Detection and virulence of *Ralstonia solanacearum* the causal of potato brown rot disease

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Abstract-This work aimed to detect and determine the virulence in addition to the host range of *Ralstonia solanacearum* the causal of potato brown rot disease. Isolation from naturally infected potato tubers of three different cvs. (Spunta, Draga and Nicola) showing brown rot disease symptoms revealed that all isolated bacteria showed typical morphological growth of *R. solanacearum* on SMSA medium where; colonies were fluidal white with red center. Also, IFAS test (Immunoflurescence Microscope Antibody Staining) confirmed that these isolated bacteria are *R. solanacearum*. The traditional identification of the three tested pathogenic bacteria isolates exhibited similarity among them based on their cultural and morphological characteristics where, these isolates were non-sporulating short rods with weak Gram negative reaction. Also, their developed colonies on nutrient agar (NA) medium were irregularly/round, convex, smooth surface, entire margin, translucent and yellowish brown in colour. Meantime, these colonies were whitish-gray in colour on King's B (KB) medium forming brown pigments in most cases. Also, the physiological and biochemical tests of the three bacterial isolates showed oxidative metabolism of glucose and positive results with oxidase reaction, catalase reduction, H2S production, nitrate reduction. However, the three isolates were negative to Indole production, gelatin liquefaction, Arginine dihydrolase, starch hydrolysis, Voges proskauer test and Levan formation. Also, the three tested isolates of *R. solanacearum* showed virulence against potato and tomato plants. While, these isolates were avirulent to eggplant, pepper, tobacco and banana plants under artificial inoculation conditions which reveal that theses isolates belong to race 3. Also, all three tested isolates utilized maltose, lactose, cellobiose and glucose but not oxidized mannitol, sorbitol and dulcitol. As for virulence of the three tested *R. solanacearum* isolates, results exhibited different percentages of disease severity on potato plants (cv.Nicola) at 15 days of incubation period. Also, Draga- isolate was the fastest one followed by Nicola -isolate. Draga-isolate of *R. solanacearum* infected many hosts with different degrees of wilt severity where it was virulent to tomato, mallow, datura and little hogweed. Also, positive results were obtained with SMSA, IF and PCR techniques to confirm the infection with Draga-isolate.

Key words- *Ralstonia solanacearum*, potato plants, virulence, host range, morphological growth, physiological and biochemical tests

1 INTRODUCTION

Potato *(Solanum tuberosum L.)* is considered one of the four major and important food crops after wheat, maize and rice around the world (Hawkes, 1992). Potato brown rot, caused by *Ralstonia solanacearum* (Yabuuchi *et al*., 1995) has been reported in Egypt many years ago (Briton-Jones, 1925). The disease has created a lot of quarantine problems during the course of exportation of table potatoes to Europe (Farag, 2000). The disease is known to be favored by warm climates; however, serious outbreaks in Europe have been reported (Walker, 1992 and Grousset *et al*., 1998). Therefore, the origin of the disease in Egypt is thought to be the potato seeds imported from Europe (Balabel, 2006). Hayward (1991) stated that, tuber symptom was described as brown rot, cut tubers showed brownish discoloration of the vascular ring, and slight squeezing forces opus-like slime out of the ring, or it may exude naturally. Shekhawat *et al*., (1992a) reported that the brown rot pathogen affects both above and below ground plant parts and damage can occur in two ways; premature wilting of foliage top growth namely bacterial wilt of potato plants, and rotting the tubers in the soil or during storage namely brown rot of potato tubers. Engelbrecht (1994) improved a semi selective medium (SMSA) in South Africa then modified by Elphinstone *et al.,* (1996 and 1998) to detect the pathogen from soil, water and potato tuber tissues. The limit of detection of the modified SMSA medium was 10^2 CFU/mL infected tuber homogenate (Elphinstone *et al*., 1996). The described colony appearance on SMSA was found similar to that on triphenyltetrazolium chloride (TTC) medium (Kelman, 1954). Elphinstone *et al.,* (2000) stated that symptomless (latent) infections are common, particularly at low temperatures. In this phase, the pathogen can survive in stored potato tubers for long distance during transport and cause the disease in new environments whenever conditions favor its multiplication. Balabel (2006) suggested that plating bacterial suspensions of *R. solanacearum* from different sources revealed virulent and avirulent forms. The first is described as milky, white, flat, irregular and fluidal with red coloration in the center. Avirulent form developed less fluidal or a fluidal colony which is completely pink to red. Dean *et al.,* (2006) suggested that early symptoms were wilting of the lower leaves with rolling of the leaf margins, subsequently leaves showed sectorial chlorosis and eventually papery brown necrosis. Sometimes only one part of the stem showed wilting symptoms. Bader (2012) stated that infected

potato plants with brown rot disease (caused by *Ralstonia solanacearum*) under greenhouse and field conditions exhibited yellow leaves or sudden wilting of leaves then dead plants, whitish exudates seen on the cut surface on tubers, a wet breakdown inhibited at the point of attachment of the stolon and the eyes of tubers. A light-brown breakdown of water-conducting tissues could be seen in tuber crosses. Milky fluid light is squeezed from this discolored area in infected potato tubers.

The bacterium *Ralstonia solanacearum* is highly motile, bear 1-4 polar flagella (Kelman and Hruschka, 1973). Hayward (1991) reported that *Ralstonia solanacearum* is a strictly aerobic, gram-negative, short rod, non-spore forming and non capsulating bacterium. The bacterium's colony has an irregularly round form with fluidal, white and red center slime on SMSA and TTC media (Elphinstone *et al.*, 1996). Shambhu *et al.*, (2001) performed the characterization of *Ralstonia solanacearum* strains, the causal agent of potato bacterial wilt disease from Nepal and Thailand based on pathogenicity, biochemical, physiological and serological tests. Fifteen *R. solanacearum* strains isolated from wilt infected potato plants and tubers grown in Nepal were characterized as race 3, biovar 2 based on the pathogenicity on different host plants. Atta (2008) stated that detection methods by plating on the SMSA medium showed that colonies with irregularly round shape and slimy white color with pink centers was considered the typical morphology of bacterial colony. Siri *et al.*, (2011) evaluated 28 strains of *R. solanacearum* isolated from major potato-growing areas in Uruguay, including 26 strains isolated from potato tubers and 2 from soil samples. All strains belonged to phylotype IIB, sequevar 1 (race 3, biovar 2). Bader (2012) isolated ten isolates of *R. solanacearum* (*Rs*) from the diseased potato tubers and soil collected from the tested fields in Qalubiya (Beltan and El-Hadaden) and Beheira (El-Tawfikia and Hosh-Eysa) governorates during growth seasons 2009-2011. All test trails of bacteria identification based on morphological, physiological and biochemical characteristics confirmed that all ten isolates were *R. solanacearum*. Massart *et al.*, (2014) reported that *Ralstonia solanacearum* race 3 is the causal agent of brown-rot of potato. This disease represent a serious threat to potato production in temperate climates.

This work aimed to detect and determine the virulence of *Ralstonia solanacearum* the causal of potato brown rot disease. Investigating the host plants of *Ralstonia solanacearum*, the causal of potato brown rot.

2 Materials and Methods

Isolation of *Ralstonia solanacearum* from naturally infected potato tubers:

Naturally infected samples of potato tubers cvs. Spunta, Nicola, Draga showing external and internal symptoms of potato brown rot disease were collected from the production of potato cultivations of Talia village, Menoufya governorate for investigation by the Potato Brown Rot Project (PBRP), Agric. Res. Center, Giza – Egypt. These infected samples were used in isolation trials of *Ralstonia solanacearum* the causal agent of potato brown rot disease (Youssef, 2013)

Infected potato tubers were washed in running tap water, surface sterilized with 90% alcohol by flaming and the stolon ends were aseptically removed. Cores of 5-10 mm diameter and 5 mm length, containing mainly vascular and cortical tissues were macerated in 1.0 mL of sterile phosphate buffer; the suspension then transferred to sterile 1 mL eppendorf. The macerate was allowed to stand for 30 minutes. Plating was made on modified Selective Medium South Africa (SMSA) which considered the selective medium for isolation of *R. solanacearum* as described by Engelbrecht (1994) and modified by Elphinstone, (1996). Purification was made by streaking and plating for typical colonies on basal and nutrient agar (NA) medium.

Incubation was held at 28^{O}C and daily observed for developing fluidal, slightly raised, irregular white or white with pink center colonies, typical for virulent colonies of *Ralstonia solanacearum*. Colonies were selected, picked up and streaked on glucose nutrient agar medium, incubated for 48hr at 28°C (Dowson, 1957) for further studies.

Identification of isolated bacteria:

Cultural and morphological characteristics, physiological and biochemical tests, immunofluorescence (IFAS), bioassay test (pathogenicity test), were used for identification of the isolated bacteria as follows:

Morphological characteristics:

Cultural and morphological characteristics of the isolated bacterium were studied by re-inoculating them on nutrient agar (NA), King's B (KB) and SMSA media and were compared by description of Engelbrecht (1994) and Elphinstone *et al.*, (1996). Shape of bacterial cells, sporulation and reaction to gram stain were recorded.

Physiological and biochemical tests:

Physiological and biochemical tests were studied according the methods described by McCarter (1991) and Bergy & Holt, (1994). Biovar identification was determined by the ability of isolates to oxidize lactose, maltose, sorbitol, mannitol, cellobios and dulcitol. Where tests were performed at 28–30^{o}C (Hayward, 1964).

Virulence of brown rot pathogen:

Pure culture of the three isolated bacteria that obtained from infected plant materials were used. This isolates were grown on casmino acid peptone glucose (CPG) agar medium at 28oC for 48hr (Kelman, 1954). Bacterial growth was suspended in sterile phosphate buffer 0.05M pH 7.2 and adjusted to a standard optical density at 590nm to 107 colony forming units (cfu/mL). Potato tubers (Nicola cv.) and tomato plants (Castle rock cv.) were examined to be free from *R. solanacearum*. Four weeks old, tomato seedlings were transplanted in 10cm diameter pots, containing sterilized sand-clay soil mixture (1:1,v/v) while, potato tubers previously stored at 4oC,were placed in trays at room temperature in dark to stimulate germination. Germinated tubers were planted in pots (30 cm diameter) containing sterilized sand- clay soil (1:1, v: v), where one germinated tuber was planted in each pot; five pots were used as replicates for each host. Tomato plants (three-weeks after transplanting) were injected at the leaf axis with a sharp needle laden with the bacterial growth of the pathogen (Janse, 1988). Potato plants were inoculated into the axel of the second and third leaf from the apical meristem by injection of bacterial suspension (10µL) using needle (Martin and El-Nashaar, 1992). Five plants of tomato and potatoes were injected with sterilized water as the control treatment, the inoculated plants were covered with polyethylene bags for three days, at 30°C and 28% relative humidity (RH) in an automated quarantine green house, then bags were removed and pots were irrigated daily. Wilt symptoms severity were recorded daily according to the scale of Kempe and Sequeira (1983) where, (0 = no symptoms, 1= up to 25 % wilt, 2 = 26-50 % wilt, 3 = 51-75 % wilt, 4 = 76-100% wilt and 5 = dead plants).

Host range

Tomato (*Solanum lycopersicum* (*Lycopersicon esculentum* L. cvs.GS 12), eggplant (*Solanum melongena* L.), pepper (*Capsicum annum* L.). bean (*Phaseolus vulgaris* L.), onion (*Allium cepa* L.), maize (*Zea mays* L.), faba been (*Vicia faba* L.), datura (*Datura inoxia P.), little* hogweed (*Portulaca oleracea* L.), and Mallow (*Malva aegyptia* L.) were used as a test plants to determine the host range of *Ralstonia solanacearum*. Seeds and/or seedlings of plants tested as host range were planted in pots (15cm in diameter, containing about 0.5 kg of clay-sandy soil each) under greenhouse conditions. The stem puncture technique injection described by Janse, (1988) was made for tomato, potato, pepper, bean, mallow datura and little hogweed at the leaf axis by a needle laden with a 1 mL bacterial suspension of *Ralstonia solanacearum* (Draga isolate) with inoculum density of 108 cfu/mL). While, maize, onion and faba bean were inoculated as described by Matter, (2008) where roots were cut using sterilized knife then the pots were inoculated with a rate of 200 mL/pot (108 cfu/mL). The inoculated plants were covered with polyethylene bags for three days, at 30oC, then bags were removed and pots were irrigated daily. Five replicates were made for each host and another five replicates was treated with sterilized water as control. Wilt symptoms were recorded daily according to the scale of Kempe and Sequeira (1983) as mentioned above.

3 RESULTS

Isolation of *Ralstonia solanacearum* from naturally infected potato tubers:

As clear in Fig. (1), naturally infected potato tubers of three different cvs (Spunta, Draga and Nicola) showing brown rot diseases were used for isolation of *R. solanacearum* pathogen. All isolates of *R. solanacearum* showed typical morphological growth on SMSA medium as showed in Fig. (2). Where, colonies were fluidal white with red center. Results showed that these isolates were *R. solanacearum* which confirmed by giving positive result using IFAS test (Immunoflurescence Microscope Antibody Staining) as clear in Fig. (3).

Fig (1) Internal brown rot symptoms in potato tubers showing bacterial ooze

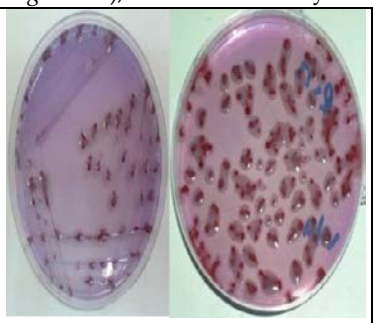

Fig.(2) Typical colonies of *R. solanacearum* on SMSA medium

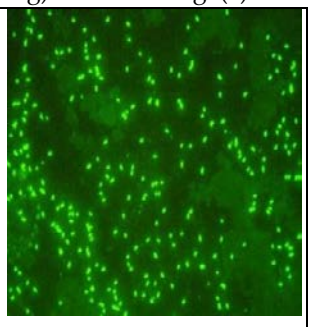

Fig. (3) IFAS test showing positive reaction of *R. solanacearum.*

Identification of isolated bacteria:

The isolates in concern were subjected to complete their identification up to species level. The results showed that all isolates recovered from different sources were similar in their morphological and physiological reactions. No strain variation could be detected.

Table (1): Morphological and cultural characteristics of isolates identified primary as *Ralstonia solanacearum* from different potato cultivars.

Identification Test	Source of *R. solanacearum* isolates		
	Spunta	Draga	Nicola
Gram reaction	-	-	-
Cell shape	Short Rods		
Spore formation	-	-	-
Motility	+	+	+
Colony shape	Irregular / Round		
Colony elevation	Convex		
Colony surface	Smooth		
Colony margin	Entire		
Colony density	Translucent		
Colour on NA media	Yellowish brown		
Colour on KB	Whitish grey		
Colour on TTC and basal SMSA	Fluidal white with red centre		

NA= nutrient agar medium, KB= King's B medium, TTC= Triphenyl tetrazolium chloride agar medium, SMSA= semi selective medium of South Africa

Cultural and morphological characteristics:

Data in Table (1) exhibit the cultural and morphological characteristics of the three tested pathogenic bacteria isolates which isolated from naturally infected potato tubers of three different cvs. (Spunta, Draga and Nicola) exhibiting brown rot disease where, stained smears of the bacteria showed non-sporulating short rods with weak Gram negative reaction. Colonies developed on nutrient agar (NA) medium were irregularly / round, convex, smooth surface, entire margin, translucent and yellowish brown in colour. Meantime, these colonies were whitish–gray in colour on King's B (KB) medium forming brown pigments in most cases as clear in Fig (4). Colonies on tetrazolium chloride (TTC) medium and semi – selective medium of South Africa (SMSA) were fluidal white with red center.

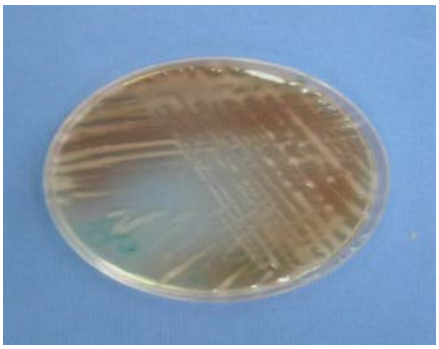

Fig. (4) Brown diffusible pigment of *R. solanacearum* on king's B medium

Physiological and biochemical tests:

Data in Table (2) show the physiological and biochemical tests of the three tested pathogenic bacteria isolates which isolated from naturally infected potato tubers of three different potato cvs (Spunta, Draga and Nicola) exhibiting brown rot disease where, all the three tested bacterial isolates showed oxidative metabolism of glucose and positive results with oxidase reaction, catalase reduction, H2S production, nitrate reduction. However, the three isolates were negative to Indole production, gelatin liquefaction, Arginine dihydrolase, starch hydrolysis, Voges proskauer test and Levan formation. Fluorescent pigment was not produced into king's medium but showed diffusible brown non fluorescent pigment. Also, the three pathogenic bacteria isolates were not able to grow on 4 or 41oC mean while they grow on 1 and 2 % NaCl.

Table (2): Physiological and Biochemical characteristics of isolates identified as *Ralstonia solanacearum* from different potato cultivars.

Identification Test	Source of *R. solanacearum* isolates		
	Spunta	Draga	Nicola
Starch hydrolysis	-	-	-
Production of fluorescent on KB	-	-	-
Diffusible non fluorescent pigment	Brown	Brown	Brown
O/F	O	O	O
Oxidase reaction	+	+	+
Arginine dihydrolase	-	-	-
Catalase reduction	+	+	+
Voges Proskauer (VP)	-	-	-
Gelatine liqufication	-	-	-
Reduction of nitrate	+	+	+
Levan production	-	-	-
Indole formation	-	-	-
H_2S production	-	-	-
Growth at $41^{O}C$	-	-	-
Growth at $4^{O}C$	-	-	-
Growth on 1% NaCl	+	+	+
Growth on 2% NaCl	+	+	+

Determination of races and biovars:

Data in Table (3) indicate that all three tested isolates of *R. solanacearum* were virulent to potato and tomato plants. On the other side, these isolates were avirulent to eggplant, pepper, tobacco and banana plants under artificial inoculation conditions which reveal that theses isolates belong to race 3. Also, data in Table (8) reveal that the three tested isolates utilized maltose, lactose, cellobiose and glucose but not oxidized mannitol, sorbitol and dulcitol (other characteristics are shown). These tests confirm that these isolates belong to biovar 2.

Table (3): Race determination of the three tested *Ralstonia solanacearum* isolates based on pathogenicity test to different host plants.

Host	Tested isolates of *R. solanacearum*		
	Cv. Spunta	Cv. Nicola	Cv. Draga
Pepper	-	-	-
Eggplant	-	-	-
Tobacco	-	-	-
Potato	+	+	+
Tomato	+	+	+
Banana	-	-	-
Proposed Race	3	3	3

Table (4): Ability of isolates identified as *Ralstonia solanacearum* from different potato cultivars to utilize some carbohydrates and biovar determination.

Identification Test	Tested isolates of *R. solanacearum*		
	Cv. Spunta	Cv. Draga	Cv. Nicola
Maltose	+	+	+
Lactose	+	+	+
Cellobiose	+	+	+
Mannitol	-	-	-
Sorbitol	-	-	-
Dulcitol	-	-	-
Salicin	-	-	-
Glucose	+	+	+
Galactose	+	+	+
Glycerol	+	+	+
Mannose	+	+	+
Fructose	+	+	+
Sucrose	+	+	+
Trehalose	+	+	+
Inositol	-	-	-
Arabinose	-	-	-
Raffinose	-	-	-
Ribose	-	-	-
L-arginine	-	-	-
Xylose	-	-	-
Proposed Bivoar	2	2	2

Virulence of the three tested *R. solanacearum* isolates:

Data in Table (5) indicate that the three tested *R. solanacearum* exhibited different percentages of disease severity ranging from 76.59% to 98.4 % infection when tested on potato plants (cv.Nicola) at 15 days of incubation period. Meanwhile, the determined disease severity of the three tested *R. solanacearum* reached 100 % on tomato plants (cv. Castle rock) to reveal that Draga- isolate was the fastest one among the three tested isolates of *R. solanacearum* where, it recorded disease severity 100% at 4 days of incubation period followed by Nicola-isolate.

Table (5): Pathogenicity test on potato and tomato plants using three isolates of causal organism under artificial inoculation conditions.

Tested isolates	Potato (cv. Nicola)		Tomato (cv. Castle rock)	
	DS %	IP (days)	DS %	IP (days)
***R. solanacearum* (cv.Spunta)**	76.59	15	100	7
***R. solanacearum* (cv.Nicola)**	87.0	15	100	5
***R. solanacearum* (cv.Draga)**	98.4	15	100	4
Control	0.0	15	0	7

DS =Disease Severity%
IP= Incubation period (days)

Host range:

It is clear from the obtained previously results of pathogenicity test trial that cv Draga- isolate of *R. solanacearum* was the more aggressive one among the three tested isolates. Thus, this isolate used in the host range trail of *R. solanacearum*. In this respect, data in Table (6) show that Draga-isolate of *R. solanacearum* could infect many hosts with different degrees of wilt severity% where it was virulent to tomato, mallow, datura and little hogweed. The highest recorded wilt severity% was 80% on mallow plants followed by 60% on tomato and little hogweed plants while they were 40% only on datura. Also, positive results were obtained with SMSA, IF and PCR techniques to confirm the infection with Draga- isolate. On the other hand, Draga-isolate was not able to exhibit any wilt symptoms on pepper, eggplant, bean, maize, faba bean and onion plant hosts with no visual latent infection on the last 4 hosts except pepper and eggplant where the detection methods using SMSA, IF and PCR techniques exhibited latent infection with Draga-isolate on it.

Table (6): Host range of *R. solanacearum* (Draga-isolate), determined as wilt severity % and latent infection which confirmed by SMSA, IF and PCR techniques.

Tested hosts	Wilt severity		Detection method		
	Draga isolate	Control	SMSA	IF	PCR
Tomato	60	0	+	+	+
Pepper	0	0	+	+	+
Eggplant	0	0	+	+	+
Bean	0	0	-	-	-
Maize	0	0	-	-	-
Faba bean	0	0	-	-	-
Onion	0	0	-	-	-
Mallow	80	0	+	+	+
Datura	40	0	+	+	+
Little hogweed	60	0	+	+	+

4 DISCUSSION

Potato brown rot, caused by *Ralstonia solanacearum* (Yabuuchi et al., 1995), has been reported in Egypt many years ago (Briton-Jones, 1925). The disease has created a lot of quarantine problems during the course of exportation of table potatoes to Europe (Farag, 2000). The disease is known to be favored by warm climates; however, serious outbreaks in Europe have been reported (Walker, 1992 and Grousset *et al*., 1998). Therefore, the origin of the disease in Egypt is thought to be the potato seeds imported from Europe (Balabel, 2006).

Results of isolation from naturally infected potato tubers of three different cvs. (Spunta, Draga and Nicola) showing brown rot diseases revealed that all isolated bacteria showed typical morphological growth of *R. solanacearum* on SMSA medium where; colonies were fluidal white with red center. Also, IFAS test (Immunoflurescence Microscope Antibody Staining) confirmed that these isolated bacteria are *R. solanacearum* by giving positive result. Repeating the IFAS test of the three tested purified *R. solanacearum* isolates verified that their cells morphology of had short rod shape stained evenly as bright green fluorescent. These results are in agreement with those of Bader (2012) who found that identification of tested bacterial isolates using immunofluorescent antibody staining technique (IFAS) gave positive results which mean that these tested isolates are *R. solanacearum*. The morphology of bacterial cells appeared as short rod shape and green fluorescent with specific fluorescent-labeled antiserum.

Also, all isolates recovered from different sources were similar in their morphological and physiological reactions. In this respect, the traditional identification of the three tested pathogenic bacteria isolates which isolated from naturally infected potato tubers of three different cvs (Spunta, Draga and Nicola) exhibiting brown rot disease using exhibited the similarity of cultural and morphological characteristics of the three tested bacterial isolates where, these isolates were non-sporulating short rods with weak Gram negative reaction. Also, their colonies developed on nutrient agar (NA) medium were irregularly/round, convex, smooth surface, entire margin, translucent and yellowish brown in colour. Meantime, these colonies were whitish-gray in colour on King's B (KB) medium forming brown pigments in most cases. Their colonies on tetrazolium chloride (TTC) medium and semi-selective medium of South Africa (SMSA) were fluidal white with red center. Also, the physiological and biochemical tests of the three bacterial isolates from naturally infected potato tubers of three different cvs. (Spunta, Draga and Nicola) exhibiting brown rot disease revealed that the three tested bacterial isolates showed oxidative metabolism of glucose and positive results with oxidase reaction, catalase reduction, H2S production, nitrate reduction. However, the three isolates were negative to Indole production, gelatin liquefaction, Arginine dihydrolase, starch hydrolysis, Voges proskauer test and Levan formation. Fluorescent pigment was not produced into king's medium but shows diffusible non fluorescent pigment. The obtained results are in agreement with those obtained by Kelman, (1954) who reported that plating the isolates having typical morphological colonies on the SMSA medium showed that colonies with irregularly round shape and slimy white color with pink centers was considered the typical morphology of *R. solanacearum*. The positive isolates (typical morphological colonies) showed that all isolates were similar in their typical morphological colonies on SMSA medium. The obtained results of identification and race determination of isolates based on pathological and bacteriological tests are similar to those obtained by Hayward (1964), Cowan & Steel (1974), Schaad (1980), Krieg and Holt (1984), Lelliot & Stead (1987), Klement *et al*., (1990) and McCarter (1991). Also, Atta (2008) stated that detection methods by plating on the SMSA medium showed that colonies with irregularly round shape and slimy white color with pink centers was considered the typical morphology of bacterial colony. While, Bader (2012) isolated ten isolates of *R. solanacearum* (Rs) from the diseased potato tubers and soil collected from the tested fields in Qalubiya (Beltan and El-Hadaden) and Beheira (El-Tawfikia and Hosh-Eysa) governorates during growth of 2009- 2011 seasons. All testes trails for identification of bacteria based on morphological, physiological and biochemical characteristics confirmed that all ten isolates were *R. solanacearum*.

All three tested isolates of *R. solanacearum* showed virulence against potato plants and tomato plants. On the other side, these isolates were avirulent to eggplant, pepper, tobacco and banana plants under artificial inoculation conditions which reveal that theses isolates belong to race 3. Also, all three tested isolates utilized maltose, lactose, cellobiose and glucose but not oxidized mannitol, sorbitol and dulcitol (other characteristics are shown). These tests confirm that these isolates belong to biovar 2. The physiological and bacteriological

characteristics were found similar to those described for Race 3, biovar 2 of *R. solanacearum* as documented based on host range studies by Buddenhagen *et al.,* (1962), Pegg & Moffett (1971) and He *et al.,* (1983). Also, Farag *et al.,* (2004) confirmed that the dominant race in Egypt of *R. solanacearum* is race 3, biovar 2. Similar results were obtained also by Shambhu *et al*., (2001) who performed the characterization of *Ralstonia solanacearum* strains, the causal agent of potato bacterial wilt disease from Nepal and Thailand based on pathogenicity, biochemical, physiological and serological tests. Fifteen

R. solanacearum strains isolated from wilt infected potato plants and tubers grown in Nepal were characterized as race 3, biovar 2 based on the pathogenicity on different host plants. On the other hand, Siri *et al.,* (2011) evaluated 28 strains of *R. solanacearum* isolated from major potato-growing areas in Uruguay, including 26 strains isolated from potato tubers and 2 from soil samples. All strains belonged to phylotype IIB, sequevar 1 (race 3, biovar 2). While, Bader (2012) stated that infected potato plants with brown rot disease (caused by *Ralstonia solanacearum*) under greenhouse and field conditions exhibited yellow leaves or sudden wilting of leaves then dead plants, whitish exudates seen on the cut surface on tubers, a wet breakdown inhibited at the point of attachment of the stolon and the eyes of tubers. A light-brown breakdown of water-conducting tissues could be seen in tuber crosses. Milky fluid is squeezed from this discolored area in infected potato tubers.

As for virulence of the three tested *R. solanacearum*, results exhibited different percentages in disease severity on potato plants (cv.Nicola) at 15 days of incubation period. Also, Draga- isolate was the fastest one among the three tested isolates of *R. solanacearum* followed by Nicola - isolate. Draga-isolate of *R. solanacearum* infected many hosts with different degrees of wilt severity where it was virulent to tomato, mallow, datura and little hogweed. Also, positive results were obtained with SMSA, IF and PCR techniques to confirm the infection with Draga-isolate. Draga-isolate was not able to exhibit any wilt symptoms on pepper, eggplant, bean, maize, faba bean and onion plant hosts with no visual latent infection on the last 4 hosts except, pepper and eggplant where the detection methods using SMSA, IF and PCR techniques exhibited latent infection with Draga-isolate. These results are in agreement with those reported by Fahy and Persley (1983), Hsu (1993), Adhikari (1993), Abd El-Ghafar *et al.,* (1995), Gabr and Saleh (1997), Shehata (2001), Kehil (2002), Zayed (2004), Abd El-Ghafar *et al.,* (2004). Also, such results were confirmed with the obtained results by Kelman (1953), Buddenhagen and Kelman (1964), Hayward (2000) and Matter (2008) who found that several hundred species, representing more than 50 plant families have been identified as hosts of *R. solanacearum*; include tomato, potato, pepper, eggplant, groundnut and bananas as well as a number of ornamental plants, woody perennials and a large group of weed species. Bader (2012) stated that all tested *R. solanacearum* isolates caused bacterial wilt disease symptoms on potato plants compared with the un- inoculated control in sterilized and un-sterilized soils. *R. solanacearum* (R6) recorded the highest infection percent and disease severity percent at 35 day post inoculation of potato plants (cv. Spunta) followed by *R. solanacearum* isolates (R1, R3 & R4). While the least infection percent was recorded by *R solanacearum* (R8) and (R5).

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