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IDENTIFICATION OF *RALSTONIA SOLANACEARUM* ISOLATED FROM POTATO TUBERS, WEEDS, WATER AND SOIL IN EGYPT

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ABSTRACT: Brown rot or bacterial wilt of potato caused by *Ralstonia solanacearum* Yabuuchi *et al.*, is one of the most quarantine disease world wide as well as in Egypt. Therefore, isolation and identification of the causal organism from different sources using rapid and accurate methods was carried out. Results revealed that, from 200 collected samples, 20 pathogenic isolates were detected from potato tubers, weeds, water and soil. Fifteen isolates were isolated from Menufiya governorate, five from El-Gharbia and non from El–Sharkia. Pathogenic capability of these isolates was confirmed on tomato seedlings. Some soil, tuber and water isolates showed the highest disease severity on tomato seedlings. The aforementioned twenty pathogenic isolates were identified as *Ralstonia solanacearum* according to their biochemical and physiological properties, plating on King's B and SMSA media, immunofluorescence antibody assay (IFAS), fatty acid assay and DNA assay using Box-PCR technique.

Key words: *Ralstonia solanacearum*, isolation, identification, fatty acids, immunoflurescence antibody assay and PCR.

INTRODUCTION

Bacterial wilt or brown rot of potato, caused by *Ralstonia* (*Pseudomonas*) *solanacearum* (Yabuuchi *et al.*, 1995) is one of the most series bacterial diseases causing problems in the world and also in Egypt. The disease affects potato plants in tropical and subtropical regions as well as it causes wilt in plants and rot in tubers. It often limit potato production in many countries (Hayward, 1991; Ramadan, 2000

and Kehil., 2002). Brown rot is a disease that is subject to plant quarantine legislation. Therefore, Egyptian quarantine measures are necessary to avoid spread of the pathogen to disease free areas. So restricted lows issued to exclude imported infected potato seed tubers since the cultivated area in Egypt is pest free areas (PFAs) which is a virgin land (Anon., 2001). Since, Egyptian potatoes are grown for consumption in European market and *R. solanacearum* is a quarantine organism for Europe. Various methods have been developed for the detection of this pathogen in infected parts (tubers, soil, water and weeds) as previously mentioned by Elphinstone *et al.*, 1996 and Janse, 1996 .Isolation from tubers and weeds is very important due to the pathogen has the ability to spread latently through infected plant parts (tubers) and weeds as mentioned by Janse, 1988. Accordingly, the aim of this research work is to isolate the pathogen from different samples (tubers, soil, water and weeds) in potato fields at various locations of El-Menufiya, El-Gharbia and El-Sharkia governorates. Also, identification was carried out using physiological and biochemical properties, selective media, immunofluorcence anti-bodies stain (IFAS), fatty acid profile and box-PCR.

MATERIALS AND METHODS

Isolation, Purification and Characterization of *Ralstonia solanacearum* from Different Sources

Potato tubers

Samples of potato tubers, picked up from wilted potato plants, showing external as well as internal symptoms of brown rot disease were collected from different potato districts at different governorates *i.e.* El-Menufiya (Tallia village), El-Gharbia (Kafr Yakope village and Mansoriate El-Farastec) and El-Sharkia (pivot Nos. 10 and 20, El-Salhia city). Ten tubers were washed in running tap water, surface sterilized by dipping in ethyl alcohol 90 % and flaming, then stolen ends were aseptically removed. Cores of 5-10 mm in diameter and 5mm in length, containing main vascular and cortical tissues were macerated in ten ml sterile phosphate buffer (Na_2HPO_4. $12H_2O$, 4.26g; KH_2PO_4 ,2.72g, in one liter distilled water to give pH 7) in sterile plastic bags. The macerated tissues obtained were allowed to stand for 30 minute then the suspension was used to inoculate plates of semi-

selective media of South Africa (SMSA) described by Engelbrecht, 1994 and modified by Elphinstone *et al.*, 1996.

Incubation was made at 28°C and daily observed to develop fluidal, slightly raised, irregular white or white with pink center colonies, typical for virulent colonies of *R. solanacearum*. Colonies were selected, picked up and inoculated on glucose nutrient agar for 48hr (Dowson, 1957).

Irrigation water

Water samples from the central irrigation system, pivot Nos.10 and 20 at El-Salhia city, El-Sharkia governorate were collected. Four replicates of 50 ml/sample, 10 minutes after a brief course of irrigation, were withdrawn in a sterile bottle. Samples from water streams at Tallia village, Monufia governorate were also done at depth about 30-40 cm from the water surface, where 50 ml water were collected in duplicate from each sampling site. Samples collected from both irrigation systems were labeled, placed in an ice box and directly transferred to the laboratory. Samples were centrifuged at 10,000 rpm for 15 minutes at 15°C. Supernatant was discarded and pellet was re-suspended in one ml phosphate buffer, vortexed for homo-genization and plated on ready SMSA plates for isolation of *R. solanacearum*.

Soil

Four samples (100 g/each) from each location of pivots Nos.10 and 20 from El-Salhia city at El-Sharkia governorate; Mansoriate El-Farastec village in El-Gharbia governorate and Tallia village in El-Monufia governorate were taken from 30 cm depth, using a sampling auger, mixed in the laboratory for homogenization. The isolation of the brown rot pathogen from the soil was made on SMSA medium according to method described by Van Der Wolf *et al.*, 1998. For this purpose decimal dilutions of the suspension was made in 90 ml sterile phosphate buffer (pH.7.0) to 10^{-6}, shacked for two hours at 15°C and the plates were incubated at 28 °C for 3-6 days.

Weeds

Different weeds, developed in potato field during growing seasons including Slender Amaranth (*Amaranthus viridis),* Smallflower umbrellaplant as *(Cyperus difformis)*, poison for chicken and rats *(Withania somnifera* [L.]Dun.), Nettle leaf, Goose foot *(Chenopodium murale)* and Common Purslane *(Portulaca olevacea*) were collected and identified botanically according to Zaki,1991 and

Pradhanang *et al.*, 2000. Isolation was carried out from roots, as described by Wenneker *et al.*, 1999.

In all the above mentioned isolation trials, *R. solanacearum* colonies were picked up and used in pathogenicity tests.

Pathogenicity Tests

Pathogenicity test of twenty *R. solanacearum* isolates exhibiting typical cultural properties of *R. solanacearum* obtained from different habitats, were confirmed by inoculating seedlings of tomato cultivar GS12 (3leaves/seedling) grown in 10cm diameter pots containing 250 g sandy-clay soil (1/1, v/v) under greenhouse conditions (30±2 °C), 85 % relative humidity "RH". Inoculation was carried out by the stem puncture technique described by Janse, 1988. In this method injection was made at the leaf axis by a needle laden with bacterial suspension of the pathogen in sterile water (10^7 cfu/ml). Control treatments were prepared using sterile water instead of bacteria. The inoculated plants were covered with polyethylene bags for three days, at 30°C, then bags were removed and pots were irrigated daily. The pathogenic potential of the isolates obtained from tubers, water, soil or weeds were determined by inoculating tomato seedling (three leaves) grown in the greenhouse with most pathogenic isolates representing each of the above mentioned origins.

Five pots (10cm in diameter, containing one seedling) were inoculated for each source as previously mentioned and control treatment was considered. Five pots were used as a replicates. The disease progress was determined according to the scale described by Kempe and Sequeira, 1983. The scale based on the visual observation of the percentage of foliage wilt (zero= no symptoms, 1=up to 25%, 2= 26-50%, 3= 51-75%, 4= 76-100%and 5= dead plant) then the disease index was calculated.

Disease index (DI) was calculated by the following formula:

$DI=\sum R \times T \times 100/5 \times N$

Where, T= total number of plants in each symptoms category.

R= disease severity scale

(R= 0- 5)

N= total number of tested plants

Re-isolation was made as mentioned above from tomato seedling exhibiting wilt symptoms to complete Koch's postulates and the most pathogenic isolates were selected.

Identification of the Pathogen

Physiological and biochemical characteristics

Physiological and biochemical tests (gram stain reaction, arginine dihydrolase, kovac's oxidase, starch hydrolysis, gelatin liquefaction test, carbon source utilization, catalase and nitrate reduction) were studied according to the methods described by Schaad, 1988.

Pigment production

On King's B medium

King's B medium King *et al.*, 1954, was used for examining the isolates under UV light. Fluorescence stain production recognize the brown pigments produced by *R. solanacearum*.

On SMSA medium

Inoculation on SMSA medium was used for detection the possible variation in morphology of *R. solanacearum* colonies. A loop of a slightly tuber bacterial suspension, prepared from three days old culture grown on nutrient agar medium, were streaked on SMSA medium and incubated at 28°C for 3-5days. The developed colonies were examined with a hand lens.

Immuno - fluorescence antibody stain (IFAS)

IFAS is a serological method for rapid detection and to confirm identification of bacteria. Pathogenic isolates of *R. solanacearum* were used to prepare a suspension containing 10^6cfu/ml as well as with the reference isolate from Potato Brown Rot Project (Phase II), Dokki, Giza, Egypt. A standard volume (20 µl for 6 mm window diameter) was pipetted on five successive windows of a ten window test slide. Slide was air dried at room temperature (or at 40°C) and gently heat fixed by flaming. All windows were covered with 25ul of the antiserum in four dilutions (1:1600, 1:3200, 1:6400 and 1:12800). Slides were incubated for 30 min at room temperature in a humid chamber. Then slides were washed with tween 80 buffer and incubated with 25 µl anti-rabbit Nordic SW/AR fluorecin isothiocyanate conjugate in a 100 fold dilution for 30 min in humid chamber. Slides were washed with tween 80 buffer and excess buffer was removed carefully by blotting with filter paper. One droplet of 0.1 ml phosphate buffer glycerin (pH 7.6) was added to each window and the slide was covered with long cover glasses. Slides were examined under fluoresnt microscope (tube factor 1.25) with an epifluerescent light source and suitable filters with Fluorescin Isothocianate (FITC) under oil immersion lens. At least 20 microscope field windows were scanned for the presence of morphologically typical fluorescing cell (Janse, 1988).

Fatty acids profile

This test was carried out using Gas chromatography system at Plant Production Service, Wageningen, Netherlands. One isolate from each of soil (S1), tuber (T7), water (W11) and weed (We 16) were used in this experiment, (Stead, 1992).

Polymerase chain reaction (PCR)

DNA technique using PCR is one of a very sensitive methods used for characterization of *R. solanacearum*. It is based on the technique described by Seal *et al.* (1993) using *R.solanacearum* specific oligonucleotide primer OLI-1(5'GGG GGT AGC TTG CTA CCT GCC 3') and non specific primer Y-2, (5'CCC ACT GCT GCC TCC CGT AGG AGT 3') under the following reaction condition steps. All PCR procedures were conducted at the Brown Rot Project (phase II) Giza Egypt.

Extraction of DNA

Crude DNA of *Ralstonia solanacearum* isolates were extracted by heating 100 ml of cell suspension (10^6 cfu/ml) to 100 °C for 5 min. Boilt samples were stored at (-20 °C) until use.

DNA amplification

Two µl from each isolate were added to 23 µl reaction mixture (15.9 µl, sterile ultra pure water (SUPW): 2.5 µl of 10X PCR buffer: 1.5 µl $MgCl_2$: 1.25 µl of each d'ATP, d'CTP, d'GTP and d'TTP: 1.25 µl primer OLI-1, 1.25µl primer Y-2 and 0.1 µl taq polymerase).

Different PCR cycles were performed one cycle of two min at 96 °C to denaturent template DNA, 50 cycles of 20 seconds each at 94 °C for denaturation, one cycle of 20 sec at 68 °C for annealing of primers and one cycle of 30 sec. each at 72 °C for extension of copy and final extension of 10 min at 72 °C (Seal *et al.*, 1993).

Analysis of the PCR product

PCR fragments were detected by agarose gel electrophoresis (AGE) and stained with ethidium promide. Agarose gel was prepared by gently bringing agarose to the boiling 1X tris acetate EDTA (TAE) buffer (Seal *et al.*, 1993). Agarose in TAE buffer was made in gel at 10-15mm from the each comb and the sealing tape was removed, the tray was placed in a large electrophoresis tank containing (1X) TAE buffer to a depth of at least 5 mm buffer above the gel. Three micro liter droplets of loading buffer on parafilm were added to 12 ml of the PCR product from either sample. The positive control and distilled water as a negative control were mixed gently. Then loaded into the wells

of the gel. Appropriate DNA marker was included as reference in at least one well.Gel was run by applying 80 voltage (V) at 400 mA (8v/1cm) until the front of tracking indicator being within one cm from the end then the power supply switched off. Gel was removed carefully and soaked in eithidium bromide solution (0.5 µgm/ -1) for 30-45min. A specific PCR product of 288bp was visualized under UV trans-illumination (at 355 nm).

Box- PCR

This procedure was based on the method described by Louws *et al.* 1994 and Murry *et al.* 1995 using specific primer Box Air (5' CTA CGG CAA GGC GAC GCT GACG 3') under the following reaction condition steps.

Extraction of DNA

DNA of *R.solanacearum* isolate was extracted as mentioned before in PCR.

DNA amplification

Two µl from each isolate DNA extracts were added to 23 µl reaction mixtures composed of 13.1µl sterile ultra pure water (SUPW): 2.5µl of 10X PCR buffer, 1.5 µl $MgCl_2$: 0.5 µl d-NTP mix: 5µl primer Box-Air and 0.4µl Ampli Taq polymerase. PCR cycles were performed (one cycle of seven min at 95°C; one cycle of one min at 94°C; 30 cycles of one min at 53°C and 8min at 65°C) with a final 16 min at 65°C.

PCR analysis

Twelve µl of the amplified DNA fragment was added to three ul from 5X loading buffer and run on a 20 cm 20% agarose gel. Gel was run in TAE buffer at 105V at room temperature for exactly 2hr. Electrophoresed gel was stained for 40min in 400ml of 20mg/ml eithidium bromide as mentioned before.

Fingerprint analysis

Analysis of the genetic fingerprint patterns generated by box-PCR was carried out using gel compare software version 4.1 and the similarity of banding patterns collected by applying the Pearson product moment correlation coefficient. Cluster analysis was performed using the un-weighted pair group method using arithmetic averages algorithm (UPMGA) and the result was presented as a dendrogram.

RESULTS AND DISCUSSION

Isolation

Among 200 samples collected from El-Menufiya, El-Gharbia and El-Sharkia governorates to isolate *Ralstonia solanacearum* from tubers, water, soil and weeds, only twenty samples revealed *R.solanacearum* colonies of the

causal organism. Data in Table 1 revealed that fifteen isolates, were isolated from El-Menufiya, five from each of water, soil and weed samples (water isolates No.W11, W12, W13 from El-Nagar canal, while W14 and W15 from El-Basha canal). The last five isolates were collected from El-Gharbia governorate. Non *R.solanacearum* isolates were detected in El-Sharkia governorate samples due to samples collected from pest free areas (PFAS). This results confirm that this area is brown rot free disease and suitable for potato production and exportation to European markets .It is interesting to note that tuber samples collected from El-Menufiya and El-Gharbia governorates revealed positive brown rot infection by visual examination with characteristic oozing from the cut vascular bundles similar to that mentioned by Kelman (1954).

Pathogenicity Tests

Twenty isolates were examined for their pathogenic capability on tomato seedlings under artificial inoculation conditions. Data in Table 2 demonstrate that isolates S1, S3, T8, and W12 showed the highest severity of disease incidence. These Isolates revealed the highest value in disease index (1.0) and the lower time (3 days) period for disease development which ranged between 3-7 days. Pathogenicity tests showed typical symptoms of bacterial wilt on tomato seedlings. Differences in pathogenic capabilities and incubation periods between isolates of *R. solanacearum* isolated from different sources were also reported by Kelman (1954), Janse (1988) and McCarter (1991).

Identification of the Pathogen

Physiological and biochemical characteristics

This part deal with the biological characteristics and the taxonomic identification of the selected isolates (twenty isolates). All the 20 isolates appeared to be non–spore formers, gram–negative and motile short-rod bacteria. All of isolates Kovac's oxidase test was positive, while catalase was negative. Activity of arginin dihydrolase was negative. The isolates could not hydrolyze starch or liquefy gelatin. Nitrate reduction was positive. All isolates produce acid from maltose, lactose and cellobiose and showed no reaction with sorbitol, mannitol and dulcitol as show in Table 3. Generally, it could be concluded that the morphological, cultural and physiological characteristics of these isolates confirm with those pathological reaction described for *R. solanacearum* by Krieg and Holt (1984).

Table 1. Isolation of *Ralstonia solanacearum* from different sources at different governorates.

Governorates	No. of positive samples			
	Soil	Tuber	Water	Weeds
El-Sharkia				
Ismailia canal	*	*	–	*
El-Salhia city	–	–	–	—
Pivot 10		–	–	
Pivot 20				
El-Gharbia				
Mansoriate El-Farastec El-Alamia	*	2+	*	*
Kafer El-Zayat Kafer Yakopc	*	3+	*	*
El-Monufia				
Tallia	5+	*	*	5+
El-Nagar	-	*	3+	*
El-Basha	-	*	2+	*

* Sample don't collected from this site - Negative reaction during isolation
+ Positive reaction during isolation

Table 2. Pathogenicity tests of *Ralstonia solanacearum* isolates inculcated in tomato seedlings and measured as disease severity, disease index and incubation period.

Isolate source		Tests parameter		
		Disease Severity	Incubation period	Re-isolation IF
Soil	S1	5	5	+
	S2	3	7	+
	S3	5	5	+
	S4	4	5	+
	S5	3	7	+
Tuber	T6	3	5	+
	T7	2	3	+
	T8	5	3	+
	T9	4	5	+
	T10	4	7	+
Water	W11	2	3	+
	W12	5	7	+
	W13	3	7	+
	W14	4	7	+
	W15	3	7	+
Weed	We16	2	7	+
	We17	3	5	+
	We18	3	5	+
	We19	2	3	+
	We20	2	5	+

Table 3. Morphological, physiological and biochemical characters of *Ralstonia. solanacearum* the pathogenic bacteria

Character	Bacterial isolates																			
	S 1	S 2	S 3	S 4	S 5	T 6	T 7	T 8	T 9	T 10	W 11	W 12	W 13	W 14	W 15	We 16	We 17	We 18	We 19	We 20
Shape of cell	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Motility	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gram reaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sporulatoin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brown pigment	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fluorescent p.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kovac's Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginin dihydrolase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin liquefaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Utilization of,																				
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dulcitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

R = short rod shap , S = soil samples , T = tubers samples , W = water samples , We = weed samples
- = negative reaction , + = positive reaction

Cultivation on media

On King's B medium

No fluorescent pigments were be detected with UV but produced brown pigment in glucose nutrient agar medium.

On SMSA medium

The virulent colonies of *R. solanacearum* on SMSA medium are milky white, irregular and fluidal with blood red coloration in the center. The selective media for detecting viable cells of *R. solanacearum* from different habitats have been recommended by many investigators and can be easily differentiated by Kelman's medium (Elphinstone *et al*., 1996). Typical colony character of virulent *R. solanacearum* isolates showed in Fig., 1.

Immuno – fluorescence antibody stain (IFAS)

All colonies developed on SMSA medium were tested by IFAS .Morphologically, cells were short rods under phase contrast microscope and the cells stained evently as bright green fluorescent Fig.,2. It is established that the detection level of polyclonal antibody in IFAS is recorded of 10^4 cells ml^{-1} where the false reaction was limited to only 2-3%. Other levels give high false reaction. These results are in harmony with those reported by Janse (1988), Ramadan (2000) and Kehil (2002).

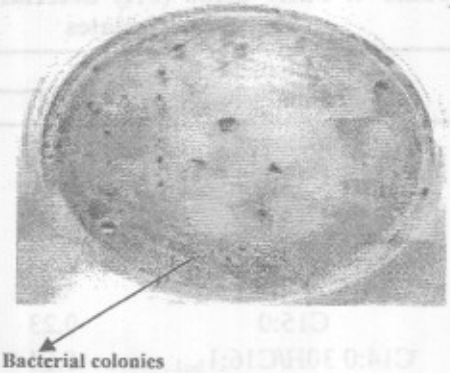

Fig. 1. Characters of virulent *Ralostonia solanacearum* isolate indicate irregular colonies and blood red color in center.

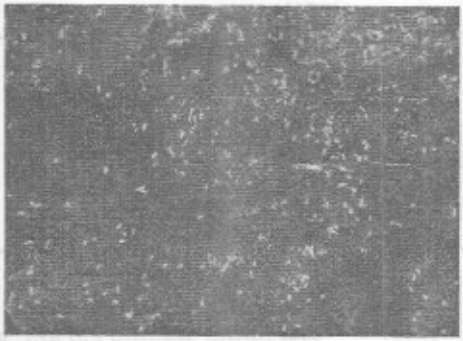

Fig. 2. *Ralstonia solanacearum* cells by IFAS under fluorcence Microscope

Fatty acid analysis of *Ralstonia solanacearum*

Tables 4 and 5 showed the fatty acid (FA) profiles of four *R. solanacearum* isolates, obtained from different sources (soil, tuber, water and weeds). The results showed that extract materials

Table 4. Fatty acids (FA) determined in estrified extracts of *Ralstonia solanacearum* isolates

No. of isolates FA analysis	Isolates No.			
	S5	T8	W13	We18
unknown	10.93	10.93	14.5	10.93
C12:0	0.09	0.12		-
C14:0	4.56	4.48	4.81	4.55
$C15:0_{Iso}$	0.22	0.09		0.18
$C15:1_{\omega 6c}$	0.2	0.7	0.27	0.24
C15:0	0.23	1.08	0.46	0.22
$C14:0\ 30H/C16:1_{Iso1}$	6.64	6.48	6.77	-
$C16:1_{\omega 7c}/C15_{Iso2oH}$	24.41	32.29	21.83	26.79
$C16:1_{\omega 5c}$	0.24	0.36	0.42	0.28
C16:0	24.66	23.78	24.48	25.16
$C17:0_{cyclo}$	8.05	9.02	10.15	7.46
C17:0	0.29	0.75	0.2	0.2
$C16:1_{2OH}$	4.6	5.56	5.88	4.53
$C16:0_{2OH}$	0.87	0.78	0.97	0.97
$C18:1_{\omega 7C/\omega 9t/\omega 12t}$	19.36	18.25	18.65	17.97
C18:0	0.35	0.3	0.23	0.28
$C19:0_{cyclo\ \omega\ 8c}$	0.53	0.43	0.62	0.37
$C18:1_{2OH}$	4.48	4.18	4.13	4.24
$C12:0_{ALDE?}$	6.87	6.95	6.77	6.67

Table 5. Percentage of homology of cellular fatty acids of four isolates of *Ralstonia solanacearum* with bacterial reference isolates

Bacterial reference Lab1(TSBA)[a].(Rev3.90)	S5	T8	W13	We18
Burkholderia	83.8	66.4	63.8	86.9
B. solanacearum	83.8	66.4	63.8	86.9
B. rickettii	77.5	60.6	63.8	69.3
Lab2(PD)[b].(Rev1.90)	62.2	49.3	41.8	68.4
Pseudomonas				
Ps. solanacearum	62.2	49.3	41.8	68.4
Ps.s.$B_{1,2,3,4}/R_1$	62.2	35.1	35.4	54.8
Ps.s.B_2R_3	62.1	49.3	41.8	68.4
Ps. pickettii	52.4	32.8	40.8	-

a and b: represent two different libraries using the microbial identification System-based of software available from MIDI DOS system (Newark, DE, USA).

TSBA and PD: two different reference labs.

contained 17 fatty acids in addition to two unknown acids one of which might be in aldehydic form with C12:0 chain. The majority of fatty acid profile was un-branched along with two cyclo-fatty acids. The extracts showed reasonable quantities of C16:0 and C16:1_{ω}7c/c15 iso 2OH and the percentage ranged between 23.78 to 25.16 and 21.83 to 32.29% for the fatty acids, respectively. Those of longer chain, however, showed similar quantities as indicated by the percentage (s) recorded. The C17:0 cyclo ranged from 7.46 to 10.15%, however, the C18:$1_{\omega 7C/\omega 9t/\omega 12t}$ ranged from 17.97 to 19.36%. Results confirmed those report by Shehata Neven, 2001 and Balabel Naglla *et al.*, 2006 who found that *R.solanacearum* extracted material contained 18 fatty acids in addition to two unknown acids one of which in aldehydic form with C12:0 chain. Cellular fatty acid analysis of four isolates tested showed various degree of homology with different bacterial references (Table, 4). All isolates tested provided the highest percentage of homology with reference bacteria *Burkholderia solanacearum*. Isolate No We18 gave 86.9% homology followed by isolate No S5 (83.8%). Isolates Nos.T8 and N13 expressed 66.4 and 63.8% homology, respectively. Otherwise reference bacteria showed weaker homology with tested isolates.

Polymerase chain reaction (PCR)

The identification results of twenty *R.solanacearum* isolates derived from different habitat were examined by PCR. The specific 288 base pairs (bp) in PCR product visualized under UV light showed very close similarity of the twenty isolates under investigation Fig., 3.

Box –PCR

Figure 4 show very close similarity of the all isolates derived from different sources. Box–PCR produced fingerprints with 10-15 well spaced bands in the approximate size region of 200-220 bp and the most suitable protocol for fingerprint analysis.

Similar result for the work of Egyptian isolates were obtained by Shehata Neven, (2001).

It is known however, that the PCR technique has been used to identify *R. solanacearum* strains/ reference and it's revealed very closed similarity of twenty isolates from different sources Fig.5.

Similar results were obtained by Seal *et al.*(1992) and Opina *et al.*(1997).

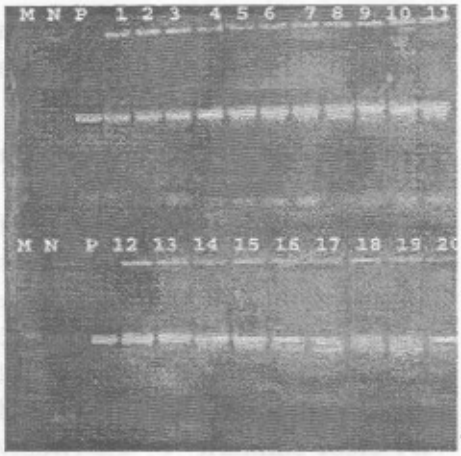

Fig. 3. PCR products amplified using two primers (Oli-1 and Y-2) from genomic DNA of different isolates from different habitats. (1-20) = No. of isolates, P =positive control N= negative control, M= marker

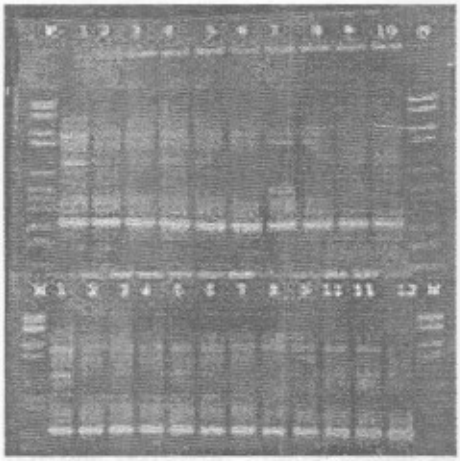

Fig. 4. POX-PCR of different isolates from different habitats (1-20) = No. of isolates, P = positive control N = negative control, M = marker

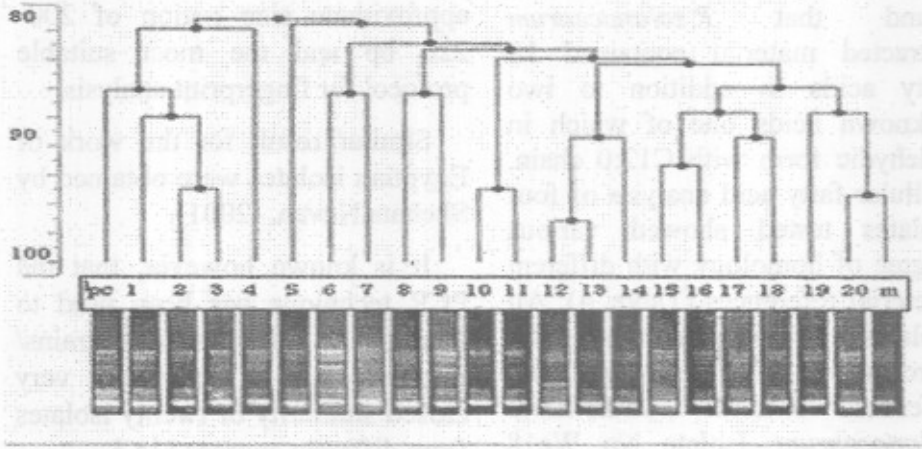

Fig. 5. A dendrogram of Box-PCR fingerprints of different *R.solanacearum* isolates from different habitats indicate the similarity degree between isolates (80%).

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تعريف رالستونيا سولاناسيرم المعزولة من درنات البطاطس ، الحشائش، الماء، و التربة في مصر

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يعتبر مرض العفن البني أو الذبول البكتيري في البطاطس من أهم أمراض البطاطس التي يجرى عليها حجرزراعي على مستوى العالم و في مصر. لذلك تم عزل وتعريف هذا المسبب المرضي من مصادر مختلفة باستخدام طرق سريعة و دقيقة.

أوضحت النتائج أنه من بين ٢٠٠ عينة، تم عزل ٢٠ عزلة ممرضة من درنات البطاطس، التربة، الماء والحشائش. و اشتملت العزلات على ١٥ عزلة من محافظة المنوفية وخمسة عزلات من الغربية ولم يتم عزل المسبب المرضي من محافظة الشرقية (الصالحية) حيث أنها منطقة خالية من هذا المرض .

تم اختبار قدرة تلك العزلات المرضية على بادرات الطماطم. وكانت عزلتي التربة (رقم ١،٢) وعزلة البطاطس رقم (٨) وعزلة الماء رقم (١٢) أكثر العزلات ضراوة في إحداث المرض على بادرات الطماطم .

أوضحت نتائج تعريف العشرين عزلة السابقة على أنها تتبع بكتيريا رالستونيا سولاناسيرم ***Ralstonia solanacearum*** وذلك طبقا لخصائصها البيوكيميائية والفسيولوجية، تنميتها على بيئة كنج B وعلى بيئة SMSA ، التحليل باستخدام الأجسام المضادة المعلمة فلورسنتيا، تحليل الأحماض الدهنية وعن طريق تحليل الحمض النووي DNA وذلك باستخدام جهاز البلمرة المتسلسل PCR .