Advancement in Detection of *Ralstonia solanacearum*

Deepika M, Madhu D and Sumitra Phurailatpam
College of Agriculture, Iroisemba, Central Agricultural University, Imphal, Manipur
Corresponding Author: sumitrapathology@gmail.com

Introduction

Ralstonia solanacearum is a highly destructive, soil- and water-borne β-proteobacterium responsible for bacterial wilt, affecting more than 310 plant species across 50 botanical families globally (Genin, 2010; Paudel *et al.*, 2020). It thrives under warm (28–35°C) and humid conditions, where it colonizes the root system, invades xylem vessels, and produces copious exopolysaccharides (EPS I and EPS II), leading to vascular occlusion and systemic wilting. The pathogen exhibits substantial genetic and phenotypic diversity, encompassing multiple phylotypes and sequevars, which enhances its adaptability, virulence, and host range (de Pedro-Jové *et al.*, 2021). Economically, *R. solanacearum* imposes severe global losses, with an estimated $848 million annual impact on potato crops alone (Charkowski *et al.*, 2020). Its persistence in various ecological niches — including deep soil layers, infected plant debris, and irrigation water — and its ability to enter a viable but non-culturable (VBNC) state complicate its management. Traditional chemical controls offer limited success, prompting a shift toward integrated approaches. In recent years, significant advancements in molecular diagnostics, including PCR, loop-mediated isothermal amplification (LAMP), and recombinase polymerase amplification with lateral flow detection (RPA-LFD), have revolutionized early detection. These innovations play a crucial role in disease forecasting, rapid surveillance, and long-term management, contributing to global food security.

Advanced Detection Technique

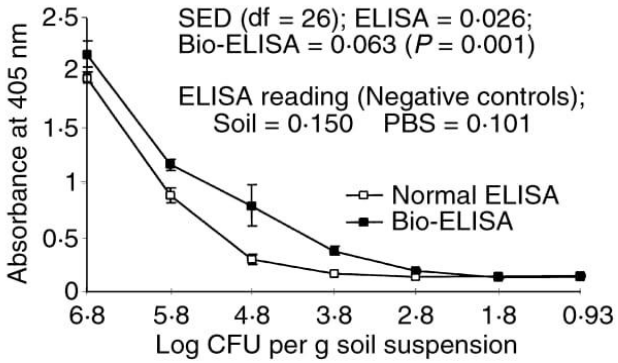
Biochemical Test

Sl.No	Biochemical tests	*R.solanacearum*
1	Gram staining	Negative
2	KOH test	Positive
3	Catalase test	Positive
4	Starch hydrolysis	Positive
5	Gelatin liquefaction	Negative
6	H_2S production	Positive
7	Methyl red test	Positive

Serological Technique

Indirect ELISA Protocol

The indirect ELISA protocol, as described by Robinson-Smith *et al.* (1995) and modified by Elphinstone *et al.* (1996), was employed to detect *Ralstonia solanacearum*. The protocol utilized polyclonal antiserum produced in rabbit against strain R 303 of *R. solanacearum* biovar 2. The antibody binding was detected using antirabbit IgG-alkaline phosphatase, and absorbance at 405 nm was measured 2 hours after substrate addition; results exceeding negative control values were considered positive.



(Source: Pradhanang, 2000)

Monoclonal Antibodies:

Monoclonal antibodies target a single epitope with high specificity but lower reactivity; combining MAbs can improve detection.

Causal organism	Mab Designation	No of target strains tested	Reference
Ralstonia solanacearum	RS 1 RS 1a	75	Alvarez et al,(1996)

Molecular Technique

1.PCR & Real-Time qPCR Advances

TaqMan multiplex real-time PCR enables simultaneous detection of broad-range R. solanacearum strains and specific biovar 2A, with a sensitivity of ~10^2 cells/mL in pure cultures. It also integrates a potato COX internal control to reduce false negatives (Weller *et al.*, 2000). A real-time qPCR assay using RSF/RSR primers (targeting the UDP-3-O-acyl-GlcNAc deacetylase gene) reliably identifies all six biovars in plant or soil tissues, with detection down to 10^2 CFU/g (Fegan & Prior, 2005).

2. Isothermal LAMP-Based Field Detection

Conventional LAMP: The IpxC-based LAMP assay selectively amplifies R. solanacearum DNA, detecting as low as 2.5 pg/µL. In Kenyan field trials, it matched qPCR accuracy and outperformed ELISA (Kubota *et al.*, 2008; Okiro *et al.*, 2023). Real-time portable LAMP: Optimized

assays targeting RSSC and phylotype I achieved detection limits of ~10^3–10^4 CFU/mL (~5–50 CFU/reaction), suitable for field use (Okiro et al., 2023). LAMP + CRISPR/Cas12a: This advanced method combines LAMP with CRISPR-based detection using Cas12a, offering rapid fluorescence or lateral-flow detection for phylotype I, reaching sensitivity as low as 2 copies (Fan *et al.*, 2023).

3. Recombinase Polymerase Amplification (RPA) & Lateral Flow

An RPA-LFD assay targeting the RipTALI-9 gene offers visual detection within ~25 minutes at 37 °C. It works efficiently in both stem and soil samples and was validated in field conditions (Huang *et al.*, 2022).

4. Emerging Biophysical & Nanopore Techniques

A novel PCR + α-hemolysin nanopore sensing method was introduced using asymmetric PCR and λ-exonuclease digestion. The resulting ssDNA is read by electrical signals through a nanopore, detecting *R. solanacearum* without purification (Liu *et al.*, 2023).

5. Immuno-Based & Optical Approaches

Improved serological kits have enhanced rapid field detection using immunostrips and ELISA with better monoclonal antibodies (Bhat *et al.*, 2024). While not yet widely applied for R. solanacearum, hyperspectral imaging and optical reflectance tools are being explored for early non-destructive detection of wilt symptoms (Zhang *et al.*, 2024).

Conclusion

The persistent threat posed by *Ralstonia solanacearum* to global agriculture underscores the urgent need for multifaceted management strategies and precise detection systems. Despite its complex ecology and genetic diversity, recent advancements in molecular diagnostics—such as LAMP, qPCR, CRISPR-based methods, and RPA—have significantly improved the speed and accuracy of pathogen identification, enabling early intervention and disease containment

References

Bhat, A. I., Singh, P., & Kumar, R. (2024). Serological tools for detection. Journal of Plant Pathology, 106(2), 290–298.

Charkowski, A., Sharma, K., Parker, M. L., Secor, G. A., Elphinstone, J. (2020). "Bacterial diseases of potato," in The potato crop (Cham: Springer).

de Pedro-Jové, R., Puigvert, M., Sebasti`a, P., Macho, A. P., Monteiro, J. S., Coll, N. S., et al. (2021). Dynamic expression of *Ralstonia solanacearum* virulence factors and metabolism-controlling genes during plant infection. *BMC Genom.* 22, 170. doi: 10.1186/s12864-021-07457-w

Fan, Y., Mei, Y., Xing, J., & Liang, Y. (2023). LAMP-Cas12a-based detection. *Plant Pathology Journal*, 39(4), 568–578.

Fegan, M., & Prior, P. (2005). How complex is the *Ralstonia solanacearum* species complex? In Bacterial Wilt Disease and the Ralstonia solanacearum Species Complex. APS Press.

Genin, S. (2010). Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. *New Phytol.* 187, 920–928. doi: 10.1111/j.1469-8137.2010. 03397.x

Huang, Q., Wang, X., Li, S., & Zhang, L. (2022). RPA-LFD assay. *Plant Disease*, 106(5), 1372–1379.

Kubota, R., Vine, B. G., Alvarez, A. M., & Jenkins, D. M. (2008). Detection of R. solanacearum by LAMP. *Phytopathology*, 98(9), 1045–1051.

Kumar, A., Hayward, A. C. (2005). "Bacterial diseases of ginger and their control," in *Ginger: the genus zingiber*. Eds. Ravindran, P. N., Nirmal Babu, K. (New York: CRC Pres), pp 341–pp 365.

Liu, C., Chen, Y., Zhao, J., & Li, H. (2023). Nanopore-based detection. *Nanomaterials,* 13(2), 332.

Louws, F. J., Rivard, C. L., Kubota, C. (2010). Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthropod and weeds. *Sci. Hortic.* 127, 127–146. doi: 10.1016/j.scienta.2010.09.023

Nion, Y. A., Toyota, K. (2008). Suppression of bacterial wilt of tomato by a *Burkholderia nodosa* strain isolated from kalimantan soils, Indonesia. *Microbes Environ.* 23, 134–141. doi: 10.1264/jsme2.23.134

Nion, Y. A., Toyota, K. (2015). Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. *Microbes Environments* 30(1),1–11. doi: 10.1264/jsme2.ME14144

Okiro, L. A., Mutitu, E. W., Mwenda, J. M., & Karanja, N. (2023). Field validation of LAMP assays. *PhytoFrontiers*, 3(1), 45–53.

Robinson-Smith A, Jones P, Elphinstone JG, Forde SMD, 1995. Production of antibodies to *Pseudomonas solanacearum*, the causative agent of bacterial wilt. Food and Agricultural Immunology 7, 67-79.

Weller, S. A., Elphinstone, J. G., Smith, N. C., Boonham, N., & Stead, D. E. (2000). Detection of *Ralstonia*

solanacearum by real-time fluorogenic PCR. *Applied and Environmental Microbiology*, 66(7), 2853–2858.

Zhang, Y., Li, Q., Chen, M., & Sun, X. (2024). Hyperspectral imaging for wilt detection. *Sensors*, 24(3), 578.

* * * * * * * * *