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Current status and future perspectives of the diagnostic of plant bacterial pathogens

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Diagnostic of plant bacterial pathogens underwent a leapfrog development from culture-based strategies to culture-free detection. Conventional diagnostics, such antibody- and PCR-based methods, are sensitive to identify pre-enriched pathogens in naturally infected crops at the late stage. However, they suffer from shortcomings relating to rapidity, signal strength, and a significant reduction in sensitivity in real plant extract. Progress has been made to address these challenges through development of labelled and non-labelled optical spectroscopy. Specifically, the micro-Raman spectroscopy enables fast, label-free, and non-invasive discrimination of viable but non-culturable pathogens at a single-cell level. A comprehensive spectroscopic database is always a prerequisite for identification, yet these spectroscopy-based methods are insufficient to detect previously unknown plant pathogens. The advance of single-cell sequencing and synthetic biology is beginning to address these crucial problems and is being used in related practical applications. Success will continue to be found at the interfaces between disciplines.

Crop pathogens and global food security

The world population is expected to rise from 7.2 billion to 9.7 billion by 2050 and 11 billion at the end of the century (Population Division of the Department of Economic and Social Affairs of the United Nations Secretaria, 2022). Global agricultural production must increase by 70% and 106% to satisfy a growing demand for food. However, the epidemic of crop pests and pathogens causes substantial economic losses and reduce food security at household, national, and global levels. Despite the difficulty in quantitative, standardized assessment of crop losses due to disease across crops, it was estimated to average 21.5% in wheat, 30.0% in rice, 22.6% in maize, 17.2% in potato, and 21.4% in soybean yield losses at a global level (Savary et al., 2019) while these crops account for half of the global human

calorie intake (Food and Agriculture Organization of the United Nations, 2024). The highest losses are associated with food-deficit regions with fast-growing populations (e.g., Indo-Gangetic Plain and Sub-Saharan Africa), and frequently with emerging or re-emerging diseases (Figure 1) (Savary et al., 2019). The situation is aggravated by global trade which intensifies the spread and distribution of quarantine pathogens (Elad and Pertot, 2014), many of which can spread or reemerge after having been under control (Bhattacharya, 2017). Secondary yield losses caused by the negative impacts of pests and diseases in the previous year was even worse (Cerda et al., 2017). Approximately 110 billion kilograms of food loss, 1.5 billion kilograms of cotton, 2.3 billion kilograms of oil, 50 billion kilograms of vegetables, and 6 billion kilograms of fruits could be saved by pest control each year, which is equivalent to an increase of 12% to 18% of the planting area. However, many countries, particularly low-income countries, are incompetent in monitoring and preventing disease spread. Therefore, to improve responses to unexpected crop disease spread and minimize the risk to food supplies, a global surveillance system for crop diseases is critical, which will extend and adapt established biosecurity practices and networking facilities (Carvajal-Yepes et al., 2019).

The occurrence and prevalence of plant diseases vary from season to season, depending on the presence of the pathogen, environmental conditions, and the crops and varieties grown. Survival improves when pathogen is detected early. However, majority of plant diseases are at an advanced stage when diagnosed. Early detection of pathogen allows early intervention to try to slow or prevent disease development and lethality. To achieve early detection of all pathogen, numerous of methods have been developed. In this article, we review the development of the plant pathogen detection technology and briefly summarize the challenges and the future directions. We see how our existing knowledges about the pathogen biology and technology development promote the sensitivity, accuracy, and throughput of detection methods for plant pathogens. Interdisciplinary collaboration is key in transforming progress in technology and biology of plant pathogens to improve early detection and plant survival.

Morphology-based techniques

Conventional methods for the detection and identification of plant pathogens mainly rely on recognizing specific morphological features, which involves a time-consuming and labor-intensive process for pathogen cultivation (Camargo and Smith, 2009; Li et al., 2012; Li et al., 2014b; Li et al., 2014a) (Table 1). The period of time from the infection of pathogens to the observation of the syndromes of suspected infectious etiology is variable ranging from days for fast-growing bacteria to weeks for slower growing species (Qinhua et al., 2011). On the other hand, individual pathogens may
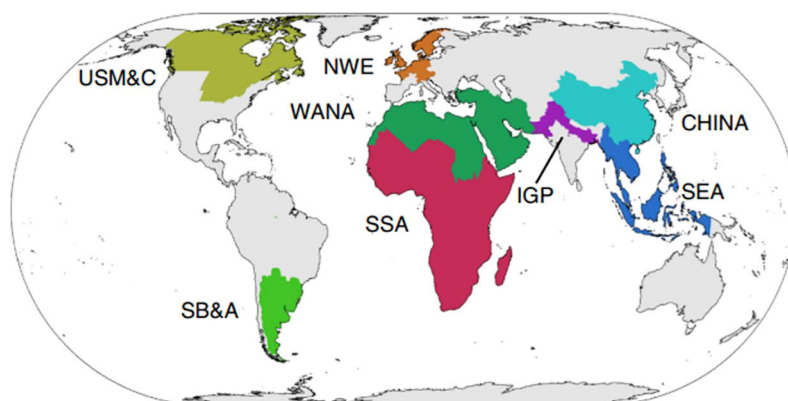
Crop	Global	USM&C	SB&A	NWE	WANA	SSA	CHINA	IGP	SEA
Wheat	19.73	16.98	21.54	24.37	10.14	23.12	24.35	13.06	-
Rice	23.88	-	-	-	-	23.88	22.38	31.6	21.04
Maize	17.63	13.92	15.12	-	-	15.48	-	37.11	-
Potato	15.46	5.6	-	5.89	11.67	10.56	-	18.98	-
Soybean	15.70	14.84	24.58	-	-	9.15	-	-	-

FIGURE 1
Global variations in crop losses and production. The global map shows the location of the eight food security hotspots where there were sufficient survey responses to estimate the loss (Hijmans et al., 2018). The bottom table shows losses for wheat, rice, maize, potato, and soybean globally or specifically to each food security hotspot. It can be see that crop losses are lower in hotspots generating food surpluses (USM&C, SB&A and NWE, except for soybean) and higher in hotspots located in food insecure regions (SSA and IGP) frequently. Food security hotspot: USM&C, US Midwest and Canada; SB&A, South Brazil, Paraguay, Uruguay and Argentina; NWE, Northwest Europe; WANA, West Asia and North Africa; SSA, Sub-Saharan Africa; CHINA, Mainland China; IGP, Indo-Gangetic Plain; SEA, Southeast Asia. [Adapted from previous study (Savary et al., 2019)].

TABLE 1 Summary of various techniques used for plant bacterial pathogen detection.

Approaches	Advantages	Limitations	Detection limit (CFU/mL)	Instrumentation	Cost
Morphology-based (Camargo and Smith, 2009; Qinhua et al., 2011; Li et al., 2012; Li et al., 2014b; Li et al., 2014a)	Low cost	Experience-dependent, time-consuming, very low sensitivity and accuracy	10^7-10^8	Simple	Low
Immunology-based (Holgate et al., 1983; Van Laere et al., 1985; Wyatt et al., 1989; Mazarei and Kerr, 1990; Franken et al., 1992; Gallo et al., 2000; Anil et al., 2008; Yao et al., 2009; Braun-Kiewnick et al., 2011; Lattanzio et al., 2012; Charlermroj et al., 2013; Gan et al., 2013)	More specific, sensitive, reproducible, and reliable	Antigen-dependent, time-consuming sample preparation, and incapable of detecting unknown pathogens	10^5-10^6	Simple	Low
Nucleic-acid-based (Bereswill et al., 1992; De Boer, 1995; Mcmanus and Jones, 1995; Pulawska, 1997; Hélias et al., 1998; Manulis et al., 1998; Zaccardelli et al., 2003; Kong et al., 2004; Zaccardelli et al., 2005; Kumagai and Fabritius, 2008; Kumagai and Fabritius, 2008; Ning et al., 2008; Shenge et al., 2008; Haan E.G.d and Bovenkamp G.W.v.d, 2009; Pang et al., 2009; Park et al., 2009; Pirc et al., 2009; Shao et al., 2009; Qinhua et al., 2011; Shao et al., 2011; Araujo et al., 2012; Gašić et al., 2012; Gehring and Geider, 2012; Li et al., 2012; Štajner et al., 2013; Feng et al., 2013; Gan et al., 2013; Liu et al., 2013; Gan et al., 2014a; Gan et al., 2014b; Gan et al., 2014c; Li et al., 2014b; Li et al., 2014a; Luqi et al., 2014; Feng et al., 2015; Silva et al., 2015; Wang et al., 2015a; Wang et al., 2015b; Fan et al., 2016; Ilicic et al., 2016; Meng et al., 2016; Zhao et al., 2016; Yang et al., 2025)	Rapid, specific, and highly sensitive	Instrument-dependent and low sensitivity for pathogens in the presence of plant extract	10^3-10^4	Simple	Low
Nanotechnology-based (Meng et al., 2005; Yao et al., 2009; Sharon et al., 2010; Etefagh et al., 2013)	High sensitivity	High cost	1-10	Complicate	High
Fluorescent hybridization-based (Franken et al., 1992; Anil et al., 2008; Yao et al., 2009; Braun-Kiewnick et al., 2011; Lattanzio et al., 2012)	High sensitivity, rapid, straightforward, and capable to detect viable but non-culturable microbes	Label/device-dependent and difficult to find proper labels and determine proper labelling conditions	10^3	Complicate	High
Noninvasive optical spectroscopy techniques (D'Haese and Nelis, 2002; Cools et al., 2005; Guicheteau et al., 2008; Jarvis and Goodacre, 2008; Blackie et al., 2009; Vanhee et al., 2009; Cam et al., 2010; Kang et al., 2010; Tebaldi et al., 2010; Kloss et al., 2013; Li AY. et al., 2014; Kloß et al., 2015; Kubryk et al., 2015; Yüksel et al., 2015; Lau et al., 2016; Wang et al., 2016; Gan et al., 2017; Li et al., 2017)	At single-bacterium level and without pre-enrichment	Signal noise and incapable of detecting unknown pathogens	1-10	Simple	High
Single-cell sequencing (Gan et al., 2017; Li et al., 2019; Marcolungo et al., 2022)	Detect at single-cell level rapidly without enrichment	Difficult to obtain single cells	1	Simple	High
Synthetic biosensor (Frechon et al., 1998; Llop et al., 2000; Taylor et al., 2001; Puławska and Sobiczewski, 2005; Mirik and Aysan, 2009; Abd-Elsalam et al., 2011; Kositcharoenkul et al., 2011; Kang et al., 2014; Pardee et al., 2014; Streets et al., 2014; Umesha et al., 2015; Mukherjee and Schroeder, 2016; Keller and Pantel, 2019; Garg et al., 2022; Attaluri and Dharavath, 2023; Cubero et al., 2024; Cao et al., 2025)	Low-cost, practical, and simple	Incapable of detecting unknown pathogens	1-10	Simple	Low

cause a variety of plant diseases with a wide range of pathological phenotypes which are hard to standardize and have high false positive rates. Occasionally certain pathogens cause subtle symptoms in the tolerant crop cultivar but can have devastating effects in a susceptible one. The situation is among others further aggravated by the morphology similarity that may be caused by different pathogens. The diagnosis of pathogens from the plant host can be complicated by the fact that plants may be infected by multiple pathogens simultaneously or individually in nature or in a laboratory setting (Camargo and Smith, 2009). Considering the largely identical morphological symptoms, the identification of different pathogens from the same plant host can be difficult.

Moreover, the morphology-based detection methods rely heavily upon cultivation methods. Although cultivation methods have been improved considerably over the past several decades with advances in the scope and diversity of media components, it still involves a time-consuming process of culture enrichment. Moreover, certain plant pathogens do not readily grow in a laboratory environment. We would thus observe strong bias toward bacteria that are amenable to cultivation. Therefore, morphology-based techniques have very low sensitivity and accuracy and are not suitable for detection of unculturable pathogens that are also not easily viewed by light microscopes such as viruses, viroids, and phytoplasmas.

Immunology-based methods

The sensitivity for cultivation-amenable microorganisms have been dramatically improved with the development of immunological detection relying mainly on the specific binding of an antibody to an antigen. The immunogold staining (IGS) was begin to be applied in 1985 to identify the plant pathogenic bacterium *Erwinia amylovora* (Van Laere et al., 1985). It requires less primary antiserum and shows the advantage that the preparations can be conserved permanently and unchanged. An "indirect" immunohistological technique was developed by employing immunoglobulin adsorbed to colloidal gold as the secondary antiserum. The technique, the immunogold-silver staining (IGSS), is of much enhanced sensitivity (up to 200-fold) as compared with IGS (Holgate et al., 1983). To further improve the sensitivity, an enzyme-linked immunosorbent assay (ELISA) was developed where the detection limit of *Pseudomonas syringae* pv. *phaseolicola*, the agent of halo blight disease of beans, was as high as 2×10^4 cells ml^{-1} (Wyatt et al., 1989; Gan et al., 2013). Indirect ELISA based on polyclonal antibodies was also developed to distinguish pathovars of *Pseudomonas syringae* from pea (Mazarei and Kerr, 1990). The suitability of the antigen-antibody complex depends mainly on the antibodies' specificity. In order to ensure the reliable detection of pathogens, a variety of antibodies have been employed in different assay types. Most polyclonal antibodies, derived from either rabbit or goat serum, contain a collection of antibodies with different cellular origins and, therefore, somewhat different specificities. Monoclonal antibodies are often more useful than polyclonal ones for specific detection of a molecule, since they provide an indefinite supply of a single antibody. Lots of monoclonal antibodies have been commercialized. Specific polyclonal and monoclonal antibodies were developed for the identification of *Xanthomonas campestris* pv. *Campestris* and *Pseudomonas syringae* pv. *pisi*, with an enzyme immunoassay (EIA), immunofluorescence microscopy (IF), or a dot-blot immunoassay (DBI) (Franken et al., 1992).

With the development of monoclonal antibodies, immunological detection of microbial contamination has become more specific, sensitive, reproducible, and reliable (Table 1). When detecting *Pseudomonas syringae*, the immuno-assay method (competitive ELISA) is approximately 100 times more sensitive than the High Performance Liquid Chromatography (HPLC) method and requires no previous extraction (Gallo et al., 2000).

On the other hand, the influence of sample preparation and antibody reactions should be thoroughly examined and understood first, as the detection sensitivity is often altered with the variability of these parameters. For example, comparison of dilution-plating by two different sample extraction methods revealed that, for samples of *Pseudomonas syringae* pv. *Pisi* in pea seed, methods involving longer time of soak in water for pea seeds were more sensitive (Gallo et al., 2000).

With the development of the immunological methods, reliable quantitative assessments detect plant pathogens were allowed to be managed in a high-throughput manner. Immune-dipstick and immune-lateral flow assays were developed and used in laboratories and then for field application. ELISA, immunofluorescence staining test (IFST), seed immunoblot binding assay (SIBA), dyed latex bead agglutination test, lateral-flow immunoassay, fluorescent silica nanoparticles (FSNP), and strip immunoassay have been successfully used to detect plant pathogens such as *Xanthomonas axonopodis* pv. *vesicatoria* (causing bacterial spot disease in tomatoes and peppers) (Yao et al., 2009) and *E. amylovora* (Braun-Kiewnick et al., 2011), with the detection limit equivalent to the typical of pathogen concentrations in symptomatic plant material (Anil et al., 2008).

To facilitate simultaneous detection of multiple pathogens, a multiplex dipstick immunoassay was developed by determining different major *Fusarium* toxins in wheat, oats, and maize (Lattanzio et al., 2012). An antibody for each plant pathogen was linked on a fluorescence-coded magnetic microsphere set which was used to capture corresponding pathogens (Lattanzio et al., 2012). The method was optimized by employing microsphere immunoassays where four important plant pathogens (i.e., fruit blotch bacterium *Acidovorax avenae* subsp. *citrulli*, chilli vein-banding mottle virus, watermelon silver mottle virus, and melon yellow spot virus) were simultaneously detected with substantially higher sensitivity and within much shorter assay time than ELISA (Charlermroj et al., 2013). The system was also shown to be capable of detecting pathogens in naturally infected plant samples. However, antibody-based detection is antigen-dependent and is still lacking the ability to detect plant pathogen in "real-time" for early infection detection and the timeline of disease progression.

Nucleic-acid-based approaches

Nucleic-acid-based approaches are rapid, specific, and highly sensitive and are among the most useful and efficient methods available for phytopathogen detection (Li et al., 2012; Gan et al., 2014a; Li et al., 2014b; Li et al., 2014a; Wang et al., 2015a) (Table 1). Polymerase chain reaction (PCR) has been demonstrated to be rapid and accurate to detect wide array of pathogens [e.g., *E. amylovora* (Bereswill et al., 1992), *Agrobacterium tumefaciens* (Pulawska, 1997), *Pseudomonas marginalis* (Qinhua et al., 2011), *Xanthomonas campestris* pv. *Vesicatoria* (Gan et al., 2014b) and different *Pseudomonas syringae* pathovars (Zaccardelli et al., 2003; Kong et al., 2004; Zaccardelli et al., 2005)], where detection limits ranging from 250 to 500 CFU mL^{-1} were obtained (De Boer, 1995). Since the invention of PCR, numerous of derivatives have been

developed to improve the simplicity, sensitivity, accuracy of pathogen detection. Molecular methods, such as restriction enzyme digestion, have been employed to simplify the pathogen detection where PCR-RFLP (Restriction Fragment Length Polymorphism) has been applied in detection of a numerous of phytopathogens from plant tissues, soil, and water extracts (Hélias et al., 1998; Shenge et al., 2008). Sample preparation, such as genomic DNA extraction, is time-consuming while automated DNA-extraction methods have been developed. Combined with the Real-time quantitative PCR (qPCR) assays, the detection method was able to discriminate *E. amylovora* (isolated from blighted woody plant material) from the other *Erwinia* strains (isolated from Hokkaido or necrotic pear blossoms) with a limit as low as 10^3 cells mL^{-1} (i.e., four cells per reaction) (Pirc et al., 2009; Shao et al., 2009; Gan et al., 2013; Liu et al., 2013; Gan et al., 2014c). qPCR also showed high sensitivity when was used to differentiated *Verticillium wilt* on susceptible and resistant hop cultivars (*Humulus lupulus* L.) (Štajner et al., 2013), *Saccharothrix yanglingensis* Hhs.015 (a major apple Valsa canker pathogen) (Fan et al., 2016), and *Pseudomonas syringae* pv. *lachrymans* (in cucumber seeds) (Meng et al., 2016). TaqMan-PCR (Shao et al., 2011; Wang et al., 2015b) and loop-mediated isothermal amplification (LAMP) (Pang et al., 2009; Feng et al., 2013; Feng et al., 2015) have been developed to improve the sensitivity while a multiplex detection system could detect as low as 0.04 pg genomic DNA of fungal and oomycete pathogens of solanaceous crops (Ning et al., 2008). The sensitivity is able to be further enhanced by 1000-fold by employing nested PCR [is sufficient for single-cell detection in pure culture (Mcmanus and Jones, 1995)] or semi-selective medium prior to PCR [Bio-PCR, with a detection limit of five cells from pure culture (Manulis et al., 1998; Kumagai and Fabritius, 2008; Silva et al., 2015)]. Most recently, droplet digital PCR (ddPCR) began to be used for plant pathogen detection where ddPCR showed a significantly higher degree of sensitivity compared to the qPCR assay and the influence of PCR inhibitors can be reduced considerably in the ddPCR assay (Zhao et al., 2016). However, it is too instrument-dependent (Zhao et al., 2016) and technique-demanding.

PCR-based technology has been developed to detect multiple plant pathogens simultaneously. Primer design is very important, for it's a component of the development of all nucleic acid-based methods. For example, repetitive sequences were used to distinguish different genetic profiles within *P. syringae* pathovars *P. s.* pvs. *syringae*, *morsprunorum*, and *persicae* (Gašić et al., 2012). The pathovar-specific primers based on *rhs* family gene sequences were also used to simultaneously identify the *Xanthomonas* species complex associated with tomato bacterial spot, including *X. vesicatoria*, *X. perforans*, and *X. gardneri* (Park et al., 2009; Araujo et al., 2012). To facilitate routine identification of *Erwinia* species, a PCR method based on species-specific sequences of the housekeeping genes *recA* and *gpd* was developed to differentiate *E. amylovora*, *E. pyrifoliae*, *E. billingiae*, *E. persicina*, *E. rhapontici*, and *E. tasmaniensis*. Moreover, differentiation using species-specific primers could be done via either conventional PCR (cPCR) or qPCR (Gehring and Geider, 2012). Moreover, multiplex qPCR with multiple fluorescent reporter dyes were developed to facilitate the simultaneous detection of different pathogenic species of genus *Pseudomonas* (Haan E.G.d and Bovenkamp G.W.v.d, 2009) and three important rice pathogens, *Xanthomonas oryzae* pv. *oryzae*, *X. oryzae* pv. *oryzicola*, and *Burkholderia glumae* (Luqi et al., 2014). A combination of specific primers and multiplex-PCR could identify *Pseudomonas syringae* pv. *morsprunorum*, *P. s.* pv. *lachrymans*, and *P. s.* pv. *syringae* from different host plants, different cultivars of sweet cherry, and oil pumpkin grown in different locations (Ilicic et al., 2016). However, a significant reduction in sensitivity was observed for pathogens in the presence of plant extract (Mcmanus and Jones, 1995). In recent years, loop-mediated isothermal amplification (LAMP) is developed for pathogen detection and disease diagnosis (Yang et al., 2025).

Nanotechnology-based diagnostics

Immunological and molecular techniques have advanced but have some issues related to rapidity, signal strength and instrumentation (Table 1). Nanoparticles are different from their bulk counterparts when reduced to nanosize (1-100 nm). In nanosize, they possess certain properties suitable for their development as diagnostic probes (Sharon et al., 2010). Nanofabrication techniques had been used in creating artificial plant parts such as stomata and xylem vessel which are then used to detect pathogens [e.g., *Aspergillus niger* (Etefagh et al., 2013), *Xanthomonas campestris* pv. *vesicatoria* (Yao et al., 2009), and *Xylella fastidiosa* (Meng et al., 2005)] and monitor the infection process and behavior of pathogens inside host plants (Meng et al., 2005). A conjugation with a secondary antibody improves the sensitivity when detecting pathogenic bacteria causing bacterial spot on solanaceous plants (Yao et al., 2009). The integration of immunological and molecular diagnostics with nanotechnology systems offers an option where all detection steps can be accommodated on a portable miniaturized device for rapid and accurate detection of plant pathogens. However, the nanotechnology-based diagnostics for plant pathogens is still developing and the cost is relatively high.

Emerging single-cell technology

Fluorescent hybridization was first introduced to detect plant pathogens at the single-cell level. Pathogens [e.g., *Pseudomonas cinnamomi* (Li AY. et al., 2014) and *Gamma proteobacteria* (Li et al., 2017)] can be specifically detected and visualized directly using fluorescent *in situ* hybridization (FISH) via a species-specific fluorescently labelled DNA probe. The advantage of FISH is that the plant or pathogen could retain integrity without damage and there is no need for subculturing. In contrast, it is difficult to localize the hyphae and reproductive structures of pathogens within plant tissues, especially in woody tissues (Li AY. et al., 2014). An additional microscopic method is solid-phase cytometry (SPC) which allows rapid detecting plant pathogen bacteria at the single cell level, without the need for a growth phase. After filtration of the sample, the retained microorganisms are fluorescently labeled on

the membrane filter and automatically counted and identified by an epifluorescence microscope (D'Haese and Nelis, 2002). SPC shows equally accurate for the quantification of bacteria compared culture-based method (Vanhee et al., 2009). It has considerable advantages compared to the culture-based method, including its low detection limit (4 cells/m^3), rapid (within 24 h), and the straightforward microscopic identification (Vanhee et al., 2009) (Table 1). Moreover, SPC in conjunction with fluorescent viability staining is powerful to detect viable but non-culturable microbes (Cools et al., 2005). In parallel, flow cytometric analysis was developed. When combined with fluorescent labels (e.g., propidium iodide), flow cytometry could detect plant pathogens in crude seed extracts without further extraction (Tebaldi et al., 2010). However, the abovementioned methods are very device-dependent as high-resolution electron microscopies are indispensable to accurately determine the labeled single cells (Li et al., 2017). Moreover, the inherent disadvantage of these methods is the difficulty to find proper labels and determine the proper labelling conditions which is time-consuming and technique-demanding. Therefore, recent research has focused on finding alternative label-free approaches for super-sensitive pathogen identification at the single-cell level.

Comprehensive biochemical information can be provided by noninvasive optical spectroscopy techniques, e.g., infrared, hyperspectral imaging and Raman spectroscopy (Kubryk et al., 2015; Wang et al., 2016) (Table 1). With very low background noise of aqueous samples, Raman spectroscopy is especially well-suited for biological applications (Wang et al., 2016). A single-cell Raman spectrum of bacterium represents a sum of Raman spectra of all cell components. It provides comprehensive information about the cell (e.g., nucleic acids, proteins, carbohydrates, and lipids), and could enable the distinction of various strains at the unicellular level with appropriate chemometrical methods based on comprehensive reference databases (Kloss et al., 2013; Kloß et al., 2015; Kubryk et al., 2015). It has been applied to detect bacteria in plants by identifying different spectra that are unique to each bacterium, much like fingerprint analysis (Jarvis and Goodacre, 2008; Gan et al., 2017).

In particular, surface-enhanced Raman scattering spectroscopy (SERS) is able to enhance the signal by 11 orders of magnitude, which is sufficient for single-molecule detection (Blackie et al., 2009). SERS signal enhancement enables the detection of low concentrations of pathogenic bacteria in plant samples. Employing standardized protocol involving optimized parameters, such as mixing procedure of bacterial samples, concentrated colloidal suspension, and statistic analysis [e.g., principal component analysis (Guicheteau et al., 2008; Gan et al., 2017)], reproducible SERS spectra could be generated and numerous of plant pathogens have be identified from bacterial mixtures (Cam et al., 2010; Kang et al., 2010). Moreover, multiplex detection of agriculturally important plant pathogens (i.e., *Botrytis cinerea*, *Pseudomonas syringae*, and *Fusarium oxysporum*) was demonstrated by using SERS (Lau et al., 2016). Furthermore, micro-Raman spectroscopy-based bioassay could detect plant pathogens at single-bacterium level in plant tissue lesions without pre-enrichment (Gan et al., 2017a) which makes in-site

identification of pathogens from real plants possible. Species-specific detection of either a single plant pathogen (Yüksel et al., 2015) or multiple pathogens (Lau et al., 2016) from infected plant materials was achieved via label-free SERS or the multiplex detection approach. In general, compared with conventional methods, with proper sample preparation, the single-cell technologies enable a highly sensitive, pre-enrichment-free, and real-time detection of plant pathogen at a single-cell level (Figure 2).

Next-generation technologies for crop pathogen detection

Although morphology-based diagnostics (e.g., morphology-, antibody-, nucleic-acid-based methods) and emerging single-cell technology (e.g., fluorescent hybridization, solid-phase cytometry, optical spectroscopy techniques) have, in many ways, met the growing need for *in vitro* diagnostic tools for pathogens, the advance of single-cell sequencing and synthetic biology promise a revolution toward the development of new diagnostic tests for a fraction of the cost and time (Gan et al., 2017; Marcolungo et al., 2022). The development and successful application of single-cell sequencing has greatly expanded our knowledge of the diversity and phylogeny of microorganisms. It provides an alternative to culturing organisms as a prerequisite for genomic sequencing and allows discrimination between subspecies when present either individually or in combination. Pathogen detection and typing could be achieved within approximately ten minutes of sequencing owing to the use of an internal control (Marcolungo et al., 2022). However, there are still challenges facing the field, such as an efficient method to obtain single cells. To address the challenge, microfluidic platforms have been developed that enable the isolation, enrichment, and biochemical or genetic analysis of individual cells with high spatiotemporal resolution (Li et al., 2019). The combination of microfluidics and single-cell sequencing has been applied for the whole-transcriptome sequencing of animal embryonic cells (Streets et al., 2014), the diagnosis of cancers and immune system diseases (Keller and Pantel, 2019). However, its application in plant pathogen detection is just emerging.

Synthetic biology was recognized early on as an opportunity to engineer organisms that could serve as whole-cell biosensors (Attaluri and Dharavath, 2023). As synthetic biology has matured, increasing gene circuit complexity has allowed for greater sensitivity and reporter tunability of the synthetic biosensors (Cubero et al., 2024). Bacterial viruses harbor natural specificity to a wide range of bacterial pathogens and can be explored for diagnostic applications of pathogens. The correct bacterial surface epitope or pathogen-derived peptides serves as a conditional input that regulates the output of signals generated by luciferase or other reporter genes of the engineered synthetic phage (Pardee et al., 2014). Low-cost microfluidics coupled with a pathogen capture technology could further improve the sensitivity of the phage-based detection (Kang et al., 2014). Although the application of single-cell sequencing and synthetic biology in plant pathogen detection is just emerging, new frontiers in the rational design principles of these technologies have been ushered with a

direct impact on applied and foundational studies relating to pathogen biology and diagnostics (Mukherjee and Schroeder, 2016).

Field trials and commercialization

On-site diagnostic is a challenge for tangible application of plant quarantine. Progress is being made to address the challenge through transforming these diagnostic technologies into "outside-the-lab" application. For example, to identify pathogens in plant tissues (Llop et al., 2000) and soil (Puławska and Sobiczewski, 2005), a single-tube nested PCR was developed. It allowed reliable detection of pathogens with a number of as few as 100, and was unaffected by the presence of plant-tissue or soil-derived PCR inhibitors (Kositcharoenkul et al., 2011). More and more pathogens could be detected from real plant samples (Mirik and Aysan, 2009), even at the early stage of infection (Taylor et al., 2001) for different pathogens with similar symptoms (Umesha et al., 2015). PCR-based kits, such as Probelia™, for the detection of plant pathogens (e.g., *Erwinia carotovora* subsp. *atroseptica* in potatoes) have been evaluated at five laboratories in four countries. The kit was based on DNA-specific PCR amplification followed by detection of amplicons by hybridization to a peroxidase-labelled DNA probe in a microplate (Frechon et al., 1998). To increase the reproducibility, optimized protocol for DNA extraction from plants, specific primers, and procedure were used for pathogen detection in contaminated plants (Abd-Elsalam et al., 2011). Immunological assays have also been commercialized for the detection of a wide variety of pathogens due to that these methods are reliable and do not require highly trained personnel. The LAMP assay successfully detect American plum line pattern virus with crude flowering cherry extract (Garg et al., 2022). Nowadays, researchers achieved precise dual detection: One-tube reverse transcription-recombinase aided amplification (RT-RAA) combined with lateral flow strip (LFS) assay for RNA and DNA target genes from pepper mild mottle virus and Colletotrichum species in crude plant samples (Cao et al., 2025).

Synthetic phage can reproduce rapidly in fermenters (Schofield et al., 2012). Therefore, phage-based pathogen are now commercially approved products for microbial detection in industrial settings, and clinical applications have been demonstrated as well (Lu et al., 2013). An emerging trend for in-site diagnostic lies in *in vitro* synthetic biology where cellular context is completely removed and synthetic gene circuits are put on paper (Figure 3) (Pardee et al., 2014). The synthetic diagnostic systems are freeze-dried onto porous substrates to create poised genetic regulatory networks that are stable for long-term storage at room temperature and are activated by rehydration. The paper-based reactions could be merged with custom, low-cost electronics for quantification and automation of diagnostic reactions in the field. At the macroscopic scale, pathogen detection methods and plant disease forecasting in crop systems have been improved by using nanosensors which can be linked to a Global Positioning System for real-time monitoring of disease, soil conditions, and crop health (Abd-Elsalam, 2013). Despite these proof-of-concept

FIGURE 2
Comparison of conventional methods and the single-cell technologies for plant pathogen detection.
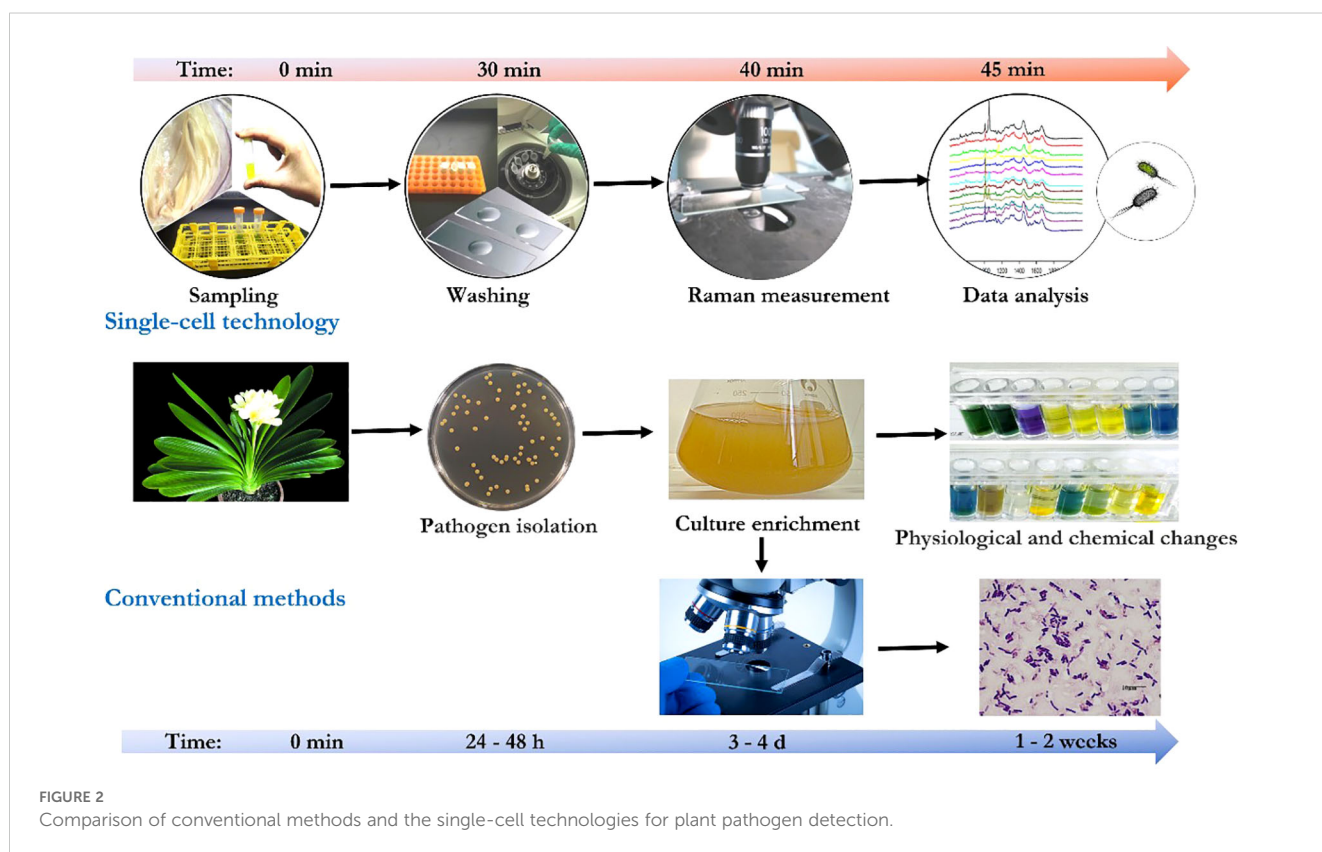
demonstrations, several challenges remain for the practical implementation of these diagnostics, including meeting the detection thresholds required for field use.

Concluding remarks and future perspectives

Detection of crop pathogens at the single-cell level is the latest goal of diagnostic technology at the present. Conventional diagnostics, such antibody- based techniques and PCR-based methods, are important and sensitive application methods. However, proper implementation of these techniques poses challenges, ranging from time-consuming bacteria enrichment to the significant cost of the technology. The most important is that these methods could not detect plant pathogenic bacteria at a single cell level.

We are witnessing a leapfrog development of pathogen detection from culture-based strategies to *in-situ* diagnostic when investigators begin to design FISH, SPC, and label-based optical spectroscopy experiments. These methods allow the detection of viable but non-culturable microbes in the field. However, the sensitivity and resolution needed for the experiments should be considered. The advent of microfluidic technologies, coupled with rapid advances in fluorescence-based molecular imaging, had spurred a revolution in biological analysis at the level of single cells (Khan, 2014). But these methods are label-dependent and technique-demanding.

The development of a micro-Raman spectroscopy-based bioassay enabled fast, label-free and non-invasive discrimination of plant pathogens, and accurate culture-free single-bacterium detection in plant tissue lesions with an identification ratio comparable to those of genetic molecular approaches (Gan et al., 2017). It signifies a trend towards the development of methods for *in situ*, real-time, and single-bacterium detection which offers exciting new opportunities in entry-exit quarantine, customs inspection, food-processing, medical diagnosis, and biological weapons inspections, crucial to national security. However, Raman spectroscopy requires capital funding for equipment. A systemic, standardized, and comprehensive spectroscopic database of cultured bacteria is always a precondition for measurements. The cultivation should include the respective environmental conditions and enough biological replicates to establish a classification model assessing the possibility to distinguish between taxa. Next generation sequencing also requires having a reference dataset of vouchered specimens for use in general diagnostics of unknown.
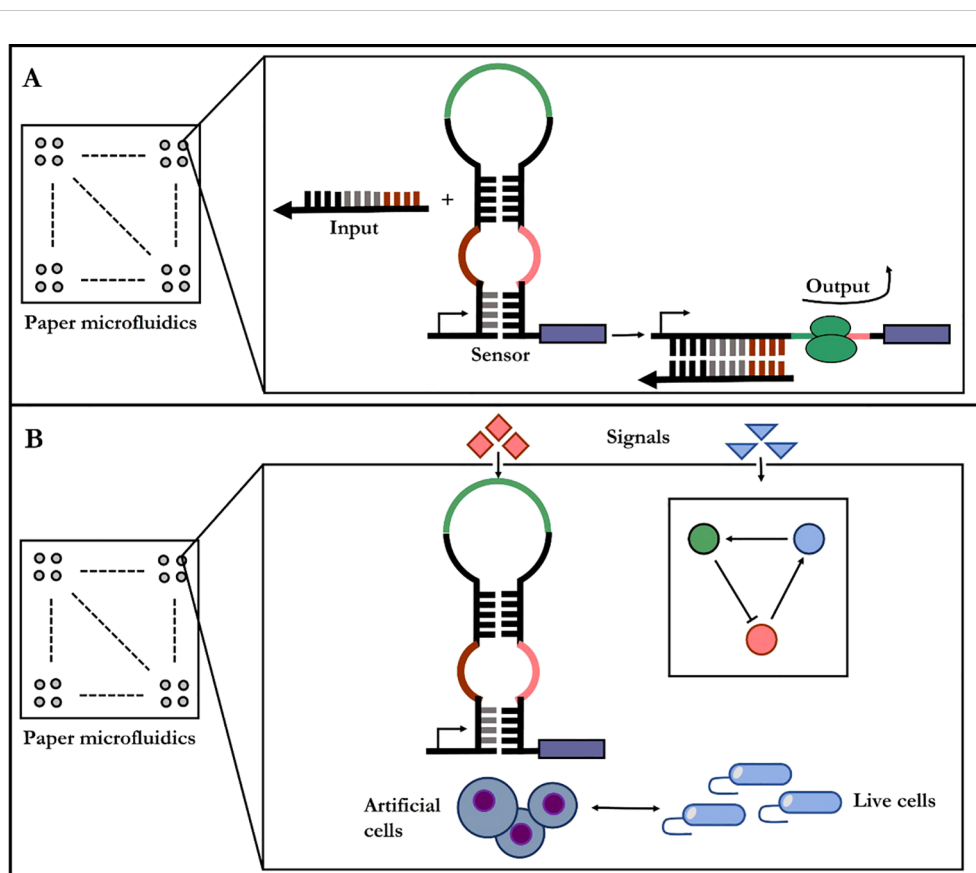

FIGURE 3

Paper-based diagnostic synthetic-biology designs. **(A)** The gene circuit becomes active when rehydrated with the test sample, containing target RNAs or small molecule. **(B)** Diagnostic gene networks and cell-free transcription/translation system are assembled into paper or other porous materials.

Therefore, the capacity of Raman spectroscopy to detect unknown plant pathogens remains challenge.

As single-cell sequencing joins ranks with other diagnostics, its combination with microfluidic enables *in situ*, real-time, and single-cell detection of unknown microbes. On the other hand, synthetic biology provides low-cost, rapidly deployable diagnostics. The engineering approaches have the potential to significantly advance the translation of promising technologies into pragmatic tools suitable for real-world applications. The paper-based synthetic biosensor and portable hybrid devices incorporating synthetic sensors or single-cell sequencing fit the need for low-cost, practical, and simple diagnostic tools for use outside of the laboratory. It is also intriguing to imagine *in vitro* synthetic biology embedded into diagnostic wearables for crops, allowing for both in-site and real-time sensing. Success will continue to be found at the interfaces between disciplines.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Abd-Elsalam, K. A. (2013). Nano platforms for plant pathogenic fungi management. *Fungal Enomics & Biology* 2, e107. doi: 10.4172/2165-8056.1000e107

Abd-Elsalam, K., Bahkali, A., Moslem, M., Amin, O. E., and Niessen, L. (2011). An optimized protocol for DNA extraction from wheat seeds and Loop-Mediated Isothermal Amplification (LAMP) to detect Fusarium graminearum contamination of wheat grain. *Int. J. Mol. Sci.* 12, 3459–3472. doi: 10.3390/ijms12063459

Anil, K., Singh, U. S., Kumar, J., and Garg, G. K. (2008). Application of molecular and immuno-diagnostic tools for detection, surveillance and quarantine regulation of Karnal bunt (Tilletia indica) of wheat. *Food Agric. Immunol.* 19, 293–311. doi: 10.1080/09540100802478194

Araujo, E. R., Costa, J. R., Ferreira, M. A. S. V., and Quezadoduval, A. M. (2012). Simultaneous detection and identification of the Xanthomonas species complex associated with tomato bacterial spot using species-specific primers and multiplex PCR. *J. Appl. Microbiol.* 113, 1479–1490. doi: 10.1111/j.1365-2672.2012.05431.x

Attaluri, S., and Dharavath, R. (2023). Novel plant disease detection techniques-a brief review. *Mol. Biol. Rep.* 50, 9677–9690. doi: 10.1007/s11033-023-08838-y

Bereswill, S., Pahl, A., Bellemann, P., Zeller, W., and Geider, K. (1992). Sensitive and species-specific detection of Erwinia amylovora by polymerase chain reaction analysis. *Appl. Environ. Microbiol.* 58, 3522–3526. doi: 10.1128/aem.58.11.3522-3526.1992

Bhattacharya, S. (2017). Deadly new wheat disease threatens Europe's crops. *Nature* 542, 145–146. doi: 10.1038/nature.2017.21424

Blackie, E. J., Ru, E. C. L., and Etchegoin, P. G. (2009). Single-molecule surface-enhanced raman spectroscopy of nonresonant molecules. *J. Am. Chem. Soc.* 131, 14466–14472. doi: 10.1021/ja905319w

Braun-Kiewnick, A., Altenbach, D., Oberhänsli, T., Bitterlin, W., and Duffy, B. (2011). A rapid lateral-flow immunoassay for phytosanitary detection of Erwinia amylovora and on-site fire blight diagnosis. *J. Microbiological Methods* 87, 1–9. doi: 10.1016/j.mimet.2011.06.015

Cam, D., Keseroglu, K., Kahraman, M., Sahin, F., and Culha, M. (2010). Multiplex identification of bacteria in bacterial mixtures with surface-enhanced Raman scattering. *J. Raman Spectrosc.* 41, 484–489. doi: 10.1002/jrs.v41:5

Camargo, A., and Smith, J. S. (2009). An image-processing based algorithm to automatically identify plant disease visual symptoms. *Biosyst. Eng.* 102, 9–21. doi: 10.1016/j.biosystemseng.2008.09.030

Cao, Y., Yan, D., Zhou, H., Han, K., Wan, Q., Peng, J., et al. (2025). Achieving precise dual detection: One-tube reverse transcription-recombinase aided amplification (RT-RAA) combined with lateral flow strip (LFS) assay for RNA and DNA target genes from pepper mild mottle virus and Colletotrichum species in crude plant samples. *Talanta* 281, 126908. doi: 10.1016/j.talanta.2024.126908

Carvajal-Yepes, M., Cardwell, K., Nelson, A., Garrett, K. A., Giovani, B., Saunders, D. G. O., et al. (2019). A global surveillance system for crop diseases. *Science* 364, 1237–1239. doi: 10.1126/science.aaw1572

Cerda, R., Avelino, J., Gary, C., Tixier, P., Lechevallier, E., and Allinne, C. (2017). Primary and secondary yield losses caused by pests and diseases: assessment and modeling in coffee. *PloS One* 12, e0169133. doi: 10.1371/journal.pone.0169133

Charlermroj, R., Himananto, O., Seepiban, C., Kumpoosiri, M., Warin, N., Oplatowska, M., et al. (2013). Multiplex detection of plant pathogens using a microsphere immunoassay technology. *PloS One* 8, e62344. doi: 10.1371/journal.pone.0062344

Cools, I., D'Haese, E., Uyttendaele, M., Storms, E., Nelis, H. J., and Debevere, J. (2005). Solid phase cytometry as a tool to detect viable but non-culturable cells of *Campylobacter jejuni*. *J. Microbiological Methods* 63, 107–114. doi: 10.1016/j.mimet.2005.02.023

Cubero, J., Zarco-Tejada, P. J., Cuesta-Morrondo, S., Palacio-Bielsa, A., Navas-Cortés, J. A., Sabuquillo, P., et al. (2024). New approaches to plant pathogen detection and disease diagnosis. *Phytopathology®* 114, 1989–2006. doi: 10.1094/PHYTO-10-23-0366-IA

D'Haese, E., and Nelis, H. J. (2002). Rapid detection of single cell bacteria as a novel approach in food microbiology. *J. Aoac Int.* 85, 979–983. doi: 10.1093/jaoac/85.4.979

De Boer, S. (1995). PCR Detection of Erwinia carotovora subsp. atroseptica Associated with Potato Tissue. *Phytopathology* 85, 854–858. doi: 10.1094/Phyto-85-854

Elad, Y., and Pertot, I. (2014). Climate change impacts on plant pathogens and plant diseases. *J. Crop Improvement* 28, 99–139. doi: 10.1080/15427528.2014.865412

Etefagh, R., Azhir, E., and Shahtahmasebi, N. (2013). Synthesis of CuO nanoparticles and fabrication of nanostructural layer biosensors for detecting Aspergillus Niger fungi. *Scientia Iranica* 20, 1055–1058. doi: 10.1016/j.scient.2013.05.015

Fan, D., Li, Y., Zhao, L., Li, Z., Huang, L., and Yan, X. (2016). Study on Interactions between the Major Apple Valsa Canker PathogenValsa Maliand Its Biocontrol AgentSaccharothrix yanglingensisHhs.015 Using RT-qPCR. *PloS One* 11, e0162174. doi: 10.1371/journal.pone.0162174

Feng, L., Li, H., Ni, X., Wang, J., Ji, Y., and Gan, Q. (2015). Loop-mediated isothermal amplification (LAMP) for rapid detection of *Xanthomonas axonopodis* pv. *manihotis*. *Plant Prot. (in Chinese)* 41, 93–99.

Feng, L., Ni, X., Li, Y., Wu, X., and Gan, Q. (2013). Detection of *Pseudomonas syringae* pv.*pisi* by LAMP (Loop-mediated isothermal amplification). *Plant Quarantine (in Chinese)* 27, 80–84.

Food and Agriculture Organization of the United Nations. (2024). *Value of agricultural production* (Statistics Database). Cham, Switzerland. Available online at: http://www.fao.org/faostat/en.

Franken, A. A. J. M., Zilverentant, J. F., Boonekamp, P. M., and Schots, A. (1992). Specificity of polyclonal and monoclonal antibodies for the identification of Xanthomonas campestris pv. campestris. *Netherlands J. Plant Pathol.* 98, 81–94. doi: 10.1007/BF01996321

Frechon, D., Exbrayat, P., Helias, V., Hyman, L., Jouan, B., Llop, P., et al. (1998). Evaluation of a PCR kit for the detection ofErwinia carotovora subsp.atroseptica on potato tubers. *Potato Res.* 41, 163–173. doi: 10.1007/BF02358439

Gallo, M., Fogliano, V., Ritieni, A., Peluso, L., Greco, M., Lops, R., et al. (2000). Immuno-assessment of Pseudomonas syringae lipodepsipeptides (syringomycins and syringopeptins). *Phytopathol. Mediterr.* 39, 410–416. doi: 10.1400/57873

Gan, Q., Chen, C., Wang, Y., Ji, Y., Wu, X., and Shao, X. (2013). Detection and identification of Tomato black ring nepovirus from imported cucumber seeds. *Food Res. Dev. (in Chinese)* 34, 88–90.

Gan, Q., Wang, Y., Wei Shao, X.. X., Liu, C., and Li, Y. (2014a). Detection and sequence analysis of Hop stunt viroid in grapevine rootstock from France. *Acta Phytophylacica Sin. (in Chinese)* 41, 346–351.

Gan, Q., Lu, Y., Shao, X., Sun, T., Li, Y., and Liu, C. (2014b). Rapid Detection and Typing of Xanthomonas campestris pv. vesicatoria by Pyrosequencing. *J. Phytopathol.* 162, 190–194. doi: 10.1111/jph.2014.162.issue-3

Gan, Q., Wang, X.., Wang, Y., Xie, Z., Tian, Y., and Lu, Y. (2017). Culture-free detection of crop pathogens at the single-cell level by micro-raman spectroscopy. *Advanced Sci.* 4, 1700127. doi: 10.1002/advs.201700127

Gan, Q., Wu, X., and Feng, L. (2014c). Shandong Bureau intercepts and capture *Hop dwarf* virus like virus from grape rootstock imported from France. *Plant Quarantine (in Chinese)* 28, 92.

Garg, N., Ahmad, F. J., and Kar, S. (2022). Recent advances in loop-mediated isothermal amplification (LAMP) for rapid and efficient detection of pathogens. *Curr. Res. Microbial Sci.* 3, 100120. doi: 10.1016/j.crmicr.2022.100120

Gašić, K., Prokić, A., Ivanović, M., Kuzmanović, N., and Obradović, A. (2012). Differentiation of Pseudomonas syringae pathovars originating from stone fruits. *Pesticidi I Fitomedicina* 27, 219–229. doi: 10.2298/PIF1203219G

Gehring, I., and Geider, K. (2012). Identification of Erwinia species isolated from apples and pears by differential PCR. *J. Microbiological Methods* 89, 57–62. doi: 10.1016/j.mimet.2012.01.018

Guicheteau, J., Argue, L., Emge, D., Hyre, A., Jacobson, M., and Christesen, S. (2008). Bacillus spore classification via surface-enhanced Raman spectroscopy and principal component analysis. *Appl. Spectrosc.* 62, 267–272. doi: 10.1366/000370208783759623

Haan E.G.d and Bovenkamp G.W.v.d (2009). Test development in Erwinia at the NAK: BioPlex real-time PCR. *Gewasbescherming* 40, 172–175.

Hélias, V., Le Roux, A., Bertheau, Y., Andrivon, D., Gauthier, J., and Jouan, B. (1998). Characterisation of Erwinia carotovora subspecies and detection of Erwinia carotovora subsp. atroseptica in potato plants, soil and water extracts with PCR-based methods. *Eur. J. Plant Pathol.* 104, 685–699. doi: 10.1023/A:1008606801839

Hijmans, R., Garcia, N., and Wieczorek, J. (2018). *Global administrative areas database (GADM) version 3.6* (New York, NY, USA: UN).

Holgate, C. S., Jackson, P., Cowen, P. N., and Bird, C. C. (1983). Immunogold-silver staining: new method of immunostaining with enhanced sensitivity. *J. Histochem. Cytochem.* 31, 938–944. doi: 10.1177/31.7.6189883

Ilicic, R., Balaž, J., Stojšin, V., Bagi, F., Pivic, R., Stanojković-Sebić, A., et al. (2016). Molecular characterization of Pseudomonas syringae pvs. from different host plants by repetitive sequence-based PCR and multiplex-PCR@Įvairių augalų-šeimininkų Pseudomonas syringae pvs. molekulinis apibūdinimas taikant pasikartojančių sekų ir jungtinę PGR. *Zemdirbyste-agriculture* 103, 199–206. doi: 10.13080/z-a.2016.103.026

Jarvis, R. M., and Goodacre, R. (2008). Characterisation and identification of bacteria using SERS. *Chem. Soc. Rev.* 37, 931–936. doi: 10.1039/b705973f

Kang, J. H., Super, M., Yung, C., Cooper, R., Domansky, K., Graveline, A., et al. (2014). An extracorporeal blood-cleansing device for sepsis therapy. *Nat. Medcine* 20, 1211–1216. doi: 10.1038/nm.3640

Kang, Y. P., Si, M. Z., Li, Q. Y., Huang, Q., and Liu, R. M. (2010). SERS spectra of Xanthomonas oryzae pv. Oryzae (Xoo) on nano silver film prepared by electrolysis method. *Spectrosc. Spectral Anal.* 30, 372–375. doi: 10.3964/j.issn.1000-0593(2010)02-0372-04

Keller, L., and Pantel, K. (2019). Unravelling tumour heterogeneity by single-cell profiling of circulating tumour cells. *Nat. Rev. Cancer* 19, 553–567. doi: 10.1038/s41568-019-0180-2

Khan, A. S. (2014). Rapid advances in nucleic acid technologies for detection and diagnostics of pathogens. *J. Microbiol. Experiment.* 1, 1–7. doi: 10.15406/jmen.2014.01.00009

Kloß, S., Rösch, P., Pfister, W., Kiehntopf, M., and Popp, J. (2015). Toward culture-free Raman spectroscopic identification of pathogens in ascitic fluid. *Analytical Chem.* 87, 937–943. doi: 10.1021/ac503373r

Kloss, S., Kampe, B., Sachse, S., Rösch, P., Straube, E., Pfister, W., et al. (2013). Culture independent Raman spectroscopic identification of urinary tract infection pathogens: a proof of principle study. *Analytical Chem.* 85, 9610–9616. doi: 10.1021/ac401806f

Kong, H., Patterson, C. D., Zhang, W., Takikawa, Y., Suzuki, A., and Lydon, J. (2004). A PCR protocol for the identification of Pseudomonas syringae pv. tagetis based on genes required for tagetitoxin production. *Biol. Control* 30, 83–89. doi: 10.1016/j.biocontrol.2003.09.002

Kositcharoenkul, N., Chatchawankanphanich, O., Bhunchoth, A., and Kositratana, W. (2011). Detection of Xanthomonas citri subsp. citri from field samples using single-tube nested PCR. *Plant Pathol.* 60, 436–442. doi: 10.1111/j.1365-3059.2010.02390.x

Kubryk, P., Kölschbach, J., Marozava, S., Lueders, T., Meckenstock, R., Niessner, R., et al. (2015). Exploring the potential of stable isotope (Resonance) raman microspectroscopy and surface-enhanced Raman scattering for the analysis of microorganisms at single cell level. *Analytical Chem.* 87, 6622–6630. doi: 10.1021/acs.analchem.5b00673

Kumagai, L., and Fabritius, A.-L. (2008a). "Detection and Differentiation of Pathogenic Agrobacterium vitis and A. tumefaciens in Grapevine using Multiplex Bio-PCr," in *Proceedings of the 2nd Annual National Viticulture Research Conference*. University of California, Davis, 42–43.

Lattanzio, V. M., Nivarlet, N., Lippolis, V., Gatta, S., Huet, A., Delahaut, P., et al. (2012). Multiplex dipstick immunoassay for semi-quantitative determination of Fusarium mycotoxins in cereals. *Analytica Chimica Acta* 718, 99–108. doi: 10.1016/j.aca.2011.12.060

Lau, H. Y., Wang, Y., Wee, E. J. H., Botella, J. R., and Trau, M. (2016). Field demonstration of a multiplexed point-of-care diagnostic platform for plant pathogens. *Analytical Chem.* 88, 8074–8081. doi: 10.1021/acs.analchem.6b01551

Li, Y., Wei, X., Gan, Q., Ji, Y., Shao, X., and Wang, Y. (2014a). Isolation and identification of soft rot bacteria on imported *Zantedeschia*. *J. Food Saf. Qual. (in Chinese)* 5, 3944–3946.

Li, A. Y., Crone, M., Adams, P. J., Fenwick, S. G., Hardy, G. E. S. J., and Williams, N. (2014). The microscopic examination of phytophthora cinnamomi in plant tissues using fluorescent *in situ* hybridization. *J. Phytopathol.* 162, 747–757. doi: 10.1111/jph.2014.162.issue-11-12

Li, J., Zhang, H., Menguy, N., Benzerara, K., Wang, F., Lin, X., et al. (2017). Single-cell resolution of uncultured magnetotactic bacteria via fluorescence-coupled electron microscopy. *Appl. Environ. Microbiol.* 83, e00409–e00417. doi: 10.1128/AEM.00409-17

Li, H., Torab, P., Mach, K. E., Surrette, C., England, M. R., Craft, D. W., et al. (2019). Adaptable microfluidic system for single-cell pathogen classification and antimicrobial susceptibility testing. *Proc. Natl. Acad. Sci.* 116, 10270–10279. doi: 10.1073/pnas.1819569116

Li, Y., Gan, Q., Feng, L., and Shao, X. (2014b). Isolation, identification, and biological characterization of *Burkholderia gladioli* in *Cymbidium* imported from Korea. *Acta Phytopathologica Sin. (in Chinese)* 44, 313–317.

Li, Y., Wang, Y., Ni, X., Gan, Q., and Feng, L. (2012). Isolation and identification of *Erwinia cypripedii* from imported Korea *Cymbidium*. *J. Anhui Agric. Sci. (in Chinese)* 31, 15243–15244.

Lin, C., Liang, C. Z., Xu, B., Sun, M., Liu, C. X., Gao, H. W., et al. (2009). Establishment of Loop-mediated Isothermal Amplification (LAMP) method for *Campylobacter jejuni* detection. *Microbiol. China (in Chinese)* 36, 923–928.

Liu, C., et al. (2013). Qualitative test of radish ingredients in food by Real-time PCR. *Food Res. Dev.* 34, 58–61.

Llop, P., Bonaterra, A., Penalver, J., and Lopez, M. M. (2000). Development of a highly sensitive nested-PCR procedure using a single closed tube for detection of

Erwinia amylovora in asymptomatic plant material. *Appl. Environ. Microbiol.* 66, 2071–2078. doi: 10.1128/AEM.66.5.2071-2078.2000

Lu, T. K., Bowers, J., and Koeris, M. S. (2013). Advancing bacteriophage-based microbial diagnostics with synthetic biology. *Trends Biotechnol.* 31, 325–327. doi: 10.1016/j.tibtech.2013.03.009

Lu, W., Pan, L., Zhao, H., Jia, Y., Wang, Y., Yu, X., et al. (2014). Molecular detection of Xanthomonas oryzae pv.oryzae, Xanthomonas oryzae pv. oryzicola, and Burkholderia glumae in infected rice seeds and leaves. *Crop J.* 2, 398–406. doi: 10.1016/j.cj.2014.06.005

Manulis, S., Kogan, N., Valinsky, L., Dror, O., and Kleitman, F. (1998). Detection of Erwinia herbicola pv. gypsophilae in gypsophila plants by PCR. *Eur. J. Plant Pathol.* 104, 85–91. doi: 10.1023/A:1008659001313

Marcolungo, L., Passera, A., Maestri, S., Segala, E., Alfano, M., Gaffuri, F., et al. (2022). Real-time on-site diagnosis of quarantine pathogens in plant tissues by nanopore-based sequencing. *Pathogens* 11, 199. doi: 10.3390/pathogens11020199

Mazarei, M., and Kerr, A. (1990). Distinguishing pathovars of *Pseudomonas syringae* on peas: nutritional, pathogenicity and serological tests. *Plant Pathol.* 39, 278–285. doi: 10.1111/j.1365-3059.1990.tb02504.x

Mcmanus, P. S., and Jones, A. L. (1995). Detection of Erwinia amylovora by nested PCR and PCR-dot-blot and reverse-blot hybridizations. *Phytopathology* 85, 618–623. doi: 10.1094/Phyto-85-618

Meng, Y., Li, Y., Galvani, C. D., Hao, G., Turner, J. N., Burr, T. J., et al. (2005). Upstream Migration of Xylella fastidiosa via Pilus-Driven Twitching Motility. *J. Bacteriology* 187, 5560–5567. doi: 10.1128/JB.187.16.5560-5567.2005

Meng, X., Chai, A. L., Chen, L., Shi, Y., Xie, X., Ma, Z., et al. (2016). Rapid detection and quantification of viable Pseudomonas syringae pv. lachrymans cells in contaminated cucumber seeds using propidium monoazide and a real-time PCR assay. *Can. J. Plant Pathol.* 38, 296–306. doi: 10.1080/07060661.2016.1216897

Mirik, M., and Aysan, Y. (2009). Detection of Xanthomonas axonopodis pv. vesicatoria in naturally infected pepper seeds in Turkey. *J. Plant Pathol.* 91, 433–436. doi: 10.4454/JPP.V91I2.974

Mukherjee, A., and Schroeder, C. M. (2016). *Microfluidic methods in single cell biology.* Ed. C. Lu, et al (Cham, Switzerland: Springer International Publishing).

Ning, Z., Meaghanl, M. C., and Christined, S. (2008). A macroarray system for the detection of fungal and oomycete pathogens of solanaceous crops. *Plant Dis.* 92, 953–960. doi: 10.1094/PDIS-92-6-0953

Pardee, K., Green, A. A., Ferrante, T., Cameron, D. E., DaleyKeyser, A., Yin, P., et al. (2014). Paper-based synthetic gene networks. *Cell* 159, 940–954. doi: 10.1016/j.cell.2014.10.004

Park, D., Shim, J. K., Kim, J. S., Lim, C. K., Shrestha, R., Hahn, J. H., et al. (2009). Sensitive and specific detection of Xanthomonas campestris pv. vesicatoria by PCR using pathovar-specific primers based on rhs family gene sequences. *Microbiological Res.* 164, 36–42. doi: 10.1016/j.micres.2006.11.005

Pirc, M., Ravnikar, M., Tomlinson, J., and Dreo, T. (2009). Improved fireblight diagnostics using quantitative real-time PCR detection of Erwinia amylovora chromosomal DNA. *Plant Pathol.* 58, 872–881. doi: 10.1111/j.1365-3059.2009.02083.x

Population Division of the Department of Economic and Social Affairs of the United Nations Secretaria (2022). *World population projected to reach 9.7 billion by 2050* (Statistics Database). Cham. Switzerland. Available online at: https://www.un.org/en/desa.

Pulawska, J. (1997). "Detection of agrobacterium tumefaciens in soil using PCR," in *Developments in plant pathology*, vol. 11 . Ed. H. W. Dehne, G. Adam, M. Diekmann, J. Frahm, A. Mauler-Machnik and P. Halteren (Springer, Dordrecht).

Puławska, J., and Sobiczewski, P. (2005). Development of a semi-nested PCR based method for sensitive detection of tumorigenicAgrobacteriumin soil. *J. Appl. Microbiol.* 98, 710–721.

Qinhua, G., Yan, L., Xiuling, S., and Yingchao, W. (2011). Isolation and identification of Pseudomonas marginalis and its biological characterization. *J. Plant Prot.* 38, 183–184.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., and Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nat. Ecol. Evol.* 3, 430–439. doi: 10.1038/s41559-018-0793-y

Schofield, D. A., Sharp, N. J., and Westwater, C. (2012). Phage-based platforms for the clinical detection of human bacterial pathogens. *Bacteriophage* 2, 105–283. doi: 10.4161/bact.19274

Shao, X., Gan, Q., Zhao, W., Li, Y., Wang, Y., and Pang, G. (2009). Detection and identification of *Xanthomonas hyacinthi* by real-time fluorescent PCR. *Plant Quarantine (in Chinese)* 23, 25–27.

Shao, X., Gan, Q., Li, Y., Zhao, W., Wu, X., and Su, Z. (2011). Detection of *Burkholderia caryophylli* by TaqMan real-time fluorescent PCR. *Acta Phytopathologica Sin. (in Chinese)* 41, 24–30.

Sharon, M., Choudhary, A. K., and Kumar, R. (2010). Nanotechnology in agricultural diseases and food safety. *J. Phytology* 2, 83–92.

Shenge, K., Stephan, D., Mabagala, R. B., Mortensen, C. N., and Wydra, K. (2008). Molecular characterization ofPseudomonas syringae pv.tomato isolates from Tanzania. *Phytoparasitica* 36, 338–351. doi: 10.1007/BF02980813

Silva, C. F., Torres, P., Oliveira, N. P. D., Marques, A. S. A., and Ferreira, M. A. S. V. (2015). PCR-based methods for detection of Erwinia psidii on guava. *Trop. Plant Pathol.* 40, 251–259. doi: 10.1007/s40858-015-0020-1

Štajner, N., Cregeen, S., and Javornik, B. (2013). Evaluation of reference genes for RT-qPCR expression studies in hop (Humulus lupulus L.) during infection with vascular pathogen verticillium albo-atrum. *PloS One* 8, e68228. doi: 10.1371/journal.pone.0068228

Streets, A. M., Zhang, X., Cao, C., Pang, Y., Wu, X., Xiong, L., et al. (2014). Microfluidic single-cell whole-transcriptome sequencing. *Proc. Natl. Acad. Sci. United States America* 111, 7048–7053. doi: 10.1073/pnas.1402030111

Taylor, R. K., Guilford, P. J., Clark, R. G., Hale, C. N., and Forster, R. L. S. (2001). Detection of *Erwinia amylovora* in plant material using novel polymerase chain reaction (PCR) primers. *New Z. J. Crop Hortic. Sci.* 29, 35–43. doi: 10.1080/01140671.2001.9514158

Tebaldi, N. D., Peters, J., Chitarra, L., Souza, R., Zouwen, and Bergervoet, J. H. W. (2010). Detection of Xanthomonas axonopodis pv. phaseoli in bean seeds by flow cytometry, immunostaining and direct viable counting. *Trop. Plant Pathol.* 35, 213–222. doi: 10.1590/S1982-56762010000400002

Umesha, S., Jyothi, N., and Roohie, R. K. (2015). Detection of bacterial and fusarium wilt pathogens in cabbage by multiplex PCR. *J. Plant Sci.* 3, 185. doi: 10.11648/J.JPS.20150304.13

Vanhee, L. M. E., Nelis, H. J., and Coenye, T. (2009). Rapid detection and quantification of *Aspergillus fumigatus* in environmental air samples using Solid-phase cytometry. *Environ. Sci. Technol.* 43, 3233–3239. doi: 10.1021/es803435a

Van Laere, O., De Wael, L., and De Mey, J. (1985). Immuno gold staining (IGS) and immuno gold silver staining (IGSS) for the identification of the plant pathogenic bacterium *Erwinia amylovora* (Burrill) Winslow et al. *Histochemistry* 83, 397–399. doi: 10.1007/BF00509198

Wang, Y., Ji, Y., Li, Y., Wang, J., Jiang, Q., Gan, Q., et al (2015a). Detection for *Alternaria porri* using PCR based on Brn1 gene. *Plant Prot. (in Chinese)* 29, 30–32.

Wang, Y., Gan, Q., Li, Y., Ji, Y., Wu, X., and Shao, X.. (2015b). Detection of *Embellisia allii* using real-time quantitative PCR based on glyceraldehyde-3-phosphate dehydrogenase gene. *Scientia Agricultura Sin. (in Chinese)* 48, 390–397.

Wang, Y., Huang, W. E., Cui, L., and Wagner, M. (2016). Single cell stable isotope probing in microbiology using Raman microspectroscopy. *Curr. Opin. Biotechnol.* 41, 34–42. doi: 10.1016/j.copbio.2016.04.018

Wyatt, G. M., Turner, J. G., and Morgan, M. R. A. (1989). Rapid and specific detection of Pseudomonas syringae pv. phaseolicola by immunological methods. *Food Agric. Immunol.* 1, 53–63. doi: 10.1080/09540108909354674

Yang, L., Sun, Y., Sun, L., Wang Feng, Z.. J., and Liang, Y. (2025). Application of loop-mediated isothermal amplification in plant pathogen detection. *Phytopathology*® 115, 6–13. doi: 10.1094/PHYTO-10-23-0391-KC

Yao, K. S., Li, S. J., Tzeng, K. C., Cheng, T. C., Chang, C. Y., Chiu, C. Y., et al. (2009). Fluorescence silica nanoprobe as a biomarker for rapid detection of plant pathogens. *Advanced Materials Res.* 79-82, 513–516. doi: 10.4028/www.scientific.net/AMR.79-82

Yüksel, S., Schwenkbier, L., Pollok, S., Weber, K., Cialla-May, D., Popp, J., et al. (2015). Label-free detection of Phytophthora ramorum using surface-enhanced Raman spectroscopy. *Analyst* 140, 7254–7262. doi: 10.1039/C5AN01156F.

Zaccardelli, M., Spasiano, A., Bazzi, C., and Merighi, M. (2005). Identification and in planta detection of Pseudomonas syringae pv. tomato using PCR amplification of hrpZ Pst. *Eur. J. Plant Pathol.* 111, 85–90. doi: 10.1007/s10658-004-2734-7.

Zaccardelli, M., Spasiano, A., Merighi, M., and Bazzi, C. (2003). *Pseudomonas syringae and related pathogens.* Ed. N. S. Iacobellis, A. Collmer, S. Hutcheson, Mansfield,, C. Morris, J. Murillo, et al (Dordrecht: Springer Netherlands), 553–558.

Zhao, Y., Xia, Q., Yin, Y., and Wang, Z. (2016). Comparison of Droplet Digital PCR and Quantitative PCR Assays for Quantitative Detection of Xanthomonas citri Subsp. citri. *PloS One* 11, e0159004. doi: 10.1371/journal.pone.0159004