AWARDS AND HONOURS

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Bacterial wilt of solanaceous crops: Diagnosis, diversity and management*

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ABSTRACT: Wilt disease of solanaceous crops caused by bacterial pathogen *Ralstonia solanacearum* is a major devastating soil borne disease in the world, limiting the production of solanaceous vegetable crops, and affecting more than 450 plant species. The loss due to this disease is very high, ranging from 2 to 100% depending on environmental conditions and crops. The affected plants show a typical wilt symptoms and browning of vascular tissue in roots, stems and tubers. The pathogen is a gram -ve, rod shaped, frequently occurs in pairs, motile with 1 to 4 polar flagella, aerobic, non-fluorescent, positive in catalase and oxidase tests, forms nitrites from nitrates and negative in levan production as well as starch hydrolysis. Virulent isolates on 2,3,5-Triphenyl Tetrazolium Chloride (TTC) medium develop fluidal, irregular colonies having peripheral white and pinkish in centre. The optimum growth of the bacterium on artificial medium occurs at 28 to 32°C. In India, race 1 and bvs 3 and 4 are prevalent in tomato chilli capsicum and brinjal. However, race 1 and 3 infect the potato. Presently, *R. solanacearum* strains are categorized into four genetic groups called phylotypes which reflect the geographical origin and also ancestral relationships between the strains. Besides cultural, morphological and biochemical tests, sero-diagnostic like ELISA (Enzyme-linked immunosorbent assay), immunofluorescent cell staining, lateral flow devices, serological kits are applied for detecting *R. solanacearum* from infected plant, tubers and soil. For detecting *R. solanacearum*, polymerase chain reaction (PCR) based techniques are usually applied using amplification of ribosomal sequences (16S rRNA) and other conserved genes of bacteria. To improve the sensitivity, various modifications have been made like enrichment in SMSA broth, nutrient broth or CPG broth to allow bacteria to multiply prior to do conventional PCR, called as BIO-PCR and two round PCR as nested- PCR. Multiplex-PCR protocols have been developed to simultaneously detect either *R. solanacearum* and *E. carotovora* sub sp. *carotovora* or *Clavibacter michiganensis* sub sp. *sepedonicus* from tubers of potato and also *R. solanacearum* along with *Xanthomonas perforans* from tomato. Management of bacterial wilt disease of solanaceous crops is very difficult due to its soil borne nature. Different workers throughout the world have made efforts to control this disease by using cultural, chemical, biological and host resistance methods, either alone or in combination of these methods.

Keywords: Bacterial wilt, diagnosis, diversity, management, *Ralstonia solanacearum*, solanaceous crops

Bacterial wilt disease of solanaceous crops incited by *Bacillus solanacearum* was first described by Smith (1896) in the USA. Presently, the causal organism of bacterial wilt is *Ralstonia solanacearum* (Smith, 1896) Yabuuchi *et al.* (1995) with synonym: *Bacillus solanacearum* Smith, 1896, *Pseudomonas solanacearum* (Smith, 1896, 1914), *Burkholderia solanacearum* (Smith, 1896; Yabuuchi and Arakawa, 1993). A very little information was available on the incidence of this disease until in early 1950's when research on this disease was done by Kelman (1954) and Smith *et al.* (2003). The bacterial pathogen, *R. solanacearum* is reported from all the climates as tropical,

subtropical, and temperate climates, and more than 80 countries around the world are affected by this disease but are not believed to survive at cold temperatures. The host range of the bacterium is unusually broad with 450 plant species and 54 plant family (Sharma *et al.*, 2016a) and major hosts are *Lycopersicon esculentum* (tomato), *Capsicum annum* (sweet pepper and hot pepper), *Solanum tuberosum* (potato), *Solanum melongena* (brinjal), *Nicotiana tabacum* (tobacco), *Arachis hypogaea* (groundnut), *Musa paradisiaca* (banana) and *Heliconia* spp. (plantain) and *Zingiber officinale* (ginger). *Davana* (*Artemisia pallens*) and *coleus* (*Coleus forskohlii*) showing typical wilt symptoms were recorded two new hosts for *R. solanacearum* (Chandrashekara and Prasannakumar, 2010).

Ralstonia solanacearum has A2 quarantine status. It is one of the most important plant diseases of solanaceous crops in entire world due to damage incurred exceed US \$950 million each year (Ephinstone,

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2005). The crop loss caused by the disease is varied from 2-100 per cent depending upon the crops, environmental conditions and strains of the pathogen. The solanaceous vegetable crops such as summer grown tomato, brinjal in the plain and tomato, potato in hills, are mostly affected by *R. solanacearum*. The losses caused by bacterial wilt varies from 20-100% in chilli (Shekhawat *et al.*, 2000), 2-95% in tomato (Singh *et al.*, 2010) and 25-100% in brinjal. Verma and Shekhawat (1991) reported nearly 37% potato loss due to brown rot disease in potato in Mukteshwar region of Uttarakhand, 13.8-55% in Kumaun hills and 75% in Karnataka (Gadewar *et al.*, 1991).

Diagnosis

Disease symptoms: The most characteristics symptoms of the disease on tomato, pepper (hot and sweet chilli) and eggplant are very rapid wilting, particularly younger and succulent plants (Singh *et al.*, 2010). The flowering stage of the plant is the most critical stage, where infected plant shows quick wilting. The flaccid appearance of the youngest leaves is first visible symptom of the disease. Besides this symptom, stunting, downward curling of leaflets and petioles are other primary indications of the disease. Usually infected plants collapse quickly. Discoloration of the vascular system from pale yellow to dark is also a symptom of the disease. Blackening of vascular system is observed at the junction of stem and leaf and further down the stem, the whole vascular system may be completely blackened. On potatoes, leaves and stems show rapid wilting and in later stages plants fail to recover, become yellow and brown necrotic and die. The tubers may show bacterial ooze on the eyes and stolon end attachment. When diseases tuber is cut, glassy to yellow-browning and eventual necrosis of the vascular tissue may be observed.

Ooze test: It is a common test for diagnosing bacterial wilt in solanaceous crops. A common sign of bacterial wilt observes as sticky, milky-white exudates at the surface of freshly-cut sections from severely infected stems are come out which indicates the presence of dense masses of bacteria in the xylem. Bacterial ooze may accumulate on the cut surface. This Ooze test is a presumptive diagnostic test which is very useful at field level. Oozing only occurs with bacterial wilt disease and not with any other type of pathogen or abiotic cause.

Morphological, biochemical and physiological characters: *Ralstonia solanacearum* is characterized by using classical methods like Gram staining, shape and size, KOH test and microscopical study. Colonies of *R. solanacearum* on solid medium (CPG medium) are usually small, irregularly round, slightly raised, glistening, white and smooth margins whereas on TTC and SMSA media, slimy dull white with pink to red centre (Schaad *et al.*, 2001; Chandrashekara *et al.*, 2012). Kelman (1954) distinguished colony variants on TTC medium and two types colonies of *R. solanacearum* were found on agar plates, as fluidal or mucoid which are irregularly round entire, white or white with light pink (Denny and Hayward, 2001; EPPO, 2004) and a non- fluidal or non-mucoid,

butyrous type, which are round, translucent, smooth, deep red with border of a narrow light bluish. The mucoid substance produced by bacteria is due to accumulation of an exopolysaccharide (EPS) and causes these mucoid colonies to show a typical irregularity of their surfaces often with characteristic whorls in the centre. *R. solanacearum* colonies are non-fluorescent, however, sometimes, the bacteria produces a diffusible brown pigment on some media (Denny and Hayward, 2001). Optimum temperature for growth of *R. solanacearum* strains isolated from tropical areas all over the world is required a high 35°C, while those strains occurring at higher altitudes in the tropics and in subtropical and temperate areas is a moderate 27°C. However, the bacterium did not grow at high temperature 40°C or at low temperature 4°C (EPPO, 2004). The minimum temperature for growth of this bacterium may be 8-10°C and maximum 37-39°C and optimum temperature 27-37°C, depending on the strains of *R. solanacearum*. It is observed that acid media inhibited the growth of *R. solanacearum* but the growth is favoured in alkaline conditions. The bacterium can grow in 1% NaCl liquid medium but, it grows little or none in 2% NaCl (EPPO, 2004).

The single cell of *R. solanacearum* is a small rod with rounded ends, non-spore forming (Smith, 1896) and average size 0.5 to 0.7 by 1.5 to 2.5 µm (Denny and Hayward, 2001). Motility is transiently expressed during growth of *R. solanacearum*. The bacterium has one to several polar flagella and has swimming motility (Tans-Kersten, 2001). However, for observing this motility, the cell density of *R. solanacearum* may be $>10^6$ and $<10^9$ cfu/ml. The bacterium is a chemo-organotrophs, aerobic, positive in catalase and Kovac's oxidase tests and nitrate is reduced to nitrite. A large number of organic compounds as a carbon source are utilized by individual strains of *R. solanacearum* but most isolates utilize glucose, fructose and sucrose. They are not utilized xylose, arabinose, arginine and starch and not hydrolyzed gelatin (Sharma *et al.*, 2016a). The bacterium produces poly-β-hydroxybutyrate granules which act as cell energetic reserve.

The Biolog technique is one of the automatic methods based on different carbon source substrates utilized by *R. solanacearum*. It is inexpensive, fast, reliable, and easy to use. The bacterial strains are characterized by determining their utilization profiles of 95 different carbon substrate with BIOLOG GN2 or BP2 on a microtiter plate. The Biolog automated system was used by Tawfik *et al.* (2008) and they proved that this system support rapid diagnosis of the brown rot disease potato. Nineteen isolates recovered from potatoes suspected of belonging to *R. solanacearum* biovar 2A based on cultural and physiological characters of bacteria.

Use of indicator plants: Since the use of the hypersensitive reaction (dark brown necrotic lesions) techniques in tobacco leaves, there has been a number of attempts made to detect *R. solanacearum*. Potato shoots cut from healthy tubers or surface sterilized tomato seeds and tomato seedlings (Elphinstone *et al.*,

1998) of susceptible cultivars also have been used as planting materials for indicator plants, which showed symptom within 4 weeks under controlled conditions when inoculated or planted in the sick soil with the pathogen. Tomato, as well as eggplant, is used as indicator plants to detect *R. solanacearum*.

Use of bacteriophages: Bacteriophages (phages) are used as recognition elements for diagnosis and detection of specific bacterial pathogens of the plant. The phages are able to identify their host by specific receptor molecules on the outside of the bacterial cell. They infect the bacterial host and use it as a factory for their own replication. The major advantage to using phages is their high sensitivity and more specificity to the bacteria. Yamada *et al.* (2007) isolated various kinds of bacteriophage with distinguished features and they may be useful for effective detection and diagnosis of the *R. solanacearum* strains belonging to different races and/or biovars in different cropping ecosystems and also in growing crops. The bacteriophages are different shape, size and genomes. For examples, a virulent phage ϕ P4282 and its related phages, have a polyhedral head (69 nm in diameter) with a short tail (20 nm in length), and circular dsDNA genome with size of 39.3-kbp (Ozawa *et al.*, 2001). Another phage, ϕ PK-101, has a linear 35-kbp dsDNA genome (Toyoda *et al.*, 1991) and they have very narrow host ranges and they infect only a few *R. solanacearum* strains. ϕ RSS1 and ϕ RSM1 phages are filamentous, Ff-like phages (Inoviridae) to infect bacteria. Coliphage ϕ M13 is a filamentous and very useful as vectors to display proteins on the virion surface in the bacteriophage-display method (Smith, 1991). Similarly, ϕ RSS1 and ϕ RSM1 have been utilized to display a tag protein on the virion surface to apply for diagnosis or monitoring (Kawasaki *et al.*, 2007). In pure *R. solanacearum* cultures, the protocol consistently detected approximately 3.3 cfu/ml with 5.3×10^2 PFU/ml M_DS1 after one hour of incubation (Kutin *et al.*, 2008).

Phospholipid fatty acid analysis: *Ralstonia solanacearum* was differentiated from *Burkholderia caryophyll* and *B. gladioli* pv. *gladioli*, which belong to ribosomal RNA group II by DNA-DNA hybridization on the basis of fatty acid compositions of oleic (18:1) and two kinds of unidentified fatty acids (Rt 8.9 and Rt 10.9). Although *R. pickettii* is very closely related with *R. solanacearum* in bacterial properties, these two species were readily differentiated by the presence or absence of oleic (18:1), 2-hydroxypalmitic (16:1 2-OH) and three kinds of unidentified fatty acids CRt 8.9, Rt 11.2 and ltt 16.4). Eight kinds of fatty acid were identified and quantified in Brazilian strains of *R. solanacearum*. They were myristic (14:0), palmitic (16:0), palmitoleic (16:1 cis 9), vaccenic (18:1 cis 11), 2-hydroxypalmitic (16:0 2-OH) and three kinds of unidentified fatty acids CRt 9.7, Rt 10.9 and Rt 16.4) (De Melo *et al.*, 1999).

Serodiagnosis

The correct diagnosis is a prerequisite for effective disease management. The more rapidly and accurately

the causal organism is identified, the sooner proper control can be instituted. Race specific antisera have been prepared against *R. solanacearum* races 1, 2 and 3 and race 2 and 3 are found more closely related. Cell surface antigens are categories such as exopolysaccharide, cell wall structural (somatic) components ("O" antigens), extra cellular glycoprotein, and flagella components ("H" antigens). Glycoproteins tend to be strain specific whereas EPS and somatic antigens are common to all strains of *R. solanacearum*. Chakrabarti (1995) produced antisera against the formalinised whole cell, glycoprotein and lipopolysaccharide (LPS) fractions of the bacterium. A protocol of alkaline phosphatase based DAS- ELISA to detect *R. solanacearum* either from infected stems or tubers using antiserum produced against formalin killed bacteria (race 1) was standardized by Chakrabarti (1993) and no race specificity was found in slide agglutination test. The antiserum against whole cells proves effective for ELISA of low concentration of pathogen from infected plants. However, strains specific monoclonal antibody against *R. solanacearum* was developed by Robinson (1993) to detect the bacterium and he reported that the indirect ELISA system can detect a minimum of 10^4 cfu/ml in pure or mixed bacterial suspension or plant extract. Both polyclonal and monoclonal antibodies have been developed by Robinson-Smith *et al.* (1995) to detect *R. solanacearum* from different samples. A recombinant single chain antibody has developed against *R. solanacearum* (biovar 2, race 3) lipopolysaccharide and improved characteristics for diagnosis of brown rot of potato. The polyclonal antibodies, when used with an indirect ELISA detected *R. solanacearum* from infected plant or soil samples, but they were not able to distinguish between *R. solanacearum*, from other species of *Ralstonia*. Reliability of ELISA- PCR is improved by incubating samples in nutrient broth or semi-selective broth medium prior to testing for increasing the population of the pathogen. Post-enrichment NCM-ELISA on nitrocellulose membrane using enriched sample has also developed and found as sensitive as the double-antibody sandwich-ELISA (Caruso, 2002). Van der Wolf (2000) described a comparative test of an immunofluorescence cell-staining and ELISA methods based on using polyclonal and recombinant monoclonal antibodies for detection of *R. solanacearum* from potato tuber and no differences in detection levels were found in based neither on polyclonal or monoclonal antibodies nor between water and potato tubers extracts.

Recently, a lateral flow device kit is developed for this bacterium based on existing technology similar to a pregnancy test kit by Central Science Laboratory, U.K. which allows detection of *R. solanacearum* in single step within 3 min. Rapid ImmunoStrip® is available from Agdia Inc. Immunofluorescence. It is the most readily available grower friendly diagnostic tools today and major advantage of this device is simple to use, quick, usually take less than 10 min.

Polymerase chain reaction (PCR) based diagnosis: DNA based techniques particularly PCR are fast, accurate, reliable and more sensitive to detect *R.*

solanacearum (Singh *et al.*, 2014; Weller *et al.*, 2000). Standardization of PCR and real time PCR analysis could play an important role for detection *R. solanacearum* from the disease in the seeds planting material, soil and irrigation water, where the pathogen survives in low number (Table 1). The bacterial pathogen causing wilt disease can be detected by using conventional PCR with detection level from 10^2 cfu/ ml (Singh *et al.*, 2014a) to 10^5 cfu/ ml (Khakvar *et al.*, 2008). PCR based methods for the detection of *R. solanacearum* are usually based

on the amplification of ribosomal sequences (16S rRNA or ITS region) and other conserved genes of bacteria (Pastrick *et al.*, 2002; Ranajn *et al.*, 2016; Singh *et al.*, 2014a; Weller *et al.*, 2000). Molecular based detection of *R. solanacearum* causing bacterial wilt of tomato, chilli, brinjal and potato has been done by using specific primers Y2 and OLII and it also distinguished *R. solanacearum* from other saprophytic bacteria grown on TTC medium (Singh *et al.*, 2010, 2014a). Various modifications have been made to improve the sensitivity

Table 1. Detection of *R. solanacearum* from seeds and planting materials of solanaceous crops, soil and water by using different PCR techniques

PCR genomic assay	Host plant	Detected from	Race/biovar of bacteria	Reference
16S rRNA	Tomato	Plant, culture	<i>R. solanacearum</i>	Singh <i>et al.</i> , 2010
PCR (RNA operon)	-	-		Pastrick <i>et al.</i> , 2002
<i>Fli C</i>	-	Soil	<i>R. solanacearum</i> , <i>R. pickettii</i>	Schonfeld <i>et al.</i> , 2003
Nested- PCR	Tomato, chilli, eggplant and tobacco	Soil	<i>R. solanacearum</i>	Khakvar <i>et al.</i> , 2008
<i>hrp</i> gene, PCR	Tomato, potato, tobacco, Eggplant, pepper	Plant and tuber	<i>R. solanacearum</i>	Poussier <i>et al.</i> , 1999;
<i>hrp</i> gene, Nested- PCR			<i>R. solanacearum</i> biovar 1, N2, 3, 4 & 5	Poussier <i>et al.</i> , 2000
BIO-PCR	Tomato	Soil	<i>R. solanacearum</i> race 1 biovar 3	Lin <i>et al.</i> , 2009; Singh <i>et al.</i> , 2014a
PCR (insertion sequence)	Eight different host	-	<i>R. solanacearum</i> race 1	Lee <i>et al.</i> , 2001
16S rRNA, Multiplex- PCR	Potato	Potato tuber	<i>R. solanacearum</i> biovar1, 2, N2, 3, 4 & 5	Pastrick and Maiss, 2000, Seal <i>et al.</i> , 1993; Ito <i>et al.</i> , 1998
PCR -RFLP	-	-	<i>R. solanacearum</i>	Poussier <i>et al.</i> , 1999, 2000
Multiplex –PCR (16S-23S r RNA ITS)	Potato	Tuber	<i>R. solanacearum</i>	Pastrick <i>et al.</i> , 2002
Real –Time PCR	Potato	Tuber and plant	<i>R. solanacearum</i>	Stead <i>et al.</i> , 2003; Weller <i>et al.</i> , 2000
Real Time Bio-PCR	Potato	Tuber and plant	<i>R. solanacearum</i> race 3 biovar 2	Ozakman and Schaad, 2003; Thammakijawat <i>et al.</i> , 2006
RNA based approaches (NASBA)	-	-	<i>R. solanacearum</i>	Betsink <i>et al.</i> , 2002
16S rRNA	Potato	Tuber, irrigation water	<i>R. solanacearum</i> race 3 bv 2	van der Wolf <i>et al.</i> , 2004
IC-PCR	Tomato, pepper, weeds (<i>Physalis minima</i> , <i>Amaranthus spinosus</i> and <i>Euphorbia hirta</i>)	Plant and soil	<i>R. solanacearum</i> race 1	Dittapongpitch and Surat, 2003
Cytochrome c1 Signal Peptide Sequences	-	-	Amplify only <i>R. solanacearum</i>	Kang <i>et al.</i> , 2007
BIO- PCR, <i>hrp</i> gene	Tomato	Plant, soil, irrigation water	<i>R. solanacearum</i> race 1, bv 3 & 4	Singh <i>et al.</i> , 2014
Multiplex – BIO- PCR	Potato	Tuber	<i>R. solanacearum</i> and <i>Erwinia carotovora</i> subsp. <i>carotovora</i>	Ranjan <i>et al.</i> , 2016
Multiplex- PCR	Potato	Tuber	<i>R. solanacearum</i> and <i>Clavibacter michiganensis</i> subsp. <i>sepedonicus</i>	Sebastien <i>et al.</i> , 2014
Multiplex- PCR	Tomato	Plant material	<i>R. solanacearum</i> and <i>Xanthomonas perforans</i>	Umesha and Avinash, 2015

of a single round PCR, viz., enrichment in semi-selective SMSA broth prior to PCR (Elphinstone *et al.*, 1996; Ranjan *et al.*, 2016; Singh *et al.*, 2014a) or real time PCR called as BIO-PCR (Ozakman and Schaad, 2003) as well as double round PCR as nested-PCR (Khakhar *et al.*, 2008). Grover *et al.* (2009) detected *R. solanacearum* as low as 1 cfu/ml of within 8 h including DNA isolation. For detection of *R. solanacearum* *hrpB*-based on *hrp* gene sequence primers (Hrp_rs2F and Hrp_rs2R) amplified at 323 bp was used in BIO-PCR to detect *R. solanacearum* from soil, asymptomatic plant and irrigation water with great accuracy and more sensitivity (Singh *et al.*, 2014a). Multiplex polymerase chain reaction (PCR) protocol was developed and validated simultaneous detection of *R. solanacearum* (bacterial wilt) and *E. carotovora* subsp. *carotovora* (soft rot disease) from potato tubers (Ranjan *et al.*, 2016). Another multiplex real-time PCR method was developed by Sebastien *et al.* (2014) to detect simultaneously *Clavibacter michiganensis* subsp. *sepedonicus* (ring rot disease) and *R. solanacearum* from potato. The detection limit of the developed duplex was at least 100 cfu/ml of potato macerate for each pathogen. Simultaneously *R. solanacearum* (RS-F-759 and RS-R-760) and *Xanthomonas perforans* (leaf spot disease) RST2 and RST3 was detected from asymptomatic and symptomatic tomato plants amplified at 381 bp and 840 bp respectively (Umesha and Avinash, 2015).

Loop-mediated isothermal amplification (LAMP):

LAMP is based on isothermal amplification without using PCR to detect plant pathogens with high sensitivity and minimum costs (Notomi *et al.*, 2000). In this method, a set of four specifically designed primers is required which recognizes six distinct sequences of the target gene of the bacteria and a DNA polymerase with strand displacement activity. The amplified products are stem-loop DNA structures with several inverted repeats of the target and cauliflower-like structures with multiple loops. The LAMP reaction takes place at 60-65°C for 60 min and may be enhanced further by the addition of loop primers. A four set of primers to replicate the gene coding for the flagellar subunit, *fliC* was developed by Kubota *et al.* (2008). The protocol was standardized at 65°C for 60 min to detect *R. solanacearum*. Magnesium pyrophosphate resulted from the amplification reaction can be detected optically due to an increase in the solution turbidity. The amplified DNA products spread in a reproducible ladder-like banding pattern after electrophoresis in an agarose gel. This method is more rapid, low cost and sensitive and its detection limit are between 10⁴-10⁶ cfu/ml. The ability to detect *R. solanacearum* LAMP products using hydroxynaphthol blue (HNB) at concentration of 150 µM (Goto *et al.*, 2009) is a colorimetric means to indicate positive reaction.

Fluorescence in situ hybridization (FISH): FISH is actually dependent on the hybridization of DNA probes to species-specific regions of the bacterial ribosome. A single cell of the bacterium can be detected, however, in practice; about 10³ cells/ml of plant extract may be detected (Ercolini *et al.*, 2006). It is a high affinity and

selectivity of DNA probes because it takes place under very stringent hybridization conditions, where a difference of one nucleotide in a 15-20 oligonucleotide probe targeted to 23S rRNA is sufficient to discriminate the bacteria. van der Wolf and Schoen (2004) was used this method to detect *R. solanacearum* race 3 bv 2 from potato peels and it was included in official diagnostic protocols in the EU and recommended in the EPPO protocol for *R. solanacearum* (EPPO, 2004).

Diversity

Ralstonia solanacearum has a lot of diversity in respect of host, utilization of carbon sources and genetic. The five races of *R. solanacearum* have wide host range, biovars and geographic distributions. Race 1 is a highly diverse group having very wide host range and causing disease in southern USA, Africa, Asia and South America and it requires optimum temperature 35°C as required by races 2, 4 and 5. Race 2 principally attacks bananas and *Heliconia* spp. and causes Moko disease and plantains (bugtok disease in Philippines). It is found mainly in Central America and Southeast Asia. Race 3 is primarily associated with potato and called 'potato race' and it is found worldwide. It is cold resistant and requires optimum temperature 27°C. Race 4 infects ginger crop in Asia and Hawaii. Race 5 affects mulberries and reported from China (Denny and Hayward, 2001; Denny, 2006). However, in India, races 1 and 3 isolated solanaceous crops, while race 1 was isolated from tomato, potato, brinjal and pepper, whereas race 3 was isolated from potato (Ranjan *et al.*, 2015; Ranjan and Singh, 2015; Singh *et al.*, 2010). *R. solanacearum* strains have variation in their metabolic activities and based their ability to utilize and/or oxidize several hexose alcohols and disaccharides, they divided into 6 different biovars (Denny, 2006; EPPO, 2004; Hayward, 1991). Biovar 1 is most dominant in USA and bvs 3 and 4 in Asian countries, whereas bvs 2 and 5 occur in Australia and China. The bv 4 occurs only in India and Indonesia. In India, The strain of *R. solanacearum* isolated potato crop from Uttarakhand belong to four bvs i.e. bvs 1, 2, 2T and 3, from Meghalaya and Odisha bvs 2, 2T and 3, from West Bengal bvs 2T and 3, from Himachal Pradesh bvs 2 and 3 and from Karnataka isolated only bv3 (Ranjan *et al.*, 2015; Ranjan and Singh, 2015). Sagar *et al.* (2014) reported Himachal Pradesh isolates belong to bvs 2, 3 and 4, West Bengal isolates belong to bvs 2 and 3 and Meghalaya isolates belong to bvs 2 and 2T. However, bv 3 of *R. solanacearum* was dominated in other solanaceous crops like tomato, brinjal and pepper and bv4 was found rarely (Singh *et al.*, 2012a). A general correlation between races and biovars cannot be drawn, but some biovar 2 strains belong to race 3 and vice versa (Hayward, 1991; Ranjan and Singh, 2015).

Genetic diversity of *R. solanacearum* has been studied by using different molecular methods such as RAPD (Silveira *et al.*, 2005; Singh *et al.*, 2014), PCR-RFLP of *hrp* gene (Poussier and Luisetti, 2000), Rep-PCR (Sapna and Ramesh, 2014; Xue *et al.*, 2011) and multilocus sequence typing (MLST) sequencing (Castillo

and Greenberg, 2007). Genetic diversity of 50 isolates of *R. solanacearum* infecting solanaceous vegetables from Western regions of India BOX- and ERIC- PCRs was done and results indicated that all the isolates belonged to Phylotype I and bv 3, except one isolate, belong to bv 6. These isolates of *R. solanacearum* are phenotypically similar, but have great variation found when the whole genome is analyzed by rep-PCR (Sapna and Ramesh, 2014). The genetic diversity of *R. solanacearum* was assessed by employing restriction arrangement length polymorphism analysis and the strains are divided into two divisions 1 (Asiaticum) and 2 (Americanum). Classical restriction fragment length polymorphism (RFLP) analysis shows fundamental heterogeneity of pathogen strains, but at large-scale DNA sequence analyses, more profoundly influences the understanding of bacterial systematics (Castillo and Greenberg, 2007; Denny, 2006; Prior and Fegan, 2005a,b). As the diversity of the strains examined by using advanced tools, it quite became clear that the *R. solanacearum* strains have four major subdivisions, denoted as phylotypes (Fegan and Prior, 2005; Prior and Fegan 2005a). To determine the phylotypes, a multiplex PCR may rapidly assign strains to a phylotype (Fegan and Prior, 2005) based on sequence analysis of the internal transcribed spacer (ITS) region, the endoglucanase (*egl*) gene and *hrpB* gene (Fagan and Prior, 2006; Lebeau *et al.*, 2011) and it corresponds roughly to the strains' geographic origin: Asia (phylotype I), the Americas (II), Africa (III), and Indonesia (IV). Phylotype II has two clearly recognizable subclusters (IIA and IIB) (Castillo and Greenberg, 2007; Fegan and Prior, 2006; Prior and Fegan, 2005a). Phylotype I contain all strains belonging to biovars 3, 4 and 5, isolated primarily from Asia. Further, each phylotype is subdivided into numbered sequevars based on *egl* gene sequence analysis.

In multilocus sequence typing, five chromosomal housekeeping genes *ppsA*- (phosphoenolpyruvate synthase), *gyrB* (DNA gyrase, subunit B), *adk* (adenylate kinase), *gdhA* (glutamate dehydrogenase oxidoreductase) and *gapA* (glyceraldehyde 3-phosphate dehydrogenase oxidoreductase) and three megaplasmid virulence-related genes such as *hrpB* (regulatory transcription regulator), *fliC* (encoding flagellin protein) and *egl* (endoglucanase precursor) are used to study genetic diversity (Castillo and Greenberg, 2007; Wicker *et al.*, 2012). Besides these housekeeping genes, some other genes such as *accD* (Acetyl-Co A carboxylase carboxyl transferase (β)), *atpG* (ATP synthase gamma chain), *coxA* (Cytochrome C oxidase polypeptide I; cytochrome AA3 subunit 1), *hutH1* (histidine ammonia-lyase), *pyrB*- (aspartate carbamoyltransferase), RSc1279 (Cytochrome C oxidase (subunit III), *sdhA* (succinate dehydrogenase; flavoprotein subunit) have also been used in MLST.

The diverse classification schemes proposed for *R. solanacearum* have great phenotypic and genotypic variation within the species, which is called "*R. solanacearum* species complex" and defined as "a cluster

of closely related isolates whose individual members may represent more than one species" (Fegan and Prior, 2005). The *R. solanacearum* species complex has divided into three groups of strains which exceed the accepted threshold for speciation. Based on extensive genetic results, two new species i.e. *R. sequeirae* and *R. haywardii* and three subspecies (*R. haywardii* subsp. *celebensis*, *R. haywardii* subsp. *solanacearum* and *R. haywardii* subsp. *syzygii*) were proposed (Remenant *et al.*, 2011) based on the limited DNA-DNA hybridization data. Another classification suggests the division of *R. solanacearum* into new species (Allen *et al.*, 2014; Remenant *et al.*, 2011; Safni *et al.*, 2014). In the first putative proposal, only phylotype II strains were named as *R. solanacearum*, whereas phylotypes I and III were included in the new species as *R. sequeirae* and phylotype IV was named as *R. haywardii*. Later Allen *et al.* (2014) and Safni *et al.* (2014) proposed new taxonomic review and they suggested the division into three species according to significant biological (phenotypic) and pathogenic differences and to genomic divergence. Thus in phylotype, I and III include *R. sequeirae* sp. nov. by Allen *et al.* (2014) and *R. pseudosolanacearum* sp. nov. by Safni *et al.* (2014). *R. solanacearum* was proposed in phylotype II by both Allen *et al.* (2014) and Safni *et al.* (2014), whereas in phylotype III, *R. sequeirae* sp. nov. was proposed by Allen *et al.* (2014) and *R. pseudosolanacearum* sp. nov. by Safni *et al.* (2014). They also divided the species *R. syzygii* into three distinct groups as *R. syzygii* subsp. *haywardii* subsp. nov. (Allen *et al.*, 2014), *R. syzygii* subsp. *indonesiensis* subsp. nov. (Safni *et al.*, 2014) and *R. syzygii* subsp. *syzygii* subsp. nov. (Allen *et al.*, 2014; Safni *et al.*, 2014) and *R. syzygii* subsp. *celebensis* subsp. nov. by Allen *et al.* (2014) and *R. syzygii* subsp. *celebesensis* subsp. nov. by Safni *et al.* (2014) under phylotype IV. However, such proposal has not been accepted by scientist community yet.

Disease management

Bacterial wilt disease in solanaceous crops is very difficult to control due to its soil borne nature. Different management strategies are applied to control and suppress the disease including host-plant resistance and biological control. Different workers throughout the world have made efforts to manage this disease amicably through cultural, chemical, biological and host resistance, either alone or in combination of these control methods.

Cultural practices: Change in planting dates is generally designed only to avoid either period of high temperature or heavy rainfall or both. Crop rotations solanaceous crops with maize or ragi crops are found effective to control the disease (Sharma and Kumar, 2004) because of these crops reduce the population of *R. solanacearum* in the soil significantly. Crop rotation, intercropping or incorporation, green manure and planting a susceptible crop such as green gram before the cultivation of the main crop have been practiced. Crop rotation for 5-7 years with a non-susceptible crop provides reduced wilt infection in potato (Smith *et al.*, 1995), but it is found less effective due to wide host range of the pathogen.

The occurrence of the wilt disease was delayed by 1-3 weeks and wilt severity was also reduced 20-26% in susceptible tomato, which was grown after corn, lady's finger, cowpea, or resistant cultivar of tomato. Poultry and farmyard manure added soils suppressed bacterial wilt in tomato and increased higher microbial activity (Islam and Toyota, 2004).

Sun and Hung (1985) used rice husks, corn stalk, cow manure, oyster shell powder and mineral ash to improve mixture called 'Sun and Hung'. The organic amendment and compost applied in soil released biologically active substances from crop residues and soil microorganisms such as allelochemicals (Wydra *et al.*, 2005). The sunhemp mixture @ 2, 6 and 10% reduced the population of *R. solanacearum* after 4 weeks of incubation. The population of *R. solanacearum* was declined by adding household compost. Compost of corn stalk, rice straw and tree bark suppressed bacterial wilt of tomato and they reduced significantly the disease severity index, however, the bacterial wilt incidence was found minimum in bark compost treated field (Masayitah, 2004). Bacterial wilt of tomato was suppressed by incorporating 20-30% of the aerial parts of pigeon pea and crotalaria crops into the soil and incubated for 30 days (Cardoso *et al.*, 2006). Although soil solarization has been applied for the control of numerous soil borne pathogens but its role for the management of bacterial wilt has not been much studied. However, the wilt incidence was slightly reduced by 8 weeks of soil solarization with 25 µm thick transparent polyethylene sheet (Sharma *et al.*, 2004; Sharma *et al.*, 2016).

Host resistance: Application of resistance cultivar of solanaceous crops is the best strategy to control bacterial wilt disease. Multiplication of *R. solanacearum* in stems of resistant cv. Hawaii 7996 of tomato was suppressed and restricts the movement of bacterial pathogen from the protoxylem or the primary xylem to other xylem tissues. Two types of resistance exist in tomato; one is polygenic, originating from *Lycopersicon esculentum* var. *ceresiformae* and needs to be sustained by additional crop management measures. The second type is a dominant resistance present in cv. Hawaii, which is of simpler heredity, like the monogenic resistance described in Hawaii 7998. Most of the resistant sources against *R. solanacearum* were found in *L. pimpinellifolium* or ecotypes of *L. esculentum* var. *ceresiformae*, which are wild tomato germplasm with small fruits and resistant to elevated temperatures. Resistant cultivars/lines of tomato are BT118-4-1-1, BT-116-8-1-1, Tomato-415, Sonali (Sel-2), DPT 38, VC 48, CRA 66 (Sel-A), BWR 1 (Asel. from VC 8-1-2-1), Sonali x SP-2-2, Utkal Pallavi, Utkal Deepti, BT12, BT 14, BT 18, Pusa Early Dwarf, Navodaya, Selection 7, Pusa Sheetal, Arka Abha, Arka Alok, EC386019, IC214633, EC386023, LA 2639A, LA 2691, 88 BWR, 83-211, 84 BWR, CR15955-223, D4 -22-0, Swarna Lalima, Swarna Naveen, CHDT-1X, CHDT-180, CHDT-195X, CHDT-180, CHDT-4XCHDT-1, (EC339074XEC-386021), (Swarna Sampada), Shakti, BT- 10, CKVT-17 and Sikkim local. Resistant cultivars/lines for brinjal such as Swarna Shyamli, Swarna

Pratibha, BB 64, JC 8, Arka Keshav and Arka Nidhi, Singnath, BB 40, BB 64 and Green round, Surya, Swetha, Annapurna, BB-7, BB13-1, BB44, F1 hybrids Surya x SM-116, Arka Keshav X SM-71, Arka Neelkanth, 95- 17, BB-46, CHES 243, IHR-12, IHR-21, IHR-54, B-7, DPL-B1, SM-6-6, BB-60-C showed resistance against the wilt disease.

The graft of susceptible cultivars of tomato on resistant rootstocks belonging to *Solanum* spp. is found effective to manage the wilt disease. The disease was controlled by grafting susceptible tomato cultivars onto resistant eggplant (Pusa Purple Cluster) rootstocks. In grafting, the wilt incidence in Ponderosa scions was less on the rootstock of Hawaii 7996 as compared to the most resistant rootstock (LS-89) and Hawaii 7996 may be used as an alternative genetic source for breeding for resistance against bacterial wilt disease (Nakaho *et al.*, 2004).

Chemical control: Soil treatment with various chemicals like chloropicrin, methyl bromide and methyl bromide-chloropicrin mixture (67: 33%) are effective in retarding the wilt development. Bleaching powder @15 kg/ha applied in the field was found effective to control the wilt disease significantly. The disease can also be controlled effectively if dazomet application is combined with soil solarization. A little effect on suppression of *R. solanacearum* is reported by using soil fumigant and antibiotics (Penicillin, Ampicillin, Tetracycline and Streptomycin). Bacterinol-100, when applied as dry seed dresser, a nursery sprays and field application resulted in 11.5% yield increase over untreated control. Streptomycin Sulphate/ Oxytetracycline, when sprayed @ 200 ppm, gave significant control of tomato wilt (Mazumdar, 1998) and Agrimycin (Streptomycin) (a) 200 ppm application suppressed the wilt disease upto 30%. Araujo *et al.* (2004) reported that avirulent bacteriocin-producing *R. solanacearum* strains were found potential to control of tomato bacterial wilt disease and it was clearly demonstrated that none of the bacteriocin-producing strains is inhibited by its own bacteriocin. Singh *et al.* (2012b) reported that bacterial wilt disease was reduced 50-52% by applying calcium chloride (0.1%) and bleaching powder (0.025%) in the soil under glass house conditions. Karanj cake along with bleaching powder reduced the population of bacteria at flowering stage and also increased the tomato yield (Sharma and Kumar, 2009; Sharma *et al.*, 2016b). Application of CaO alone significantly reduced the population of *R. solanacearum*. Pre-plant application of lime 200 lb/acre in form of CaCO₃ augmented Ca accumulation in leaf tissue and soil and reduced the rate of bacterial development on susceptible cultivars soil but had little effect on fruit yield reported that increase the calcium conc. in soil reduced the wilt disease incidence (Champoiseau *et al.*, 2009). Application of lime (CaCO₃) @ 25 q lime/ha before 15 days of transplanting resulted reduction of the initial population of bacterial pathogen in soil at 90 days after transplanting of tomato (Sharma and Kumar, 2009).

Incorporation of salts like KNO₃, NaNO₃, KCl and NaCl to soil (1:99) reduced the soil population of P.

solanacearum after four days of application, but NaNO_3 was the most effective salt and reduced the bacterial population up to 65 per cent. The population of wilt bacterium decreased with the increased calcium concentration in the nutrient solution (Yamazaki *et al.*, 2000). Ajjappalavara *et al.* (2008) evaluated the effect of gypsum with recommended dose of NPK (125: 100: 50), which decreased the wilt incidence and increase the yield of brinjal significantly. Application of copra and peanut meal has been reported to reduce the disease.

Biological control: Microbial antagonist plays an important role in suppression of bacterial wilt disease of solanaceous crops and they preserve the environment by reducing the dependency on chemicals and also maintain sustainable management practices. Soil microbes grown in the rhizosphere are ideal to use as biocontrol agents and they provide the frontline defense for roots against any attack by the pathogens. Several biological control agents like *Acinetobacter* sp. (Xue *et al.*, 2009), *Burkholderia nodosa*, *B. sacchari*, *B. tericola*, *B. pyrocinia* (Nion and Toyota, 2008), bacteriophages (Yamada *et al.*, 2007), *Bacillus thuringiensis* (Zhou *et al.*, 2008), *Bacillus subtilis* (Singh *et al.*, 2012a, 2016), *Bacillus* sp. (Ramesh *et al.*, 2009; Xue *et al.*, 2009), *Enterobacter* sp. (Ramesh *et al.*, 2009), *Flavobacterium johnsoniae* (Huang *et al.*, 2013), *Myroides odoratimimus* (Yang *et al.*, 2012), *Paenibacillus marcerans* (Li and Dong, 2013), *P. polymyxa* (Li *et al.*, 2011), *Pseudomonas fluorescens* (Sharma and Kumar, 2009; Vanitha *et al.*, 2009), *P. brassicacearum* (Zhou *et al.*, 2012), *P. aeruginosa* (Mathew *et al.*, 2007), *Pseudomonas* sp. (Ramesh *et al.*, 2009), avirulent strain of *R. solanacearum* (Trigalet and Trigalet-Demery, 1990), *Serratia* sp. (Xue *et al.*, 2013), *Stenotrophomonas maltophilia* (Messiha *et al.*, 2007), *Streptomyces rochei* (Liu *et al.*, 2013), and vesicular arbuscular mycorrhiza (Suresh and Rai, 1991), *Trichoderma viride* and *T. pseudokoningii* (Mathew *et al.*, 2007) are found effective to delay the appearance of the disease symptoms and reduced the wilt disease incidence in solanaceous crops.

Application of antagonists like *P. fluorescens* and *Bacillus* spp. are have been found to be effective at some locations (Shekawat *et al.*, 1993). Similarly, *B. subtilis* (strain B5) recovered from rhizosphere soil of potato plants from bacterial wilt infested fields of Bhowali, Uttarakhand controlled tuber borne *R. solanacearum* under different agro-climate conditions and enhanced the crop yield (Sunaina *et al.*, 2006). A bioformulations of *B. subtilis* (strain B5) has also been developed. Lemessa and Zeller (2007) used six strains of rhizobacteria and they reduced wilt disease incidence and increased plants weight of tomato. Messiha (2006) tested biological soil disinfestation (BSD) in glass vessels, microplots and in an accidentally infested commercial field and reduced the population of *R. solanacearum* in the BSD treatment (>93%).

Soil biofumigation with essential oils was very effective to reduce the bacterial wilt incidence even up to 100% (Alves *et al.*, 2014). In a greenhouse, no

symptoms of the wilt disease in tomatoes was reported when transplanted to soils biofumigated with essential oils of thyme, palmarosa, lemongrass, Greek oregano and tea tree (Pradhanang *et al.*, 2003).

Integrated approach: Integration of antagonistic rhizobacteria *i.e.* *P. fluorescens* and *B. cereus* and soil solarization for 8 and 10 weeks during March to May suppressed wilt incidence by 73.4 per cent (Kumar and Sood, 2001). Wilt incidence was not observed when bacterized (*P. fluorescens* and *B. cereus*) tomato seedlings of moderately resistant line EC 191536 were planted in 10 weeks solarized soil (Kumar and Sood, 2005). Biopesticide Biofor-Pf containing *P. fluorescens* strain PfD-1 in combination with vermicompost, carboxymethyl cellulose and mannitol applied as seed treatment, root and soil application reduced disease incidence and also increased the yield of tomato (Bora and Deka, 2007) and brinjal (Chakravarty and Kalita, 2011a,b). Arasumallaiah and Rangaswamy (2008) reported that the combination of seed treatment with streptocycline sulphate (1 g/kg seed), bleaching powder application in the soil (20 kg/ha), root dip treatment (streptocycline sulphate (1 g) + copper sulphate (1.75 g) in liter of water) on Arka Keshav (resistant variety) recorded the highest yield with minimum disease incidence in brinjal. The bacterial wilt disease in brinjal cv. Pusa Kranti caused by *Meloidogyne incognita* and *R. solanacearum* complex was minimized by integration of summer ploughing followed by half recommended dose each of carbofuran (3g), streptocycline, neem cake and full dose of *T. harzianum* under field conditions (Hussain and Bora, 2009). A combination of actigaurd (Acibenzolar-s- methyl) with *P. putida* 89B61 or Bioyield applied in tomato reduced incidence of bacterial wilt significantly as compared to control (Anith *et al.*, 2004). Chitosan along with and *Paenibacillus polymyxa* MB02-1007 has a strong antibacterial activities against *R. solanacearum* *in vitro* condition (Algam *et al.*, 2010). Microbes *viz*; *Glomus mosseae*, *T. viride* and *Azotobacter* + *Phosphobactrin* suppressed primary inoculum of *R. solanacearum* in soil and increased the yield (Sharma and Kumar, 2009). Multiple strains of rhizobacteria used as formulation have more efficiency in the biological control of the disease as compared to apply a single strain. Application of lime one month before transplanting of the crop followed by application of *P. fluorescens* as biocontrol agent were effective to minimize the bacterial wilt incidence in the field (Biswas and Singh, 2008).

Future perspectives

- Development of quick, reliable, race specific and cost effective marker to diagnose the disease.
- Develop diagnostic tools to distinguish subgroups of *R. solanacearum*, particularly race 3 bv 2 from other strains of *R. solanacearum*.
- Distribution of races of *R. solanacearum* in India.
- Bacterial behavior in natural hosts under biologically realistic conditions should be studied.

- Identification of resistance gene against bacterial wilt of tomato.
- Development of resistant varieties against bacterial wilt.
- There is need to search for new molecules of chemicals to control the disease.
- Unculturable bacterial endophytes are largely unexplored communities that affect disease development and also potential biocontrol agents.
- To understand identify hidden partnerships of plant pathogenic bacteria that are often associated with single disease and accelerate the growth and disease severity in plants.
- Screen out potential chemical and biological control products.
- To develop vegetative plant material certification schemes to exclude the *R. solanacearum* from tomato transplants, potato seeds and cuttings.
- More efforts are require knowing effects of crop rotation, cover crops and mulches on pathogen dynamics and wilt disease incidence.

REFERENCES

- Ajjappalavara PS, Dharmatti PR, Salimath PM, Patil RV, Patil MS and Krishnaraj PU (2008). Genetics of bacterial wilt resistance in brinjal Karnataka. *J. Agric. Sci.* 21: 424-427.
- Algam SA, Xie EG, Li B, Yu S, Su T and Larsen J (2010). Effects of *Paenibacillus* strains and chitosan on plant growth promotion and control of Ralstonia wilt in tomato. *J. Plant Pathol.* 92: 593-600.
- Allen C, Ailloud F, Remenant B, Sanchez B, Dalsing B, Bull, CT and Prior P (2014). *Ralstonia solanacearum* into three species: *R. solanacearum*, *R. sequeirae* sp. nov., and *R. syzygii*. In: Inter. Conf. On Plant Pathogenic Bacteria, 13, 2014, Shanghai, China. Proc Shanghai.
- Alves AO, Santos MMB, Santos TCG, Souza EB and Mariano RLR (2014). Biofumigation with essential oils for managing bacterial wilt of sweet peppers. *J. Plant Pathol.* 96: 363-367.
- Anith KN, Momol MT, Kloepper J, Marois JJ, Olson SM and Jones JB (2004). Efficacy of plant growth-promoting rhizobacteria, acibenzolar-s-methyl, and soil amendment for integrated management of bacterial wilt on tomato. *Plant Dis.* 88: 669-673.
- Arasumallaiah L and Rangaswamy SD (2008). Effect of chemicals in the management of bacterial wilt on different brinjal varieties in coastal Karnataka. *Environ. Ecol.* 26: 1445-1446.
- Araujo JS, de Rodrigues P, Ribeiro R, de LD, Goncalves KS and Polidoro JC (2004). Bacteriocin production by Brazilian isolates of *Ralstonia solanacearum* in vitro. ISHS. Acta Horticulturae International Symposium on Tomato Diseases. Brazil. <http://www.actahort.org>. =695_37.
- Bentsink L, Leone GOM, van Beckhoven JRCM, van Schijndel HB, van Gemen B and van der Wolf JM (2002). Amplification of RNA by NASBA allows direct detection of viable cells of *Ralstonia solanacearum* in potato. *J. Appl. Microbiol.* 93: 647-655.
- Biswas S and Singh NP (2008). Integrated management of wilt of tomato caused by *Ralstonia solanacearum*. *Indian J. Mycol. Pl. Pathol.* 38: 182-184.
- Bora LC and Deka SN (2007). Wilt disease suppression and yield enhancement in tomato (*Lycopersicon esculentum*) by application of *Pseudomonas fluorescens* based biopesticide (Biofor-Pf) in Assam. *Indian J. Agric. Sci.* 77: 490-494.
- Cardoso SC, Soares ACF, Brito ADS, Laranjeira FF, Ledo CAS and Santos PD (2006). Control of tomato bacterial wilt through the incorporation of aerial part of pigeonpea and crotalaria to soil. *Summa Phytopathol.* 32: 27-33.
- Caruso P, Gorris MT, Cambra M, Palomo JL, Collar J and Lopez MM (2002). Enrichment double-antibody sandwich indirect enzyme-linked immunosorbent assay that uses a specific monoclonal antibody for sensitive detection of *Ralstonia solanacearum* in asymptomatic potato tubers. *Appl. Environ. Microbiol.* 68: 3634 -3638.
- Castillo JA and Greenberg JT (2007) Evolutionary dynamics of *Ralstonia solanacearum*. *Appl. Environ. Microbiol.* 73: 1225-1238.
- Chakrabarti SK, Shekhawat GS, Gadewar AV and Gopal J (1993). Selection of bacterial wilt tolerant potato germ plasma. In: seminar on crop protection held at Shimla, India, May 1993, abstract and souvenir.
- Chakravarty G. and Kalita MC (2011a). Management of bacterial wilt of brinjal by *P. fluorescens* based bioformulation. *ARPN J. Agric. Biol. Sci.* 6: 1-11.
- Chakravarty G and Kalita MC (2011b). Comparative evaluation of organic formulations of *Pseudomonas fluorescens* based biopesticides and their application in the management of bacterial wilt of brinjal (*Solanum melongena* L.). *Afri. J. Biotechnol.* 10: 7174-7182.
- Champoiseau P, Jones JB, Allen C and Momol T (2009). Description and strategies for best management of *Ralstonia solanacearum* Race 3 biovar 2 as a potential incitant of bacterial wilt of tomato. Proce. 24th Annual Tomato Disease Workshop Held on, Nov. 3-5, State College, Pennsylvania, pp. 1-35.
- Chandrashekara KN and Prasannakumar MK (2010). New host plants for *Ralstonia solanacearum* from India. *New Dis. Repor.* 22-26.
- Chandrashekara KN, Prashannakumar MK, Deepa M, Vani A and Khan ANA (2012). Prevalance of races and biotypes of *Ralstonia solanacearum* in India. *J. Plant Protec.* 52: 53-58.
- de Melo MS, Furuya N, Matsunoto M and Matsuyama N (1999). Comparative studies on fatty acid composition of the whole cell and outer membrane in Brazilian strains of *Ralstonia solanacearum*. *J. Fac. Agr., Kyushu Univ.* 44: 17-23.
- Denny TP (2006). Plant Pathogenic *Ralstonia* Species. In: *Plant-Associated Bacteria*, Gnanamanickam SS (Ed.). Springer Publishing, Dordrecht, The Netherlands, pp. 573-644.
- Denny TP and Hayward AC (2001). *Ralstonia Solanacearum*. In: *Laboratory Guide for Identification of Plant Pathogenic Bacteria*, ed. Schaad NW, Jones JB and Chun W (Eds.). 3rd Edn., APS Press, St. Paul, MN, pp. 151-173.
- Dittapongpich V and Surat S (2003). Detection of *Ralstonia solanacearum* in soil and weeds from commercial tomato fields using immunocapture and the polymerase chain reaction. *J. Phytopathol.* 151: 239-246.
- Elphinstone JG (2005). The current bacterial wilt situation: a global overview. In: Bacterial wilt disease and the *Ralstonia solanacearum* species complex. eds. Allen C, Prior P, Hayward AC. St. Paul, MN: APS Press, 9.

- Elphinstone JG, Hennessy J, Wilson JK and Stead DE (1996). Sensitivity of different methods for the detection of *Ralstonia solanacearum* in potato tuber extracts. *EPPO Bull.* 26: 663-678.
- EPPO (2004). EPPO Standards PM 7/21. Diagnostic protocols for regulated pests: *Ralstonia solanacearum*. *EPPO Bull.* 34: 173-178.
- Fegan M and Prior P (2005). How complex is the *Ralstonia solanacearum* species complex? In: *Bacterial Wilt Disease and the Ralstonia solanacearum Species Complex*. Eds. Allen C, Prior P and Hayward AC, American Phytopathological Society Press, St. Paul, MN., USA., IS
- Fegan M and Prior P (2006). Diverse members of the *Ralstonia solanacearum* species complex cause bacterial wilts of banana. *Australas. Plant Pathol.* 35: 93-101.
- Goto M, Honda E, Ogura A, Nomoto A and Hanaki KI (2009). Colorimetric detection of loop mediated isothermal amplification reaction by using hydroxy naphthol blue. *BioTechniques*. 46: 67-172.
- Grover A, Azmi M, Gadewar AV, Pattanayak D, Naik PS, Shekhawat GS and Chakrabarti SK (2009). Genotypic diversity in a localized population of *Ralstonia solanacearum* as revealed by random amplified polymorphic DNA markers. *J. Appl. Microbiol.* 101: 798-806.
- Guo J, Guo H, Guo YH, Zhang LX, Qi HY and Fang ZD (2001). Screening for biocontrol agents against cayenne pepper bacterial wilt. *Chin. J. Biol. Control* 17: 101-106.
- Hayward AC (1991). Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Annu. Rev. Phytopathol.* 29: 65-87.
- Huang J, Wei Z, Tan S, Mei X, Yin S, Shen Q, and Xu Y (2013). The rhizosphere soil of diseased tomato plants as a source for novel microorganisms to control bacterial wilt. *Appl. Soil Ecol.* 72: 79-84.
- Hussain Z and Bora BC (2009) Interrelationship of *Meloidogyne incognita* and *Ralstonia solanacearum* complex in brinjal. *Indian J. Nematol.* 39: 41-45.
- Islam TM and Toyota K (2004). Suppression of bacterial wilt of tomato by *Ralstonia solanacearum* by incorporation of composts in soil and possible mechanisms. *Microbes Environ.* 19: 53-60.
- Ito S, Ushijima Y, Fujii T, Tanaka S, Kameya-Iwaki M, Yoshiwara S and Kish F (1998). Detection of visible cells of *Ralstonia solanacearum* in soil using a semi selective medium and a PCR technique. *J. Phytopathol.* 146: 379-384.
- Kang, Man Jung, Mi HL, Jae KS, Sang TS, Rosemary S, Min SC, Jang HH and Park DS (2007). PCR-based specific detection of *Ralstonia solanacearum* by amplification of cytochrome c1 signal peptide sequences. *J. Microbiol. Biotechnol.* 17: 1765-1771.
- Kawasaki T, Nagata S, Fujiwara A, Satsuma H, Fujie M, Usami S and Yamada T (2007) Genomic characterization of the filamentous integrative bacteriophage FRSS1 and FRSM1, which infect *Ralstonia solanacearum*. *J. Bacteriol.* 189: 5792-5802.
- Kelman A (1954). The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on tetrazolium medium. *Phytopathology* 44: 693-695.
- Khakvar R, Sijan K, Wong MY, Radu S and Thong KL (2008). Improving a PCR-based method for identification of *Ralstonia solanacearum* in natural sources of West Malaysia. *Am. J. Agric. Bio. Sci.* 4: 490-449.
- Kubota R, Vine BG, Alvarez AM and Jenkins DM (2008). Detection of *Ralstonia solanacearum* by loop-mediated isothermal amplification. *Phytopathology* 98: 1045-1051.
- Kumar P and Sood AK (2001). Integration of antagonistic rhizobacteria and soil solarization for the management of bacterial wilt of tomato caused by *Ralstonia solanacearum*. *Indian Phytopath.* 54: 12-15.
- Kumar P and Sood AK (2005). An ecofriendly approach for the management of bacterial wilt of tomato. *Plant Dis. Res. (Ludhiana)*. 20: 55-57.
- Kutin RK, Alvarez A and Jenkins DM (2008). Detection of *Ralstonia solanacearum* in natural substrates using phage amplification integrated with real-time PCR assay. *J. Microbiol. Methods* 76: 241-246.
- Lebeau A, Daunay MC, Frary DA, Palloix FA, Wang JF, Dintinger J, Chiroleu F, Wicker E and Prior P (2011). Bacterial wilt resistance in tomato, pepper, and eggplant: Genetic resources respond to diverse strains in the *Ralstonia solanacearum* species complex. *Phytopathology* 101: 154-165.
- Lee YA, Fan SC, Chiu LY and Hsia KC (2001). Isolation of an insertion sequence from *Ralstonia solanacearum* Race 1 and its potential use for strain characterization and detection. *Appl. Environ. Microbiol.* 67: 3943-3950.
- Lemessa F and Zeller W (2007). Screening rhizobacteria for biological control of *Ralstonia solanacearum* in Ethiopia. *Biol. Control* 42: 336-344.
- Li JG, and Dong YH (2013). Effect of a rock dust amendment on disease severity of tomato bacterial wilt. *Anto. van Leeuw.* 103: 11-22.
- Li B, Yu RR, Tang QM, Su T, Chen XL, Zhu B, Wang YL, Xie GL and Sun GC (2011). Biofilm formation ability of *Paenibacillus polymyxa* and *Paenibacillus macerans* and their inhibitory effect against tomato bacterial wilt. *Afr. J. Microbiol. Res.* 5: 4260-4266.
- Lin CH, Hsu ST, Tzeng KC and Wang JF (2009). Detection of race 1 strains of *Ralstonia solanacearum* in field samples in Taiwan using a BIO-PCR method. *Eur. J. Plant Pathol.* 124: 75-85.
- Liu Y, Shi J, Feng Y, Yang X, Li X, and Shen Q (2013). Tobacco bacterial wilt can be biologically controlled by the application of antagonistic strains in combination with organic fertilizer. *Biol. Fertil. Soils* 49: 447-464.
- Masyitah S (2004). The development of disease suppressive compost and potting mix for the control of bacterial wilt of tomato. *M.Sc. Thesis, Unversiti Putra Malaysia*.
- Mathew SK, Manimala R, Gopal K S and Devi SN (2007). Effect of microbial antagonists on the management of bacterial wilt in tomato. Recent trends in horticultural biotechnology, Vol. II. ICAR Nati. Symp. on Biotechnol. Interv. for Improvement of Hortil. crops: issues and strategies, Vellanikkara, Kerala, India, 10-12 January, 2005. 823-828.
- Mazumder N (1998). Managing *Ralstonia solanacearum* wilt of tomato. *J. Mycol. Plant Pathol.* 28: 189-192.
- Messiha NAS (2006). Bacterial wilt of potato (*Ralstonia solanacearum* race 3, biovar 2): disease management, pathogen survival and possible eradication. *Ph.D. Thesis, Wageningen University, The Netherlands*.
- Messiha NAS, van Diepeningen AD, Farag NS, Abdallah SA, Janse JD and van Bruggen AHC (2007). *Stenotrophomonas malthophilia*: a new potential biocontrol agent of *Ralstonia solanacearum*, causal agent of potato brown rot. *Eur. J. Plant Pathol.* 118: 211-225.
- Nakaho K, Inoue H, Takayama T and Miyagawa H (2004). Distribution and multiplication of *Ralstonia solanacearum* in tomato plants with resistance derived from different origins. *J. Gen. Plant Pathol.* 70: 115-119.

- Nion YA and Toyota K (2008). Suppression of bacterial wilt of tomato by a *Burkholderia nodosa* strain isolated from Kalimantan soils, Indonesia. *Microbes Environ.* 23: 134-141.
- Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N and Hase T (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.* 28: E63 10.1093/nar/28.12.e63.
- Ozakman M and Schaad NW (2003.) A real-time BIO-PCR assay for detection of *Ralstonia solanacearum* race 3, biovar 2, in asymptomatic potato tubers. *Can. J. Plant Pathol.* 25: 232-239.
- Ozawa H, Tanaka H, Ichinose Y, Shiraishi T and Yamada T (2001). Bacteriophage P4282, a parasite of *Ralstonia solanacearum*, encodes a bacteriolytic protein important for lytic infection of its host. *Mol. Genet. Genomics.* 265: 95-101.
- Pastrik KH and Maiss E (2000). Detection of *Ralstonia solanacearum* in potato tubers by polymerase chain reaction. *J. Phytopathol.* 148: 619-626.
- Pastrik KH, Elphinstone JG and Pukall R (2002). Sequence analysis and detection of *Ralstonia solanacearum* by multiplex PCR amplification of 16S-23S ribosomal intergenic spacer region with internal positive control. *Eur. J. Plant Pathol.* 108: 831-842.
- Posas BM, Toyota K and Islam TM (2007). Inhibition of bacterial wilt of tomato caused by *Ralstonia solanacearum* by sugars and amino acids. *Microb. Environ.* 22: 290-296.
- Poussier S and Luisetti J (2000). Specific detection of biovars of *Ralstonia solanacearum* in plant tissue by nested PCR. *Eur. J. Plant Pathol.* 106: 255-265.
- Poussier S, Vandewalle P and Luisetti J (1999). Genetic diversity of African and worldwide strains of *Ralstonia solanacearum* as determined by PCR-restriction fragment length polymorphism analysis of the *hrp* gene region. *Appl. Environ. Microbiol.* 65: 2184-2194.
- Pradhanang PM, Momol MT, Olson SM and Jones JB (2003). Effects of plant essential oils on *Ralstonia solanacearum* population density and bacterial wilt incidence in tomato. *Plant Dis.* 87: 423-427.
- Prior P and Fegan M (2005a). Recent development in the phylogeny and classification of *Ralstonia solanacearum*. In: Momol T and Jones JB (ed.), *Proc. First Inter. Symp. Tomato Diseases*, vol. 695. *ISHS-Acta Horticulturae*. p. 127-136
- Prior P and Fegan M (2005b). Diversity and molecular detection of *Ralstonia solanacearum* race 2 strains by multiplex PCR, p. 405-414. In *Bacterial wilt disease and the Ralstonia solanacearum* species complex. (ed.), Allen C, Prior P and Hayward AC. *APS Press*, Madison.
- Ramesh R and Phadke GS (2012). Rhizosphere and endophytic bacteria for the suppression of eggplant wilt caused by *Ralstonia solanacearum*. *Crop Prot.* 37: 35-41.
- Ramesh R, Joshi AA and Ghanekar MP (2009). Pseudomonads: major antagonistic endophytic bacteria to suppress bacterial wilt pathogen, *Ralstonia solanacearum* in the eggplant (*Solanum melongena* L.). *World J. Microbiol. Biotechnol.* 25: 47-55.
- Ranjan RK, Singh D, Sharma P and Dhar Shri (2015). Characterization and genetic diversity of *Ralstonia solanacearum* causing brown rot disease of potato. *Indian Phytopath.* 68: 368-374.
- Ranjan RK and Singh D (2015). Occurrence of biovars, races and phylotyping of *Ralstonia solanacearum* causing brown rot disease of potato under different agro-climatic conditions. *J. Pure Appl. Microbiol.* 9: 2931-2941.
- Ranjan RK, Singh D, Singh and Baranwal, VK (2016). Simultaneous detection of brown rot- and soft rot-causing bacterial pathogens from potato tubers through multiplex PCR. *Curr. Microbiol.* 01 10.1007/s00284-016-1110-0.
- Remenant B, de Cambiaire JC, Cellier G, Jacobs JM, Mangenot S, Barbe V, Lajus A, Vallenet, D, Medigue C, Fegan M, Allen C and Prior P (2011). *Ralstonia syzygii*, the blood disease bacterium and some Asian *R. solanacearum* strains form a single genomic species despite divergent lifestyles. *PLoS ONE* 6: e24356.
- Robinson A (1993). Serological detection of *Pseudomonas solanacearum* by ELISA. In: *Bacterial Wilt Proc. Inter. Symp.* eds Hartman GL and Hayward AC, Kaohsiang T aiwan, ROC, 28-30 October 1993.
- Robinson-Smith A, Jones P, Elphinstone JG and Forde SMD (1995). Production of antibodies to *Ralstonia solanacearum*, the causative agent of bacterial wilt. *Food Agric. Immunol.* 7: 67-79.
- Sagar V, Jeevalatha A, Mian S, Chakrabarti SK, Gurjar MS, Arora RK, Sharma S, Bakade RR and Singh BP (2014.) Potato bacterial wilt in India caused by strains of Phylotype I, II and IV of *Ralstonia solanacearum*. *Eur. J. Plant Pathol.* 138: 51-65.
- Safni I, Cleenwerck I, De Vos P, Fegan M, Sly L and Kappler U (2014). Polyphasic taxonomic revision of the *Ralstonia solanacearum* species complex: proposal to emend the descriptions of *R. solanacearum* and *R. syzygii* and reclassify current *R. syzygii* strains as *Ralstonia syzygii* subsp. *syzygii*, *R. solanacearum* phylotype IV strains as *Ralstonia syzygii* subsp. *indonesiensis* subsp. nov., banana blood disease bacterium strains as *Ralstonia syzygii* subsp. *celebesensis* subsp. nov. and *R. solanacearum* phylotypes I and III strains as *Ralstonia pseudosolanacearum* sp. nov. *Inter. J. Syst. Evolu. Microbiol.* 64: 3087-3103.
- Sapna G and Ramesh R (2014). Genetic characterization of *Ralstonia solanacearum* infecting eggplant *Solanum melongena* L. from Goa and Western region of India. *Int. J. Curr. Sci.* 12: 128-139.
- Seal SE, Jackson LA, Young JPW and Daniels MJ (1993). Differentiation of *Pseudomonas solanacearum*, *Pseudomonas syzygii*, *Pseudomonas pickettii* and the blood disease bacterium by partial 16S rRNA sequencing: construction of oligonucleotide primers for sensitive detection by polymerase chain reaction. *J. Gen. Microbiol.* 139: 1587-1594.
- Sebastien M, Catherine Nagy and Haissam Jijakli M (2014). Development of the simultaneous detection of *Ralstonia solanacearum* race 3 and *Clavibacter michiganensis* subsp. *sepedonicus* in potato tubers by a multiplex real-time PCR assay. *Eur. J. Plant Pathol.* 138: 29-37.
- Schaad NW, Jones JB and Chun W (2001). *Laboratory Guide for the Identification of Plant Pathogenic Bacteria* (3rd ed.). St. Paul, MN USA:APS Press
- Schonfeld J, Gelsomino A, van Overbeek LS, Gorissen A, Smalla K and van Elsas JD (2003). Effects of compost addition and simulated solarization on the fate of *Ralstonia solanacearum* biovar 2 and indigenous bacteria in soil. *FMES Microbiol. Ecol.* 43: 63-74.
- Sharma JP and Kumar S (2009). Management of *Ralstonia* wilt of tomato through microbes, plant extract and combination of cake and chemicals. *Indian Phytopath.* 62: 417-423.
- Sharma JP, Kumar S and Singh Dinesh (2016b). *Bacterial Wilt of Solanaceous Vegetables*. IK International Publishing House Pvt. Ltd. New Delhi, 269 pp.

- Sharma JP, Kumar S and Singh Dinesh (2016a). Integrated management of bacterial wilt of tomato caused by *Ralstonia solanacearum*. *Indian J. Agric. Sci.* 86: 956-960.
- Sharma MP, Gaur A, Tanu U and Sharma OP (2004). Prospects of arbuscular mycorrhiza in sustainable management of root and soil-borne diseases of vegetable crops. In: Disease management of fruits and vegetables. Vol. I. Fruit and vegetable diseases. Ed. Mukerji KG Kluwer Academic Publishers, The Netherlands. pp. 501-539.
- Shekhawat GS, Gadewar AV and Chakrabarti SK (2000). Potato bacterial wilt in India. Tec Bull. No. 389 Revised, CPRI, Shimla, India.
- Singh D, Sinha S, Yadav DK, Sharma JP, Srivastava DK, Lal HC, Mondal KK and Jaiswal, RK (2010). Characterization of biovar/races of *Ralstonia solanacearum*, the incitant of bacterial wilt in solanaceous crops. *Indian Phytopath.* 63: 261-265.
- Singh D, Yadav DK, Sinha S and Upadhyay BK (2012a). Utilization of plant growth promoting *Bacillus Subtilis* isolates for the management of bacterial wilt incidence in tomato caused by *Ralstonia solanacearum* Race 1 Biovar 3. *Indian Phytopath.* 65: 18-24.
- Singh D, Yadav DK, Sinha S and Singh Harshita (2012b). Effect of safe chemicals and bleaching powder on bacterial wilt incidence in tomato caused by *Ralstonia solanacearum* race 1 bv. 3. *Ann. Plant. Protec. Sci.* 20: 426-429.
- Singh D, Sinha S and Yadav DK (2014a). Detection of *Ralstonia solanacearum* from asymptomatic tomato, Irrigation water and soil through non-selective enrichment medium with *hrp* Gene-based Bio-PCR. *Curr. Microbiol.* 69: 127-134.
- Singh D, Sinha S, Chaudhary G, Yadav DK and Mondal KK (2014b). Genetic diversity of biovar 3 and 4 of *Ralstonia solanacearum* causing bacterial wilt of tomato using BOX-PCR, RAPD and *hrp* gene sequences. *Indian J. Agric. Sci.* 84: 391-395.
- Singh D, Yadav DK, Chaudhary G, Rana VS and Sharma RK (2016). Potential of *Bacillus amyloliquefaciens* for biocontrol of bacterial wilt of tomato incited by *Ralstonia solanacearum*. *J. Plant Pathol. Microbiol.* 7: 327.
- Smith EF (1896). A bacterial disease of the tomato, eggplant and Irish potato (*Bacillus solanacearum* nov. sp.). *Div. Veg. Phys. Path. Bul.* 12. U. S. Dept. Agr. 1.
- Smith EF (1914). Bacteria in relation to plant disease. Washington: Carnegie Institution, 309 p.
- Smith GP (1991). Surface presentation of protein epitopes using bacteriophage expression systems. *Curr. Opin. Biotechnol.* 2: 668-673.
- Smith JJ, Murimi KR, Gouws J, Sassler GS, Trigalet A and Simons S (2003). 'Biocontrol of potato bacterial wilt in Kenya,' In Biotechnology and Sustainable Development: Voices of The South and North. . Eds. Serageldin I and Persley GJ CABI., pp 129-142.
- Smith JJ, Offord LC, Holderness M and Saddler GS (1995). Genetic diversity of *Burkholderia solanacearum* (Synonym *Pseudomonas solanacearum*) race3 in Kenya. *Appl. Environ. Microbiol.* 61: 4263-4268.
- Stead DE, Simpkins SA, Elphinstone JE, Smith NC, Hennessy J and Weller SA (2003). Real - Time PCR for *Ralstonia solanacearum*. In: *Pseudomonas syringae* and related pathogens. Eds. Iacobellis NS et al. Springer, Dordrecht
- Sun SK and Huang JW (1985). Formulated soil amendment for controlling Fusarium wilt and other soilborne diseases. *Plant Dis.* 69: 917-920.
- Sunaina V, Pandey SK and Singh BP (2006). Bio-B5, an ecofriendly biopesticide-cum-biofertilizer for management of soil and tuber borne diseases of potato and other crops. *Central Potato Research Institute Campus, Modipuram, Meerut, Extension Bull.*, pp. 10.
- Suresh CK and Rai PV (1991). Interaction of *Pseudomonas solanacearum* with Antagonistic Bacterium and VAM Mycorrhiza. *Curr. Res. Univ. Agric. Sci.* 20: 36-37.
- Tan H, Zhou S, Deng Z, He M and Cao L (2011). Ribosomal sequence- directed selection for endophytic Streptomycete strains antagonistic to *Ralstonia solanacearum* to control tomato bacterial wilt. *Biol. Control* 59: 245-254.
- Tans-Kersten J, Huang HY and Allen C (2001). *Ralstonia solanacearum* needs motility for invasive virulence on tomato. *J. Bacteriol.* 183: 3597-3605.
- Thammakijawat P, Thaveechai N, Kositratana W, Chunwongse J, Frederick RD and Schaad NW (2006). Detection of *Ralstonia solanacearum* in ginger rhizomes by real-time PCR. *Can. J. Plant Pathol.* 28: 391-400.
- Trigalet A and Trigalet-Demery D (1990). Use of a virulent mutants of *Pseudomonas solanacearum* for the biological control of bacterial wilt of tomato plants. *Physiol. Mol. Plant Pathol.* 36: 27-38.
- Toyoda H, Kakutani K, Ikeda S, Goto S, Tanaka H and Ouchi S (1991). Characterization of deoxyribonucleic acid of virulent bacteriophage and its infectivity to host bacteria, *Pseudomonas solanacearum*. *J. Phytopathol.* 131: 11-21.
- Tawfik AE, Mahdy AMM and El Hafez AAO (2008). Sensitive detection of *Ralstonia solanacearum* using serological methods and biolog automated system, In: Epidemiology and Genomics, *Pseudomonas syringae* pathovars and related pathogens – identification, epidemiology and genomics, Edited by M'Barek F, Collmer A, Iacobellis NS, Mansfield JW, Murillo J, Schaad NW and Ullrich M, Springer Netherlands, 45-53.
- Umesha S and Avinash P (2015). Multiplex PCR for identification of *Ralstonia solanacearum* and *Xanthomonas perforans*. *3 Biotech.* 5: 245- 252.
- Van der Wolf JM and Schoen CD (2004). Bacterial Pathogens: Detection and identification methods. *Encyclopedia of Plant and Crop Science Feb 2004*, 84-88.
- Van der Wolf JM, Vriend SGC, Kastelein O, Nijhuis EH, Van Bekkum PJ and Van Vuurde JWL (2000). Immunofluorescence colony-staining (IFC) for detection and quantification of *Ralstonia (Pseudomonas) solanacearum* biovar 2 (race 3) in soil and verification of positive results by PCR and dilution plating. *Eur. J. Plant Pathol.* 106: 123-133.
- Van der Wolf JM, Van Beckhoven JR, De Haan EG, Van den Bovenkamp GW and Leone GOM, (2004). Specific detection of *Ralstonia solanacearum* 16S rRNA sequences by AmpliDet RNA. *Eur. J. Plant Pathol.* 110: 25-33.
- Vanitha SC, Niranjana SR, Mortensen CN and Umesha S (2009). Bacterial wilt of tomato in Karnataka and its management by *Pseudomonas fluorescens*. *BioControl.* 54: 685-695.
- Verma RK and Shekhawat GS (1991). Effect of crop rotation and chemical soil treatment on bacterial wilt of potato. *Indian Phytopath.* 44: 5-8.
- Weller SA, Elphinstone JG, Smith NC, Boonham N and Stead DE (2000). Detection of *Ralstonia solanacearum* strains with a quantitative, multiplex, real-time, fluorogenic PCR (TaqMan) assay. *Appl. Environ. Microbiol.* 66: 2853-2858.
- Wicker E, Lefevvre P, de Cambiaire JC, Lemaire C, Poussier S and Prior P (2012). Contrasting recombination patterns and demographic histories of the plant pathogen *Ralstonia solanacearum* inferred from MLSA. *ISME J.* 6: 961-974.

- Wydra K, Diogo R, Dannon E and Semaru J (2005). Soil Amendment with silicon and bacterial antagonists induce resistance against bacterial wilt caused by *Ralstonia solanacearum* in tomato. Proc. Inte. Conf. Agric. Res. Deve. Tropentag, October 11-13, 2005, Stuttgart-Hohenheim, Germany, pp. 1-5.
- Xue QY, Chen Y, Li SM, Chen, LF, Ding GC, Guo DW and Guo JH (2009). Evaluation of the strains of *Acinetobacter* and *Enterobacter* as potential biocontrol agents against *Ralstonia* wilt of tomato. *Biol. Control* 48: 252-258.
- Xue QY, Yin YN, Yang W, Heuer H, Prior P, Guo JH and Smalla K (2011). Genetic diversity of *Ralstonia solanacearum* strains from China assessed by PCR-based fingerprints. *J. Plant Pathol.* 94: 505-516.
- Xue QY, Ding GC, Li SM, Yang Y, Lan CZ, Guo JH and Smalla K (2013). Rhizocompetence and antagonistic activity towards genetically diverse *Ralstonia solanacearum* strains an improved strategy for selecting biocontrol agents. *Appl. Microbiol. Biotechnol.* 97: 1361-1371.
- Yabuuchi E and Arakawa M (1993). *Burkholderia pseudomallei* and melioidosis: Be aware in temperate area. *Microbiol. Immunol.* 37: 823-836.
- Yabuuchi E, Kosako Y, Yano I, Hotta H and Nishiuchi Y (1995). Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* Gen. Nov.: Proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) Comb. Nov., *Ralstonia solanacearum* (Smith 1896) Comb. Nov. and *Ralstonia eutropha* (Davis 1969) Comb. Nov. *Microbiol. Immunol.* 39: 897-904.
- Yamada T, Kawasaki T, Nagata S, Fujiwara A, Usami S and Fujie M (2007). New bacteriophages that infect the phytopathogen *Ralstonia solanacearum*. *Microbiology* 153: 2630-2639.
- Yamazaki H, Kikuchi S, Hoshina T and Kimura T (2000). Calcium uptake and resistance to bacterial wilt of mutually grafted tomato seedlings. *Soil Sci. Plant Nutr.* 46: 529-534.
- Yang W, Xu Q, Liu HX, Wang YP, Wanga YM, Yang HT and Guo JH (2012). Evaluation of biological control agents against *Ralstonia* wilt on ginger. *Biol. Control* 62: 144-151.
- Zhou Y, Choi YL, Sun M and Yu Z (2008). Novel roles of *Bacillus thuringiensis* to control plant pathogens. *Appl. Microbiol. Biotechnol.* 80: 563-572.
- Zhou T, Chen D, Li C, Sun Q, Li L, Liu F, Shen Q and Shen B (2012). Isolation and characterization of *Pseudomonas brassicacearum* J12 as an antagonist against *Ralstonia solanacearum* and identification of its antimicrobial components. *Microbiol. Res.* 167: 388-394.