Bacterial wilt and its management

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Identification of the cause of disease is the most important step towards its eradication, cure and management. India being an agro-based country, plant diseases alone cause immense economic loss to the tune of rupees 500 crores every year. In this study, we focus on reportedly one of the most devastating diseases called bacterial wilt. Though each bacterial type has a set of host range, recent study shows frequent overlapping of susceptible host plants. Besides, several new species have been identified in recent times that cause wilt in plants. There are lots of similarities in the disease manifestation and molecular identification has been quite effective in proper identification of the pathogens. In this study, management of wilt mainly briefs the methods adopted against *Ralstonia solanacearum*. Other than physical and chemical methods, management of the disease by use of antagonistic bacteria and fungi has been found to be the recent trend.

Keywords: Biological control, host range, identification, pathogen virulence.

WILTING refers to the loss of rigidity of non-woody parts of plants due to lowering of water present in the cells. This may be due to several reasons: drought conditions, extreme low temperature due to which the vascular bundles fail to function, high salinity, saturated soil or infection by bacteria, fungi and nematode. Sometimes it is a combination of two or more factors that result in the manifestation of wilt.

Wilt caused by pathogens (bacteria, fungi, nematodes) involves infection of the vascular system. The pathogen enters the water-conducting xylem vessels of a plant, then proliferates within the vessels, causing water blockage. The typical symptoms include wilting and death of the leaves, followed often by death or serious impairment of the whole plant. Visual symptoms of bacterial wilt and fungal wilt are somewhat similar. The method to distinguish bacterial wilt in field is known as 'bacterial streaming'. Large populations of bacteria that exude from the cut surface of infected plant tissue can be viewed through naked eye as cloudy ooze when the cut end of infected stem is dipped into water.

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Wilt causing bacteria

The following are the reported bacteria that cause wilt in plants (Table 1).

Ralstonia solanacearum

Bacterial wilt of tomato, pepper, eggplant and Irish potato caused by *Ralstonia solanacearum*[1] (formerly called *Pseudomonas solanacearum*) is among the first diseases proved to be caused by a bacterial pathogen[2]. *R. solanacearum* was considered a 'species complex' due to significant variation within the group[3]. It attacks almost 450 plant species in 54 different plant families[4]. This constitutes one of the largest known host ranges for any plant pathogenic bacterium. Besides Solanaceae, several dicotyledonous and monocotyledonous families have members susceptible to *R. solanacearum*[5]. The initial symptom is wilting of terminal leaves, followed by a sudden and permanent wilt. Additional symptoms are vascular browning, water soaking of pith followed by browning and browning of cortex near the soil line during the later stages of infection. Bacterial streaming occurs when a freshly cut stem is suspended in water. The pathogen can survive for long periods of time in a nutrient-depleted environment[6].

Xanthomonas campestris pv. musacearum

This bacterium causes Banana bacterial wilt (BBW). It is known to affect banana in Uganda since 2001 (refs 7, 8). All banana cultivars in the affected areas are susceptible to BBW. It has been found to be very destructive with an incidence of 70–80% in many plantations. First symptom is dull green colouration of the lamina which assumes a scalded appearance and wilting back on its midrib. The disease has been reported to cause symptoms on hot pepper, tobacco, sesame, cabbage, wheat and barley, *Datura stramonium* (in Ethiopia), banana relatives (*Musa zebrina* and *Musa ornata*) and Canna-lily, an ornamental plant (in Uganda). There is uneven and premature ripening of the fruit. When fruits are cut, the sections show unique yellowish blotches and dark brown placental scars.

Xanthomonas translucens pv. graminis

Bacterial wilt from bentgrass was identified in the 1970s to be caused by this pathogen. Symptoms include etiolation, small to medium-sized patches of weak turf, turf with excessive senescence and death.

Table 1. List of bacteria that cause wilt in plants

Pathogen	Classification	Host	Symptoms	Reference
Ralstonia solanacearum	Kingdom: Bacteria Phylum: Proteobacteria Class: Beta Proteobacteria Order: Burkholderiales Family: Ralstoniaceae Genus: *Ralstonia* Species: *Ralstonia solanacearum*	Solanaceous and non-solanaceaous crops and weeds	Wilting, vascular browning	2
Xanthomonas campestris pv. *musacearum*	Kingdom: Bacteria Phylum: Proteobacteria Class: Gamma Proteobacteria Order: Xanthomonadales Family: Xanthomonadaceae Genus: *Xanthomonas* Species: *Xanthomonas campestris*	Banana, other *Musa* sp., hot pepper, tobacco, sesame, cabbage, wheat and barley and *Datura stramonium*	Wilting, chlorosis	7, 8
Xanthomonas translucens pv. *graminis*	Kingdom: Bacteria Phylum: Proteobacteria Class: Gamma Proteobacteria Order: Xanthomonadales Family: Xanthomonadaceae Genus: *Xanthomonas* Species: *Xanthomonas translucens*	Bentgrass	Etiolation, patches of weak turf, senescence	84
Curtobacterium flaccumfaciens subsp. *flaccumfaciens*	Kingdom: Bacteria Phylum: Actinobacteria Order: Actinomycetales Suborder: Micrococcineae Family: Microbacteriaceae Genus: *Curtobacterium* Species: *Curtobacterium flaccumfaciens*	Beans	Wilting, discolouration and defoliation	85
Erwinia tracheiphila	Kingdom: Bacteria Phylum: Proteobacteria Class: Gamma Proteobacteria Order: Enterobacteriales Family: Enterobacteriaceae Genus: *Erwinia* Species: *Erwinia tracheiphila*	Cucurbits, corn, Johnson grass		9
Pantoea stewartii	Kingdom: Bacteria Phylum: Proteobacteria Class: Gamma Proteobacteria Order: Enterobacteriales Family: Enterobacteriaceae Genus: *Pantoea* Species: *Pantoea stewartii*	Maize	Wilting in seedling	13
Erwinia chrysanthemi	Kingdom: Bacteria Phylum: Proteobacteria Class: Gammaproteobacteria Order: Enterobacteriales Family: Enterobacteriaceae Genus: *Erwinia* Species: *Erwinia chrysanthemi*	Tomato, pepper, eggplant, Irish potato, tobacco, petunia	Wilting, chlorosis, rotting of roots, lesions at the infection site	14
Enterobacter mori	Kingdom: Bacteria Phylum: Proteobacteria Class: Gammaproteobacteria Order: Enterobacteriales Family: Enterobacteriaceae Genus: *Enterobacter* Species: *Enterobacter mori*	*Morus alba*	Browning of vascular tissues, leaf wilt, defoliation, and tree decline	20

Curtobacterium flaccumfaciens subsp. flaccumfaciens

Curtobacterium flaccumfaciens subsp. *flaccumfaciens* is the causal pathogen of bacterial wilt of beans. The wilt kills young seedlings by plugging the vascular tissue in stems. Larger plants that become infected may survive the entire season and produce seed. However, leaves wilt during periods of moisture, stress and during warmer parts of the day. Golden brown, irregularly shaped leaf lesions occur and the affected leaves may drop off. Infected seeds may turn bright yellow, orange, or purple,

depending on the strain of the infecting bacterium. The disease is not spread easily by rain or contact with wet foliage when compared with other bean diseases.

Erwinia tracheiphila

The preferred hosts of this pathogen are in the cucurbit family (wild and cultivated species), of which cucumbers are 'the most susceptible hosts, followed by muskmelon, squash, and pumpkin'[9]. Watermelon, however, is extremely resistant to bacterial wilt. *E. tracheiphila* is reported to attack non-cucurbit hosts like corn[10], but apparently with no significant losses. Golden rod (*Solidagone moralis* and *S. altissima* L.) and Johnson grass (*Sorghum halepense* L.) are believed to be asymptomatic hosts of *E. tracheiphila* during winter[11,12].

Pantoea stewartii

The disease Stewart's wilt of corn caused by the bacterium, was first recorded (in the USA) in 1895 in Long Island[13]. This bacterium affects plants, particularly types of maize or corn such as sweet, flint, dent, flower and popcorn. The disease manifests in two phases – seedling wilt (when the growing point dies) and leaf blight (white lesions on the leaves of older plants).

Erwinia chrysanthemi (syn. Pectobacterium chrysanthemi, *Dickeya dadantii)*

It is known for soft rot, brown rot or blackleg disease and has been identified to be the cause of severe wilt and root rot in sweet potato from Georgia. The symptoms include rotting of the roots along with wilt, water-soaked lesions at the site of infection and gradually expanding chlorotic leaves. The pathogen grows intercellularly, degrading cells through pectinolytic activity and finally reaches xylem. Though host range studies have shown that tobacco, petunia, tomato, pepper, eggplant and Irish potato were infected and often killed at high temperature and humidity, disease in these hosts was not determined in field conditions[14].

Enterobacter mori

This is a plant-pathogenic enterobacterium responsible for the bacterial wilt of *Morus alba*[15]. Mulberry (*Morus alba* L.), an important sericulture plant, widely grown in China, Japan, Egypt and southeast Asian countries, had been reported to be infected by *Pseudomonas syringae* pv. *mori*[16], *Pseudomonas solanacearum*[17,18] and *Erwinia carotovora*[19]. In the year 2006, a severe bacterial wilt was noted in mulberry orchards in Hangzhou, Zhejiang Province, China, which was identified to be caused by *Enterobacter mori*. 16S rDNA and *rpoB* gene sequences of the pathogens showed <97% and <98% similarity respectively to the existing species of *Enterobacter*, and thus they were considered to be novel species[20,21].

Typical symptoms of the disease include browning of vascular tissues, leaf wilt, defoliation and tree decline. Unlike the symptoms of bacterial wilt disease caused by *R. solanacearum*, symptoms of Mulberry wilt disease generally start from the bottom of the plants and move upward.

R. solanacearum, diversity and identification

Of all the wilts, *R. solanacearum* has an unusually wide host range comprising important vegetables, fruits and cash crops with a continuously increasing number of host species. It behaves as a complex of variants, variously described as groups, races, biovars, biotypes, sub-races and strains[22]. The pathogen is subdivided into races based on host. Race 1 has a wide host range of solanaceous plants and weeds, race 2 is restricted to triploid banana and *Heliconia*, race 3 (potato race) affects potato, race 4 infects ginger, and race 5 is pathogenic to mulberry[18].

Based on biochemical tests on the ability to oxidize sugar/sugar alcohol, viz. maltose, lactose, cellobiose mannitol, sorbitol and dulcitol, *R. solanacearum* is classified into five biovars[23]. Recently an improved biovar test has been introduced that requires fewer days and uses phenol red instead of bromothymol blue as the pH indicator[24]. Biovar 1 is negative for utilization/oxidation tests. Biovar 2 utilizes disaccharides but does not oxidize the sugar alcohols. Biovar 3 utilizes all the sugars and oxidizes the sugar alcohol. Biovar 4 oxidizes all the sugar alcohols but does not utilize the disaccharides. Biovar 5 utilizes all the disaccharides, oxidizes mannitol but not dulcitol and sorbitol. Biovar 2 isolates have been differentiated into metabolically less active Andean phenotype Biovar 2A and metabolically more active tropical lowlands Biovar 2T based on utilization of L(+) tartarate and L(–) tryptophan and production of acid from D (–) ribose and D (+) trehalose[25]. While Biovar 2A is negative, Biovar 2T is positive for the tests. Variation in the ability of isolates to utilize sugars such as dulcitol is also reported[26,27] designating such strains as biovar 3A. Similarly, differences among the strains infecting potato in India and the ability of these strains to utilize sugars have also been found[28]. One strain that could not utilize mannitol and maltose has been designated as a typical strain. Recently, three isolates from Kerala have been found to be unable to utilize lactose and dulcitol. They have been designated as Biovar 3B[27].

In India bacterial wilt of potato was first reported in 1892 by Cappel[29]. The Moko Disease of banana in India was first reported in West Bengal caused by *R. solanacearum* Race 2/Biovar[30]. Wilt by this pathogen has also been reported in chilli, tomato, potato, eggplant, sesame and peanuts from Andaman and Nicobar Islands[31].

Indian farmers were long aware of this disease which was locally called Rassa (moisture disease), Bangle Blight or Bangdi, Parraya, Ghera and Ukta.

The disease is now endemic in the west coast of Thiruvananthapuram in Kerala to Gujarat, Karnataka, Western

Maharashtra and Madhya Pradesh, the eastern plains of Assam, Orissa and West Bengal, the Chhota Nagpur Plateau and the Andaman and Nicobar islands. It is also endemic in the North Western Hills up to 2200 m, the eastern hills of West Bengal, Meghalaya, Manipur, Mizoram and Nagaland, Tripura and Arunachal Pradesh and in the Nilgiris, Annamalai and Palani Hills of Tamil Nadu.

In North India, the soil is not suitable for *R. solanacearum* as it does not retain water over the year and has an average soil temperature of >40°C. In hills also, the temperature is not suitable as it can go below 0°C. This supports the presence of alternate hosts in India that play a role in survival of the bacterium.

Recent studies have shown that pathogen survives a symptomless infection in the alternate weed hosts or in presumed non-host plants[32]. Disease severity mostly increases if *R. solanacearum* is found in association with root nematodes. In tobacco, nematode infestation changes the physiology of the plants, causing susceptibility to bacterial wilt[33]. Experiments in India showed that the combined pathogenic effects of *R. solanacearum* and *Meloidogyne javanica* were greater than their independent effects[34].

It is evident that all races and biovars of the pathogen *R. solanacearum* exist in India and the strains prevalent in India appear to be the most virulent. Molecular identification of the Indian strains and a complete screening from different geographical locations is essential for wilt management.

The Indian Council of Agricultural Research, recognizing the importance of three plant pathogens, viz. *Phytophthora*, *Fusarium* and *Ralstonia* affecting large number of crops ranging from vegetables, fruits, spices, plantation crops, ornamental, pulses and oil seeds, undertook a research initiative 'PhytoFuRa[35].

The wilt bacteria move in the vascular bundles, which is followed by colonization of the xylem[36], where the bacteria adhere to the vessel walls or invade the lumen. Blocking of the vessels by bacteria is the major cause of wilting. The symptoms of *R. solanacearum* borne wilt are as follows: wilting of the leaves at the ends of the branches during the heat of the day with recovery at night. The youngest leaves are usually the first to be affected; a brown discolouration of the stem; and bacterial oozing from cut end of stem (bacterial streaming).

A selective medium (SMSA) modified by Elphinstone *et al.*[37] is generally used to distinguish *R. solanacearum* from other bacteria based on the pink coloured colonies of the former. This medium is used to identify virulent and avirulent strains based on colony morphology[38]. While virulent colonies are fluidal, irregular, white with pink centre, the avirulent colonies are less fluidal, round and red in colour.

ELISA and polymerase chain reaction (PCR), based on 16S rRNA gene targeted primers, have been successfully used to identify the pathogen. DNA sequences from which the primers are designed come from three main origins: pathogenicity/virulence genes, ribosomal genes, and plasmid genes[39]. At least 24 different primers pairs had been designed to detect *R. solanacearum*[40]. Real-time (RT) PCR has also been proposed for detection of *R. solanacearum*[41,42].

Molecular typing methods are useful to study intraspecific diversity. Several reliable molecular techniques including AFLP, BOX, ERIC and rep-PCR, IS-RFLP, MLST, and macrorestriction-PFGE are available[43]. Recent molecular studies have revealed high diversity among strains and the group is therefore considered a species complex, a heterogeneous group of related strains[44,45]. Molecular analyses using the nucleotide sequences of genes *egl* (encoding endoglucanase, a conserved virulence factor), *mutS* (a DNA mismatch repair enzyme), *hrpB* (a regulator of type 3 secretion) and the ITS region between the 16S and 23S ribosomal RNA genes have generated a phylogenetically meaningful subdivision of the species complex into four phylotypes – Phylotype I strains come from Asia, phylotype II strains from the Americas, phylotype III strains from Africa and phylotype IV strains from Indonesia (which is the probable origin of the group)[44,45]. A multiplex PCR reaction distinguishes these four phylotypes. Within phylotypes, strains are further clustered into sequevars based on nucleotide sequences of the *egl* and *mutS* genes[44–46].

Fatty acid profiling is also considered a valuable tool for classification of Gram-negative bacteria, especially the Pseudomonas group. It has been used to identify *R. solanacearum* and its related allies[47].

Bacterial wilt and Enterobacteriaceae

According to Brenner and Farmer III[48], the family Enterobacteriaceae incorporates a group of Gram-negative, facultatively anaerobic rod-shaped bacteria. They are generally motile with peritrichous flagella. Most of the bacteria in this family are metabolically active at 25–35°C.

The major classification studies on the family Enterobacteriaceae were based on phenotypic traits[49–54] such as biochemical reactions and physiological characteristics. However, phenotypically distinct strains may be closely related by genotypic criteria and may belong to the same genospecies[55,56]. Also, strains which are phenotypically close (biogroup) may belong to different genospecies e.g. *Klebsiella pneumoniae* and *Enterobacter aerogenes*[57]. Hence, identification and classification of certain species may be ambiguous with techniques based on phenotypic tests[58–60]. The coliform group of Enterobacteriaceae is considered to be a depository, because the traditional IMViC tests do not allow complete identification of species. The IMViC formula comprises indole production, methyl red reaction, Voges-Proskauer and citrate utilization tests. The environmental group of coliform comprises novel species of the genera *Klebsiella* (*K. planticola* and *K. terrigena*), *Enterobacter* (*E. amnigenus* and *E. intermedium*),

Serratia (*S. fonticola*), and *Yersinia*, or novel genera, *Budvicia*, *Buttiauxella*, *Leclercia*, *Rahnella*, and probably many species from the genera *Erwinia* and *Pantoea*. They are frequently isolated from freshwater supplies, can originate from small animals (e.g. *Buttiauxella* strains isolated from molluscs), or are commonly associated with plants, such as soft rot erwinias (carotovora group; e.g. *Pantoea agglomerans*, previously *Enterobacter agglomerans*)[61]. Extensive studies on the family Enterobacteriaceae, which contains the coliform group, and related taxa have led to the recommendation that genomic species should encompass strains with approximately 70% or greater DNA–DNA relatedness and with 5 ± C or less ΔT_m.

Enterobacteria occupy a variety of ecological niches, including both plant and animal hosts. Genome sequences of members of this family are available but taxonomically biased as a majority of genera are not represented by complete or ongoing genome projects. Besides, lateral gene transfer is extensive in some lineages of enterobacteria which creates discordant phylogenetic signals for some combinations of loci and taxa. A recent project at the University of Wisconsin-Madison targets for complete genome sequences of some previously neglected genera and species to provide a much-needed link between molecular phylogenetics and classical prokaryotic systematics[62]. Enterobacteriaceae contains over 44 genera and 176 species. The plant pathogens included in this family are *Pantoea*, *Pectobacterium*, *Erwinia*, *Brenneria* and *Dickeya* and the list is increasing.

Mulberry (*Morus alba*), where bacterial wilt is known to be caused by *R. solanacearum* phylotype was reported to be infected by a pathogen from *Enterobacter*, based on Biolog metabolic profiles, fatty acid methyl ester analysis (FAME) and sequence analysis of the partial 16s rDNA and *rpoB* genes. This was later assigned the name *E. mori*. This study found that *Enterobacter asburiae* also caused mulberry wilt disease (MWD)[20]. The observation of wilt, proceeding from the bottom of the plant to the top distinguishes this disease from bacterial wilt caused by *R. solanacearum*[21].

Several strains of *Enterobacter* had been previously known to be associated with plants like *Enterobacter cloacae* subsp. *dissolvens* from poplar, *E. cancerogenus* from maize, *E. pyrinus* from pear, *E. nimipressuralis* from elm and *E. agglomerans* from *Pyrus communis*. A pair of primers (Em-rpoBF and EmrpoBR) has been designed specifically for *E. mori* which amplifies a 307-bp portion of the RNA polymerase α-subunit gene, *rpoB*[63].

In Zingiberaceae family, *Curcuma alismatifolia* (pathumma), an ornamental plant with a worldwide market is susceptible to bacterial wilt. The pathogenic bacteria isolated from infected pathumma rhizomes were identified as *Enterobacter* sp. by morphological, biochemical and molecular methods[64]. In recent studies, wilt in solanaceous crops has been reported to be caused by Enterobacterial strains[22].

Management of bacterial wilt

Since *R. solanacearum* is a soil-borne pathogen and host resistance is limited, bacterial wilt is difficult to control[65]. Some highly aggressive strains have been reported to cause severe symptoms, even to tomato varieties classified as resistant[66]. Crop resistance has also been observed to be overcome due to high genetic diversity of the bacteria[67]. Other methods like intercropping and crop rotation are often hampered due to a wide range of pathogens[68]. Chemical control is nearly impossible to apply though use of antibiotics to control bacterial wilt started as early as 1952 (ref. 69). Soil fumigants showed either slight or no effects[70]. Antibiotics such as streptomycin, ampicillin, tetracycline and penicillin showed hardly any effect[71]. This is mainly because *R. solanacearum* is a soil-borne pathogen and is systemic in its action[72]. The concept of biocontrol of plant diseases includes disease reduction or decrease in inoculum potential of a pathogen brought about directly or indirectly by other biological agencies[73]. Plant growth promoting rhizobacteria are known to exhibit bio-control of parasitic pathogens. Recent studies indicate that biological control of bacterial wilt disease could be achieved using antagonistic bacteria[74]. Among PGPRs, fluorescent pseudomonads have been reported to be effective against a broad spectrum of plant pathogens[75]. Similarly the sporulating Gram-positive bacteria like *Bacillus* spp. have also been used successfully for plant disease control[76]. Amongst fungi, *Trichoderma* spp. is known to be effective biological means to control soil borne diseases[77].

Bacillus subtilis has been reported to be effective in the management of bacterial wilt disease in tomato[78]. *P. aeruginosa* KUCd1, a cadmium tolerant strain reported to have PGP effect shows antagonistic effect towards several plant pathogens[79–81] though its effectiveness in controlling bacterial wilt has not been reported. *Trichoderma* spp. has proved to be useful in the control of phytopathogens affecting different crops[82,83].

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