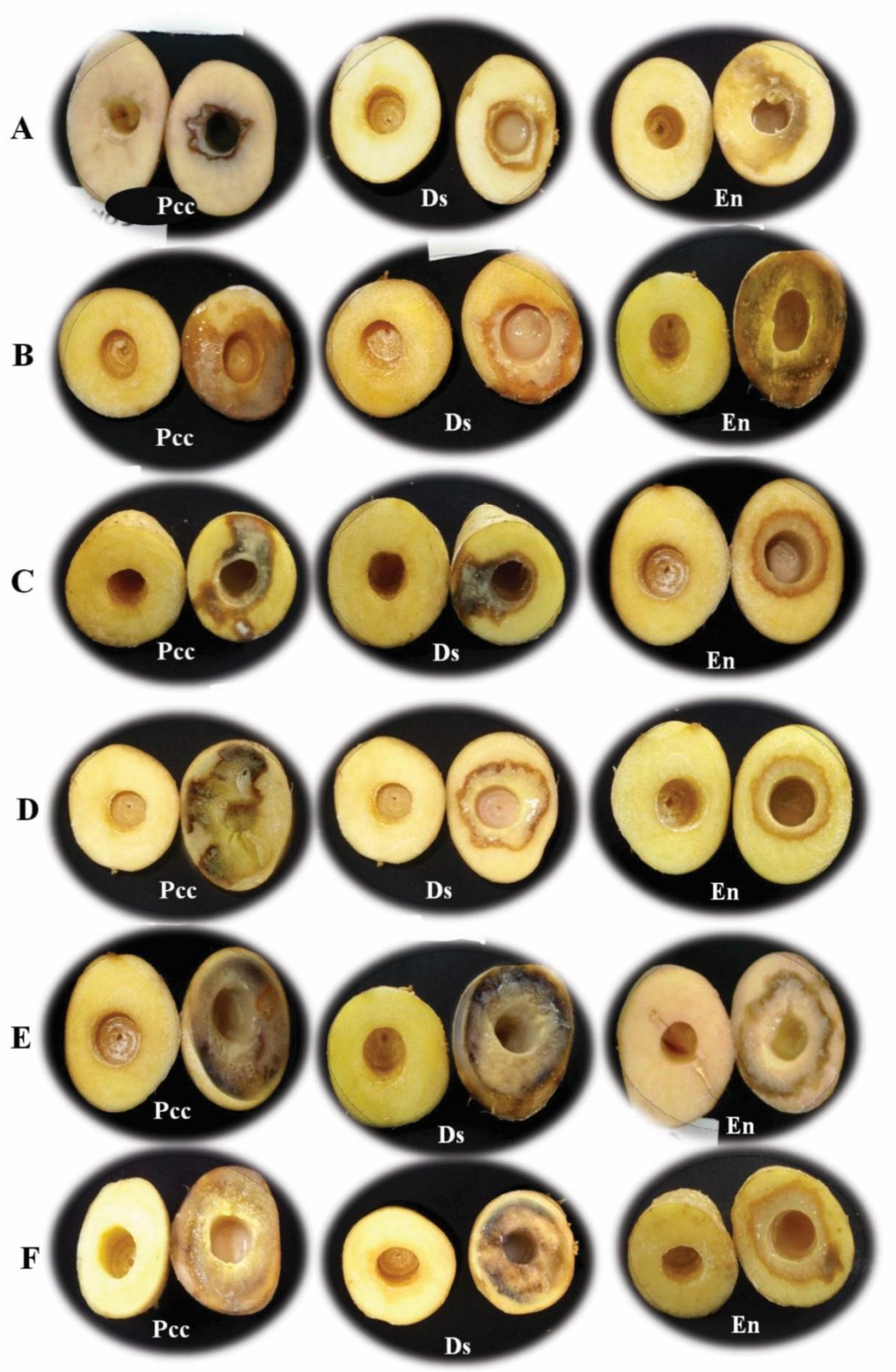

Fig. 3. Artificially inoculated potato tubers with different sot rot bacterial isolates: *Pectobacterium cartovorum* (Pcc), *Dickeya* sp. (Ds) and *Enterobacter* (En) showed soft rot symptoms on different cultivars: A, Cara; B, Agria; C, Bern; D, Diamont; E, Ladybalfor and F, Valor

Table 3: Artificially inoculated tubers of potato cultivars interaction with *Pectobacterium cartovorum* isolates

Potato Cultivars / Isolates code	Cara	Agria	Bern	Diamont	Lady balfor	Valor	Isolates Mean
AS1	*8 ^{IJ}	28.1 ^{ej}	21.53 ^{oy}	33.8 ^{ac}	30.13 ^{df}	35.7 ^{a}	26.21 ^{a}
AS2	9.2 ^{IJ}	33.03 ^{ad}	10.76 ^{EK}	17.93 ^{xc}	33.1 ^{ad}	33.03 ^{ad}	22.84 ^{bc}
AK3	7.56 ^{JK}	19.83 ^{rz}	24 ^{jr}	23 ^{lu}	24.5 ^{iq}	28.73 ^{di}	21.27 ^{bcde}
AK4	8.9 ^{IJ}	18.63 ^{tb}	9.43 ^{HK}	13.9 ^{CH}	29.5 ^{cg}	32.23 ^{ae}	18.76 ^{de}
AC5	10.26 ^{EK}	32.16 ^{ae}	20.83 ^{oz}	16.8 ^{zc}	29.33 ^{ch}	30.66 ^{bf}	23.34 ^{ab}
AC6	9 ^{IJ}	32.96 ^{ad}	10.33 ^{EK}	27.26 ^{fl}	21.3 ^{oz}	25.1 ^{go}	20.99 ^{bcde}
AH7	7.96 ^{IJ}	22.73 ^{lv}	11.16 ^{EK}	32 ^{ae}	26.93 ^{fm}	36.3 ^{a}	22.85 ^{bc}
AH8	10 ^{HK}	17.9 ^{yc}	26.33 ^{fm}	18.7 ^{ta}	29.73 ^{cf}	19.06 ^{sa}	20.28 ^{bcde}
KM9	10.2 ^{HK}	20.83 ^{oz}	10.13 ^{HK}	21.13 ^{oz}	19.7 ^{rz}	27.76 ^{ek}	18.29 ^{e}
KM10	8.33 ^{IJ}	29.26 ^{ch}	18.6 ^{tb}	21.03 ^{oz}	22 ^{ny}	20.1 ^{qz}	19.88 ^{cde}
KM11	10.7 ^{EK}	25 ^{gp}	16.73 ^{zd}	24.1 ^{jr}	24.86 ^{hp}	28.23 ^{ej}	21.6 ^{bcd}
KM12	8.4 ^{IJ}	21.96 ^{ny}	14.66 ^{AF}	14.76 ^{AE}	18.43 ^{uc}	31.9 ^{ae}	18.36 ^{de}
KM13	9.86 ^{HK}	22.6 ^{mw}	9.66 ^{HK}	22.5 ^{mx}	23.43 ^{ks}	34.9 ^{ab}	20.49 ^{bcde}
KM14	8.96 ^{IJ}	21.16 ^{oz}	22.66 ^{mw}	18.93 ^{sa}	19.73 ^{rz}	24.53 ^{iq}	19.33 ^{de}
KM15	7.23 ^{K}	14.06 ^{BG}	18.1 ^{wc}	18.36 ^{vc}	30.33 ^{bf}	20.96 ^{oz}	18.17 ^{e}
KM16	8.3 ^{IJ}	20.5 ^{pz}	11.86 ^{EJ}	27.06 ^{fm}	20.66 ^{oz}	30.3 ^{cf}	19.78 ^{cde}
KM17	11.46 ^{EK}	17.7 ^{yc}	19 ^{sa}	17.63 ^{yc}	21 ^{oz}	26.13 ^{fm}	18.82 ^{de}
MS18	8.33 ^{IJ}	29.56 ^{cg}	9.36 ^{HK}	19.36 ^{sz}	23.13 ^{lt}	28.56 ^{dj}	19.27 ^{cde}
MS19	12.16 ^{DI}	24.46 ^{iq}	12.2 ^{DI}	18.4 ^{vc}	22.2 ^{ny}	27.73 ^{ek}	19.52 ^{de}
Control	0.00	0.00	0.00	0.00	0.00	0.00	
Cultivar Mean	9.20 ^{e}	23.81 ^{b}	15.65 ^{d}	21.4 ^{c}	24.73 ^{c}	28.52 ^{a}	

* Data were average of three replicates.
Means with the same letter are not significantly different

Table 4: Artificially inoculated tubers of potato cultivars interaction with *Dickeya* sp. isolates.

Potato Cultivars / Isolates code	Cara	Agria	Bern	Diamont	Lady balfor	Valor	Isolates Mean
MS20	* 9.8 ^{j}	28 ^{abcd}	10.63 ^{hij}	27.66 ^{abcd}	30.56 ^{ab}	21.66 ^{defg}	20.77 ^{a}
MS21	7.83 ^{j}	13.26 ^{hij}	11.83 ^{hij}	24.16 ^{bcde}	33.66 ^{a}	16.43 ^{fghi}	21.38 ^{a}
MS22	10.63 ^{hij}	23.3 ^{cdef}	17.36 ^{efgh}	29.63 ^{abc}	16.16 ^{ghi}	27.56 ^{abcd}	17.86 ^{a}
Control	0.00	0.00	0.00	0.00	0.00	0.00	
Cultivars Mean	9.42 ^{b}	21.52 ^{a}	13.27 ^{b}	26.80 ^{a}	27.15 ^{a}	21.88 ^{a}	

* Data were average of three replicates.
Means with the same letter are not significantly different

Table 5: Artificially inoculated tubers of potato cultivars interaction with *Enterobacter* isolate.

Potato Cultivars / Isolates code	Cara	Agria	Bern	Diamont	Lady balfor	Valor
KM23	11 ^{d}	30.4 ^{a}	24.93 ^{ab}	17.5 ^{cd}	21.03 ^{bc}	20.7 ^{bc}
Control	0.00	0.00	0.00	0.00	0.00	0.00

* Data were average of three replicates.
Means with the same letter are not significantly different.

DISCUSSION

Twenty three soft rot bacteria were isolated from potato tubers and stems showing symptoms of potato soft rot and blackleg which collected from different Governorates in Egypt .Nineteen out of 23 isolates were classified into *P. carotovorum* subsp. *carotovorum* (Pcc), 3 isolates with typical tuber soft rot and stem blackleg symptoms were *Dickeya* sp. and one isolate *Enterobacter,* according to their cultural, morphological characteristics and biochemical tests. In the present study, all the tested isolates were short rods, non sporic, gram negative, motile and exhibited positive reaction for catalase activity, gelatin liquefaction. The same results were matched with El-Kazazz, (1984), Abdel-Alim, (1996) and Behiry, (2009) and (2013). In Egypt *Pectobacterium carotovorum* and *Pectobacterium atrosepticum* have been listed as the major pathogens which cause blackleg and tuber soft rot diseases respectively (El-Kazazz, 1984; Ahmed, 2009; Behiry, 2009 and Ashmawy *et al*, 2015) . In our study new clades of isolates were identified by cultural, morphological characteristics and biochemical tests as *Dickeya* and *Enterobacter* has been isolated from potato tubers with typical soft rot symptoms. According the available literature this is the third report of a soft rot caused by new genera on potato crop in Egypt. On the basis of the results obtained from pathogenicity test were performed on tuber slices of Diamont cultivar, results concluded that all of tested isolates can able to make soft rot symptoms during 24h. after inoculation. Also the results indicated that the infection by *P. carotovorum* AS1 and *Dickeya* sp. MS21 isolates caused various degrees of soft rot and blackleg symptoms on potato tuber and stem cultivars Cara, Spunta and Ladybalfor in greenhouse compared to other tested isolates.

The present data of disease severity revealed that Cara cv. was more resistant towards to all tested soft rot bacterial isolates than other cultivars. It was also noticed that Valor and Ladypalfor cv. were more susceptible, while Bern, Agria and Diamont were moderately susceptible. The obtained results were in agreement with those obtained by Nabhan *et al.,* (2006); Wegener and Jansen, (2007); El-Shiekh *et al*., (2011) and Mustafa and Alawami, (2012).

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الملخص العربى

التعريف والقدرة الامراضية للبكتيرات الممرضة للنبات والمرتبطة بمرض العفن الطرى فى بعض أصناف البطاطس

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إختبرت القدرة الامراضية للاجناس البكتيرية البكتوبكتيريم، الديكيا والانتيروباكتر مسببات مرضية للعفن الطرى والساق السوداء فى البطاطس التى تم دراستها. تم عزل ٢٣ عزلة بكتيرية من أماكن مختلفة من شمال غرب مصر. قدرت القدرة الامراضية للعزلات المختبرة عن طريق عدوى شرائح من درنات البطاطس وتم إجراء القدرة الامراضية بعدوى شتلات البطاطس لاصناف كارا، سبونتا وليدى بالفور. أظهرت نتائج العدوى لشرائح البطاطس المختبرة ظهور عفن طرى. كما أوضحت نتائج عدوى الشتلات إصابة للدرنات بالعفن الطرى متبوعآ باصفرار وذبول الأوراق وأيضا ظهور أعراض الساق السوداء على الشتلات المعداه. أجريت الاختبارات التفريقيةالتى اشتملت على إختبارات مورفولوجية، فيزيائية، كيموحيوية والبيئات التفريقية التى أثبتت أن العزلات المدروسة تنتمى الى البكتوبكتيريم كاروتوفورم تحت النوع كاروتوفورم، الديكيا والانتيروباكتر. تم التعبير عن شدة المرض كنسبة مئوية لوزن الدرنات المصابة بالعفن فى الاصناف التالية (كارا، أجريا، بيرن، دايمونت، ليدى بالفور وفالور). أظهرت النتائج أن عزلتى من البكتوبكتيريم AS و AC5 كانتا أشد العزلات قدرة إمراضية حيث أعطت أعلى نسبة إصابة تقدر بـ ٢٣.٣٤,٢٦.٢١%على التوالى، بينما كانت العزلة KM15 أقلهم فى شدة الاصابة و قدرت بنسبة١٨.١٧ %. فى حين أنه لم تظهر أى إختلافات معنوية بين عزلات الديكيا فى شدة الاصابة. وجد أن صنف الاجريا كان أكثر قابلية للاصابة على عكس صنف كارا الذى كان أكثر مقاومة لعزلة الانتيروبكتر .KM23 وأظهرت النتائج أيضآ أن الصنف كارا يعتبر أكثر الاصناف مقاومة للاصابة بجميع العزلات المختبرة مقارنة بالاصناف الاخرى. ومن ناحية الاخرى وجد أن الصنف فالور وليدى بالفور يعدان أكثر الاصناف قابلية للاصابة بالعزلات البكتيرية المسببة للعفن الطرى.