Novel Research in Microbiology Journal (2018), 2(6): 138-146
Research Article

(Print) (ISSN 2537-0286)
(Online) (ISSN 2537-0294)
https://nrmj.journals.ekb.eg/
DOI: 10.21608/NRMJ.2018.22706

Race and biovar determination of *Ralstonia solanacearum* in the north west of Pakistan

Muhammad Junaid[1*]; Musharaf Ahmad[1]; Saifullah[1]

[1]Department of Plant Pathology, University of Agriculture, Peshawar, Pakistan

[*]Corresponding author E-mail: mjunaid27@yahoo.com

Received: 27 November, 2018; Accepted: 9 December, 2018; Published online: 25 December, 2018

Abstract

Multiple comprehensive surveys were conducted during 2012, in order to know the current status of bacterial wilt (BW) of tomato caused by *Ralstonia solanacearum* (*R. solanacearum*), in the commercial tomato growing districts of Khyber Pakhtunkhwa (KP), Pakistan. A total of 74 locations covering all the 26 districts of the 7 divisions of KP were visited for the presence of tomato plants showing BW symptoms. According to Polymerase chain reaction (PCR), the expected 281bp band was amplified from 25 candidates of *R. solanacearum* isolates, thus genetically confirming them to be *R. solanacearum*. These *R. solanacearum* isolates were subjected to race identification and biovar determination tests. Race differentiation was done using hypersensitive response (HR) test on tobacco plants; whereas 10% solutions of six different sugars including disaccharides (i.e. sucrose, lactose and maltose), and sugar alcohols (i.e. manitol, sorbitol and dulcitol) in Triphenyl tetrazolium chloride (TTC) medium, were used for biovars determination. Results indicated that all PCR-confirmed *R. solanacearum* isolates belonged to race-1 and biovar-3. However, two isolates i.e. (*R. solanacearum*11-DIK1 and *R. solanacearum*10-MDN2) were found to belong to race-3, biovar-2.

Keywords: Tomato, *Ralstonia solanacearum*, PCR, race, biovar determination

1. Introduction

Tomato (*Solanum lycopersicum* L.), is one of the most important vegetable crops of the world, and is a member of Solanaceae family. It is perennial in nature, but sometimes it grows as an annual plant in temperate climates. Tomato can be considered as sack of nutrients with 2.9-3.9% sugars and carbohydrates contents; respectively. It contains 0.9% proteins, 1.2 % dietary fiber, and 0.2 % fats. In addition, different vitamins (vitamin A, C and E), elements (e.g. Potassium), zeaxanthin, carotene, lycopene and lutein are also present in a considerable amount in this crop (USDA. 2011).

According to Zhang *et al*., (2009); Schidfar *et al*., (2011), tomato is believed to beneficial to the heart thus reducing cardiovascular diseases, as well

as playing a positive role in the control of cancer and neurodegenerative diseases. The powerful natural anti-oxidant lycopene; is also present in tomatoes. Lycopene helps protects humans against UV-skin damage and prostate cancer (Allen, 2008). The lead producers of tomatoes are China, India, United States, and Turkey; producing 50.55, 18.23, 12.57, and 11.82 million tons annually; respectively. With annual production of 0.57 million tons, Pakistan ranked 34th in the world in terms of tomato production, and 142th in terms of per hectare yields of tomatoes (FAOSTAT. 2015). In addition to other reasons; various tomato diseases caused by viruses, fungi, nematodes and bacteria play major roles in reducing the per hectare yield of tomatoes.

Tomato bacterial wilt (BW) caused by *R. solanacearum* (Smith) is considered to be one of the most important diseases due to its economic impact. *R. solanacearum* is a Gram-negative rod; and is a major threat to the production of tomatoes, potatoes, tobacco and other solanaceous plants in both tropical and temperate zones. Up to 30% annual losses in tomatoes and potatoes were common in Pakistan. More than 200 different plant species were attacked by this pathogen including; ground nuts, chillies, cotton, rubber, cassava, caster beans, brinjals, ginger and many weeds.

R. solanacearum is a species complex consisting of groups, races, biovars, bio-types, sub-races and strains (Hossain, 2013; She *et al*., 2015, Yuan *et al*., 2015). It is highly variable and consists of at least six biovars based on biochemical properties; and five races based on host range (Fegan and Prior, 2005). *R. solanacearum* race 3 biovar 2 is classified as a select agent, and strict quarantine regulations were in place to exclude it from disease-free zones of the world including the continental U.S. It is critical to develop a clear understanding of the pathogen strains and their variability's in order to allow accurate detection and diagnosis; as well as application of effective management strategies such as deployment of disease-resistant varieties.

Since no scientific work has been carried out on BW in tomato crop in KP; there was no information regarding the races and biovars of this pathogen (A. Bibi, personal communication). To fill the vacuum of this valuable information about pathogenic *R. solanacearum*, the present research was initiated with the objective to identify all races and biovars of this pathogen consistently present in north west of Pakistan.

2. Materials and methods

2.1. Collection of diseased tomato plant samples

Tomato growing districts of the KP (Table 1) were surveyed multiple times throughout the growing period (i.e. April-August, 2012) for collection of samples of diseased plants showing typical symptoms of BW. KP was divided into seven administrative divisions; thus a total of 74 sites were surveyed for sampling. Three to ten fields were surveyed within each location. In each field, two-three spots (each having about 8-10 plants) were randomly selected.

2.2. PCR-based confirmation of R. solanacearum isolates

R. solanacearum isolates were confirmed by PCR using species-specific primers i.e. 5'GTCGCCGTCAACTCACTTTCC3' and 5'GTCGCCGTAGCAATGCGGAATCG3'; with amplified 281bp fragment (Umesha and Avinash, 2014).

2.2.1. Isolation of nucleic acids (DNA) from *R. solanacearum* isolates

DNA was extracted as described by Pastrik and Maiss, (2000). For the isolation of bacterial genomic DNA, a loopful of a bacterial culture was suspended in 1ml PBS buffer (0.14 M NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH_2PO_4; pH 7.4), and then centrifuged for 2 min. at 13,000 Xg and 4°C.

Table 1: List of administrative divisions of Khyber Pakhtunkhwa (KP) surveyed for bacterial wilt (BW) of tomato plant

S. no.	Administrative Division	Districts
1	Bannu Division	Bannu, LakkiMarwat
2	D.I. Khan Division	Dera Ismail Khan, Tank
3	Hazara Division	Abbottabad, Battagram, Haripur, Mansehra, Tor Ghar, Lower Kohistan, Upper Kohistan
4	Kohat Division	Hangu, Karak, Kohat
5	Malakand Division	Buner, Chitral, Lower Dir, Upper Dir, Malakand, Shangla, Swat
6	Mardan Division	Mardan, Swabi
7	Peshawar Division	Charsadda, Nowshera, Peshawar

The pellet was re-suspended in 320 µl lysis-buffer (100 mM NaCl; 10 mM Tris-HCl, 1mM EDTA, pH 8.0), placed on a heating block at 95°C for 10 min. and then cooled on ice for 5 min. 80 µl of lysozyme stock solution (50mg/ mllysozyme in 10mM Tris-HCl, pH 8.0) was added, and then the sample was incubated for 30 min. at 37°C. The DNA was purified using the Easy-DNA-Extraction-kit (Invitrogen, DeSchelp, Netherlands), containing two solutions (i.e. A and B). 220µl of the solution A was added; and then the mixture was incubated for 30 min. at 65°C. After addition of 100 µl of solution B and mixing; 500 µl of chloroform was added and the mixture was centrifuged for 20 min. at 20,000 Xg. The aqueous phase was separated and DNA was precipitated by addition of 70 %ethanol; the resulting pellet was washed with 80 % ethanol. After the final centrifugation, the DNA was re-suspended in 100 µl of sterile ultra-pure water.

2.2.2. PCR Amplification with species-specific primers

The extracted genomic DNA from each isolate of *R. solanacearum* was PCR-amplified using specific primers according to (Bibi *et al*., 2015). The volume of the PCR mixture used was 25 µl each. This mixture per tube consisted of 1 µl of each primer (0.2 µM); 1 µl of Taq-DNA polymerase, 3 µl template DNA (1ng), 2.25 µl buffer (KCL-50mM+Tris-HCl-2mM, pH 8.4), 2 µl $MgCl_2$ (1 mM); and 2.5 µl (100 µM) of each dNTP. A 30 PCR cycle was performed for all candidate isolates. The conditions used for this PCR were: 3 min. at 95°C (one time at the start); 20 sec. at 94°C; 50 sec. at 50°C; 30 sec. at 72°C; 5 min. at 72°C (one time at the end); and then stored at 4°C. A template-less negative control was also included.

2.2.3. Gel electrophoresis

To separate the PCR-amplified DNA bands, 2 % agarose gel (0.67 g agarose dissolved in 30ml TBE buffer) was prepared, cooled, tray-solidified and sub-merged in TBE buffer. Blue tracking (3 µl/ sample) was added to each PCR-product sample (25 µl), and each sample was carefully loaded to the wells of the gel. A 1 kb size ladder was also used. Electrophoresis was performed as per procedure of Bibi *et al*., (2015) at 200 volts for 30 min.

2.2.4. Gel staining and imaging

To visualize the different bands; 0.5 µg methediumbromide solution was used for gel staining for 15 min., and then washed in sterile water for another 15 min. UV tech machine was used to observe the bands under UV light; while images were saved for future use.

2.3. Race determination and tobacco hypersensitive reaction (HR)

The race differentiation was carried out based on tobacco hypersensitive response (HR). One month old tobacco seedlings were transplanted into plastic pots containing sterilized soil. Four weeks after transplantation; tobacco leaves were infiltrated at their lower surfaces with 1 ml of *R. solanacearum* suspension (10^8 cells/ ml) using a disposable plastic syringe. Control leaves were infiltrated with autoclaved suspension of the bacterium. Leaf reactions were examined as early as possible on the day following infiltration and again 24 h later (Gebreel *et al*., 2000).

2.4. Biovar determination of *R. solanacearum* isolates through carbohydrate utilization tests

Biovars determination was done using carbohydrate utilization test according to Chaudhry and Rashid, (2011). Solutions of 10% of six different sugars including; disaccharides such as: sucrose, lactose, maltose, and sugar alcohols such as: manitol, sorbitol and dulcitol; were prepared and then sterilized by heating for 30 min. at 100°C. (TTC) medium was prepared and then 10 ml of this medium were aseptically added in each test tube already containing 10 ml of the sterilized 10% sugar solution. About 50 µl of 48 h old bacterial suspension containing about 10^8 cells\ ml, were inoculated into each tube of TTC-sugar mixture. The tubes were incubated at 28°C and examined after 72 h for change in pH indicated by changing color of medium from green to orange.

3. Results

3.1. Molecular identification of *R. solanacearum* isolates

Using species-specific primers, PCR was used to finally confirm the identity of *R. solanacearum* isolates. The 281bp specific band was amplified from 25 isolates, confirming that they all belong to the genus *R. solanacearum* (Fig. 1.).

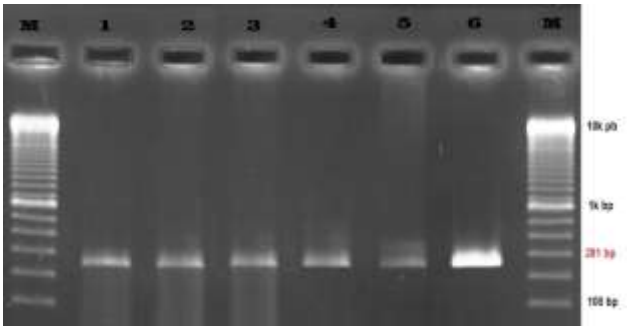

Fig. 1: Amplification of amplified *R. solanacearum*-specific 281bp fragment

3.2. Race determination using tobacco hypersensitive reaction (HR)

The PCR-confirmed *R. solanacearum* isolates were subjected to race identification test. HR test was negative for 23 isolates. All isolates except (RS11-DIK1 and RS10-MDN2); produced dark brown lesions 24 h after leaves infiltration (Table 2). After 48 h the dark brown lesions on leaves became necrotic and were surrounded by yellow halos. The two isolates (RS11-DIK1 and RS10-MDN2) produced no visible symptoms on the inoculated tissue; however, they caused yellowing of this tissue, indicating that they belong to Race-3. After 72 h; yellowing and reversible wilting symptoms were observed in tissues inoculated by both isolates. After one week, (RS11-DIK1 and RS10-MDN2) inoculated leaves showed wilting symptoms and thus confirming that they belong to Race-3, whereas, all the remaining 23 isolates exhibited complete wilting of plants thus designed as Race-1. After one month, the 23 isolates killed the inoculated plants; however, those plants inoculated with (RS11-DIK1 and RS10-MDN2) showed severe wilting symptoms. The same pathogenic *R. solanacearum* isolates were re-isolated and then re-identified from the infested plants, confirming their pathogenicity.

3.3. Biovar determination of *R. solanacearum* isolates

Results of biovar determination tests shown in Table (3) revealed that all the 25 isolates of *R. solanacearum* oxidized disaccharides (i.e. sucrose, lactose, and maltose) and sugar alcohols (i.e. mannitol, sorbitol and dulcitol). The oxidation reaction was indicated by the change of color of TTC medium from green to yellow. All isolates were designed as biovar III except isolates (RS10-MDN2 and RS11-DIK1) which failed to oxidize sugar alcohols; and were thus classified as biovar II (Table 4). All the biovar III isolates belong to Race-1, while the 2 biovar II isolates belong to Race-3.

Table 2: Race determination of 25 *R. solanacearum* isolates recovered from different locations of Khyber Pakhtunkhwa (KP)

S. no	Isolates	Location	Symptoms produced on tobacco plants					Race
			24 h	48 h	72 h	One week	One month	
1	*RS1-SGL1*	*Shangla*	DBL	NYH	Y+W	CPW	WD	1
2	*RS2-BAN1*	*Bannu*	DBL	NYH	Y+W	CPW	WD	1
3	*RS3-HAR1*	*Haripur*	DBL	NYH	Y+W	CPW	WD	1
4	*RS4-KAR1*	*Karak*	DBL	NYH	Y+W	CPW	WD	1
5	*RS5-SWBI*	*Swabi*	DBL	NYH	Y+W	CPW	WD	1
6	*RS6-SWT1*	*Swat*	DBL	NYH	Y+W	CPW	WD	1
7	*RS6-SWT2*	*Swat*	DBL	NYH	Y+W	CPW	WD	1
8	*RS7-NSH1*	*Noshera*	DBL	NYH	Y+W	CPW	WD	1
9	*RS7-NSH2*	*Noshera*	DBL	NYH	Y+W	CPW	WD	1
10	*RS8-BNR1*	*Buneer*	DBL	NYH	Y+W	CPW	WD	1
11	*RS8-BNR2*	*Buneer*	DBL	NYH	Y+W	CPW	WD	1
12	*RS9-KOH1*	*Kohat*	DBL	NYH	Y+W	CPW	WD	1
13	*RS10-MDN1*	*Mardan*	DBL	NYH	Y+W	CPW	WD	1
14	*RS10-MDN2*	*Mardan*	NVS	YIT	Y+RW	W	SW	3
15	*RS10-MDN3*	*Mardan*	DBL	NYH	Y+W	CPW	WD	1
16	*RS11-DIK1*	*D.I. Khan*	NVS	YIT	Y+RW	W	SW	3
17	*RS12-CHD1*	*Charsadda*	DBL	NYH	Y+W	CPW	WD	1
18	*RS12-CHD2*	*Charsadda*	DBL	NYH	Y+W	CPW	WD	1
19	*RS12-CHD3*	*Charsadda*	DBL	NYH	Y+W	CPW	WD	1
20	*RS13-DLO1*	*Dir Lower*	DBL	NYH	Y+W	CPW	WD	1
21	*RS13-DLO2*	*Dir Lower*	DBL	NYH	Y+W	CPW	WD	1
22	*RS14-PES1*	*Peshawar*	DBL	NYH	Y+W	CPW	WD	1
23	*RS15-MKD1*	*Malakand*	DBL	NYH	Y+W	CPW	WD	1
24	*RS15-MKD2*	*Malakand*	DBL	NYH	Y+W	CPW	WD	1
25	*RS16-HAN1*	*Hangu*	DBL	NYH	Y+W	CPW	WD	1

Where; NVS = No visible symptoms, DBL = Dark brown lesion, YIT =Yellowing of inoculated tissue, NYH = Necrosis surrounded by yellow halo, W= wilting, Y = Yellowing, RW = reversible wilting, CPW = Complete plant wilting, SW= severe wilting WD= wilted and dead.

Table 3: Biovar determination of 25 *R. solanacearum* isolates recovered from different locations of Khyber Pakhtunkhwa (KP)

S. no	Isolates	Location	Disaccharides			Sugar Alcohols			Biovars
			Sucrose	Lactose	Maltose	Mannitol	Sorbitol	Dulcitol	
1	*RS1-SGL1*	*Shangla*	+	+	+	+	+	+	III
2	*RS2-BAN1*	*Bannu*	+	+	+	+	+	+	III
3	*RS3-HAR1*	*Haripur*	+	+	+	+	+	+	III
4	*RS4-KAR1*	*Karak*	+	+	+	+	+	+	III
5	*RS5-SWBI*	*Swabi*	+	+	+	+	+	+	III
6	*RS6-SWT1*	*Swat*	+	+	+	+	+	+	III
7	*RS6-SWT2*	*Swat*	+	+	+	+	+	+	III
8	*RS7-NSH1*	*Noshera*	+	+	+	+	+	+	III
9	*RS7-NSH2*	*Noshera*	+	+	+	+	+	+	III
10	*RS8-BNR1*	*Buneer*	+	+	+	+	+	+	III
11	*RS8-BNR2*	*Buneer*	+	+	+	+	+	+	III
12	*RS9-KOH1*	*Kohat*	+	+	+	+	+	+	III
13	*RS10-MDN1*	*Mardan*	+	+	+	+	+	+	III
14	*RS10-MDN2*	*Mardan*	+	+	+	-	-	-	II
15	*RS10-MDN3*	*Mardan*	+	+	+	+	+	+	III
16	*RS11-DIK1*	*D.I. Khan*	+	+	+	-	-	-	II
17	*RS12-CHD1*	*Charsadda*	+	+	+	+	+	+	III
18	*RS12-CHD2*	*Charsadda*	+	+	+	+	+	+	III
19	*RS12-CHD3*	*Charsadda*	+	+	+	+	+	+	III
20	*RS13-DLO1*	*Dir Lower*	+	+	+	+	+	+	III
21	*RS13-DLO2*	*Dir Lower*	+	+	+	+	+	+	III
22	*RS14-PES1*	*Peshawar*	+	+	+	+	+	+	III
23	*RS15-MKD1*	*Malakand*	+	+	+	+	+	+	III
24	*RS15-MKD2*	*Malakand*	+	+	+	+	+	+	III
25	*RS16-HAN1*	*Hangu*	+	+	+	+	+	+	III

Where; (+): Color of TTC medium changed from green to yellow, (-): Color of the medium did not change

Table 4. Over all PCR identification, race and biovar determination of the 25 *R. solanacearum* isolates

S. no	Location	PCR confirmed *R. solanacearum* isolates	Race	Biovar
1	Shangla	*RS1-SGL1*	1	III
2	Bannu	*RS2-BAN1*	1	III
3	Haripur	*RS3-HAR1*	1	III
4	Karak	*RS4-KAR1*	1	III
5	Swabi	*RS5-SWBI*	1	III
6	Swat	*RS6-SWT1*	1	III
7	Swat	*RS6-SWT2*	1	III
8	Noshera	*RS7-NSH1*	1	III
9	Noshera	*RS7-NSH2*	1	III
10	Buneer	*RS8-BNR1*	1	III
11	Buneer	*RS8-BNR2*	1	III
12	Kohat	*RS9-KOH1*	1	III
13	Mardan	*RS10-MDN1*	1	III
14	Mardan	*RS10-MDN2*	3	II
15	Mardan	*RS10-MDN3*	1	III
16	D.I. Khan	*RS11-DIK1*	3	II
17	Charsadda	*RS12-CHD1*	1	III
18	Charsadda	*RS12-CHD2*	1	III
19	Charsadda	*RS12-CHD3*	1	III
20	Dir Lower	*RS13-DLO1*	1	III
21	Dir Lower	*RS13-DLO2*	1	III
22	Peshawar	*RS14-PES1*	1	III
23	Malakand	*RS15-MKD1*	1	III
24	Malakand	*RS15-MKD2*	1	III
25	Hangu	*RS16-HAN1*	1	III

4. Discussion

To confirm the identity of microbial isolates which give fallacy results, it is necessary to use molecular identity-confirmation methods such as PCR (Champoiseau and Jones, 2009). Thus to confirm the identity of current isolates as *R. solanacearum*; we used species-specific primers that amplified the specific 281bp fragment confirmed from previous reports of Kyaw *et al*., (2014); Umesha and Avinash, (2014); Ying *et al*., (2017).

As a highly diversified pathogen; *R. solanacearum* is considered as a species complex in a heterogeneous group consisting of hundreds of genetically distinct strains (Fegan and Prior, 2005). The pathogen has developed several races, biovars (Tahir *et al*., 2014). According to Denny and Hayward, (2001); this species complex was subdivided into races based on host range, and into biovars based on its ability to produce acid from a panel of sugars. Umesha and Avinash, (2014) added that; a more phylogenetically meaningful classification scheme based on sequences of several genes, divided this species complex into four phylotypes. This scheme previously grouped strains according to geographical origin such that; strains from Asia were in phylotype I, those from the Americas were in phylotype II, those from Africa were in phylotype III, and those from Indonesia which is the apparent center of diversity, were in phylotype IV (Fegan and Prior, 2005). Phylotypes themselves can be sub-grouped into sequevars which were clusters of isolates with highly conserved DNA sequences.

Race 1 biovar 1 (R_1B_1) isolates of *R. solanacearum* were considered to be the most virulent. Members of R_3B_2 typically infect potatoes; but they can also infect tomatoes and geranium (Ornamental) plants (Swanson *et al*., 2005). In the present study, we obtained two R_3B_2 strains (RS11-DIK1 and RS10-MDN2) isolated from tomato plants samples collected from D.I. Khan and Mardan districts; respectively, which were pathogenic to tomatoes. These R_3B_2 strains were widely distributed in Asia Africa, South and Central America; and were found in some soils and waterways in Europe (Elphinstone, 2005). According to Alvarez *et al*., (2005), strains of R_4B_4 have been reported from North America, while R_2B_1were reportedly found in some coastal parts of West Africa.

Results of the current study revealed that; 23 isolates of *R. solanacearum* belonged to Race 1, while two only belonged to Race 3. Race 1 strains attack tobacco, tomato, many other solanaceous crops; and certain diploid bananas. However, Race 3 causes wilting in potato, tomato and rarely other solanaceous plants. Race 2 and 4, fortunately were not common in Asia. Race 2 (found in Africa) was known to infect triploid banana (*Musa acuminata*) and *Heliconia* spp., while Race 4 attacks mulberry (OEPP/EPPO. 2004). Five races have been described so far; but they differ in their host range, geographical distribution and in ability to survive under different environmental conditions (Popoola *et al*., 2015).

Conclusions

Race and biovar determination of 25 *R. solanacearum* isolates recovered from different BW infected tomato plants growing areas of KP; Pakistan, indicated that 92% of these isolates belonged to race 1, biovar 3; while 8 were race 3, biovar 2.

Conflict of interests

The authors declare no conflict of interests.

5. References

Allen, N.E.; Key, T.J. and Appleby, P.N. (2008). Animal foods, protein, calcium and prostate cancer risk: the European Prospective Investigation into Cancer and Nutrition. British Journal of Cancer. 98:1574-1581.

Alvarez, A.M.; Trotter, K.J.; Swafford, M.B.; Berestecky, J.M.; Yu, Q.; Ming, R.; Hepperly, P.R. and Zee, F. (2005). Characterization and detection of *Ralstonia solanacearum* strains causing bacterial wilt of ginger in Hawaii. pp. 471-478, In: Bacterial Wilt: The Disease and the *Ralstonia solanacearum* Species Complex. C. Allen, P. Prior, and A. C. Hayward, eds. American Phytopathological Society, St. Paul, MN.

Bibi, A.; Junaid, M.; Ahmad, M.; Ali, A.; Wahid, F.; Naz, R.; Ullah A.; Mahar, A. and Kalhoro, S.A. (2015). Use of Polymerase Chain Reaction for Identification of the Pathogen and Management of Potato Soft Rot with Zinc Application. American Journal of Plant Sciences. 6: 1138-1143.

Champoiseau, P.G. and Jones, J.B. (2009). *Ralstonia solanacearum* race 3 biovar 2 causes tropical losses and temperate anxieties. Plant Health Progress. Online available at: http://www.apsnet.org/publications/apsnetfeatures/Documents/2009/Ralstonia.pdf

Chaudhry, Z. and Rashid, H. (2011). Isolation and characterization of *Ralstonia solanacearum* from infected tomato plants of soanskesar valley of Punjab. Pakistan Journal of Botany. 43(6): 2979-2985.

Denny, T.P. and Hayward, A.C. (2001). *Ralstonia*. In: Laboratory guide for identification of plant pathogenic bacteria, 3rd (Edn. Schaad NW, Jones JB, Chun W). APS Press, St. Paul, pp. 151-174.

Elphinstone, J.G. (2005). The current bacterial wilt situation: a global overview. In: Bacterial Wilt: The Disease and the *Ralstonia solanacearum* Species Complex. (Eds. C. Allen, Prior, P. and Hayward, A. C.). pp. 9-28. American Phytopathol. Soci., St. Paul, MN, USA.

FAOSTAT. (2015). FAO (Food and Agricultural Organization of the United Nations). http://faostat.fao.org.

Fegan, M and Prior, P. (2005). How complex is the '*Ralstonia solanacearum* species complex'. In: Bacterial wilt the disease and the *Ralstonia solanacearum* species complex (Eds. Allen C., Prior P., Elphinstone J.). pp. 449-462. APS Press, St. Paul, MN, USA.

Gebreel, H.M.; Madbouly, A.K.; Tawfik, A.A. and Youssef, Y.A. (2000). Serological, physiological and biochemical studies on *Burkholderia solanacearum* (*Pseudomonas solanacearum*) the bacterium causing potato brown rot and wilt disease. Al-Azhar Journal of Microbiology. 48: 41-60.

Hossain, M.A. (2013). Determination of Races and Biovars of *Ralstonia solanacearum* Causing Bacterial Wilt Disease of Potato. Journal of Agricultural Science. 5: 86-93.

Kyaw, W.W.H.; Aye, S.S.; Furuya, N. and Tsuchiya, K. (2014). Characterization of *Ralstonia solanacearum* isolates in Myanmar. Proceedings of the eight agricultural research conferences in commemoration of the 90th anniversary of yezin agricultural unversity Nay pyi taw Myanmar. pp. 157-167.

OEPP/EPPO. (2004). Diagnostic protocols for regulated pests: *Ralstonia solanacearum*. OEPP/EPPO Bulletin. 34: 173-178.

Pastrik, K.H. and Maiss, E. (2000). Detection of *Ralstonia solanacearum* in potato tubers by polymerase chain reaction. Journal of Phytopathology. 148(11-12): 619-626.

Popoola, A.R.; Ganiyu, S.A.; Enikuomehin, O.A.; Bodunde, J.G.; Adedibu, O.B.; Durosomo, H.A. and Karunwi, O.A. (2015). Isolation and Characterization of *Ralstonia solanacearum* Causing Bacterial Wilt of Tomato in Nigeria. Nigerian Journal of Biotechnology. 29: 1-10.

She, X.; Tang, Y.; He, Z. and Lan, G. (2015). Genome sequencing of *Ralstonia solanacearum* race 4, biovar 4, and phylotype I, strain YC45, isolated from *Rhizoma kaempferiae* in southern China. Genome Announcements. 3(5): 01110-15.

Shidfar, F.; Froghifar, N.; Vafa, M.; Rajab, A.; Hosseini, S.; Shidfar, S. and Gohari, M. (2011). The effects of Tomato consumption on serum glucose, Apolipoprotein B, Apolipoprotein A-I, Homocysteine and blood pressure in Type 2 Diabetic patients. International Journal of Food Sciences and Nutrition. 62(3): 289-294.

Swanson, J.K.; Yao, J.; Tans-Kersten, J.K. and Allen, C. (2005). Behavior of *Ralstonia solanacearum* race 3 biovar 2 during latent and active infection of geranium. Phytopathology. 95: 136-143.

Tahir, M.I.; Haq, M.I.; Ashfaq, M. and Abbasi, N.A. (2014). Surveillance of *Ralstonia solanacearum* infecting potato crop in punjab. Pakistan Journal of Phytopathology. 26 (01): 45-52.

Umesha, S. and Avinash, P. (2014). Multiplex PCR for simultaneous identification of *Ralstonia solanacearum* and *Xanthomonas perforans*. Biotech. 5(3): 245-252.

USDA. (2011). Tomatoes, red, ripe, raw, year round average, agriculture research services. Nutrient data for 11529, USDA commodity food A 238, A 233.

Ying, L.; Wu, D.; Liu, Q.; Zhang, S.; Tang, Y.; Jiang, G.; Li, S. and Ding, W. (2017). The sequevar distribution of *Ralstonia solanacearum* in tobacco-growing zones of China is structured by elevation. European Journal of Plant Pathology. 147: 541-551.

Yuan, K.X.; Cullis, J.; Lévesque, C.A.; Tambong, J.; Chen, W.; Lewis, C.T.; De Boer, S.H. and Li, X.S. (2015). Draft Genome Sequences of *Ralstonia solanacearum* Race 3 Biovar 2 Strains with Different Temperature Adaptations. Genome announcements. 3(4): 815-15.

Zhang, C.X.; Ho, S.C.; Chen, Y.M.; Fu, J.H.; Cheng, S.Z. and Lin, F.Y. (2009). Greater vegetable and fruit intake is associated with a lower risk of breast cancer among Chinese women. International Journal of Cancer. 125 (1): 181-188.