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Advances, limitations, and prospects of biosensing technology for detecting phytopathogenic bacteria

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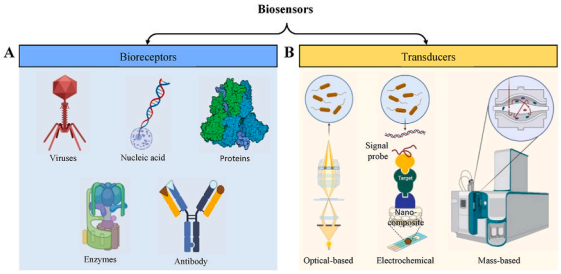
HIGHLIGHTS

- Early detection of a disease is essential to prevent crop loss.
- The conventional techniques are sensitive and specific for pathogenic identification.
- Biosensor-based techniques are innovative and promising alternatives.
- Biosensor-based systems are attractive and efficient for early detection of pathogen.

GRAPHICAL ABSTRACT



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ABSTRACT

Phytopathogenic bacteria cause severe economic losses in agricultural production worldwide. The spread rates, severity, and emerging plant bacterial diseases have become serious threat to the sustainability of food sources

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and the fruit industry. Detection and diagnosis of plant diseases are imperative in order to manage plant diseases in field conditions, greenhouses, and food storage conditions as well as to maximize agricultural productivity and sustainability. To date, various techniques including, serological, observation-based, and molecular methods have been employed for plant disease detection. These methods are sensitive and specific for genetic identification of bacteria. However, these methods are specific for genetic identification of bacteria. Currently, the innovative biosensor-based disease detection technique is an attractive and promising alternative. A biosensor system involves biological recognition and transducer active receptors based on sensors used in plant-bacteria diagnosis. This system has been broadly used for the rapid diagnosis of plant bacterial pathogens. In the present review, we have discussed the conventional methods of bacterial-disease detection, however, the present review mainly focuses on the applications of different biosensor-based techniques along with point-of-care (POC), robotics, and cell phone-based systems. In addition, we have also discussed the challenges and limitations of these techniques.

Abbreviations

POC	Point-of-care
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
HLB	Huanglongbing
PCR	Polymerase chain reaction
IC-PCR	Immune-capture polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
RT-PCR	Real-time polymerase chain reaction
VOC	Volatile organic compounds
DTBIA	Direct dot-blot immunoassay
LFD	Lateral flow dipstick assay
ELISA	Enzyme-linked immunosorbent assay
ACP	Asian citrus psyllid
CTV	*Citrus tristeza virus*
LAMP	Loop-mediated isothermal amplification
LIBS	Laser-induced breakdown spectroscopy
XRF	X-ray-fluorescence
TEM	Transmission electron microscopy
LM	Light microscopy
FISH	Fluorescence *in situ* hybridization
IF	Immunofluorescence
UAV	Unmanned aerial vehicle
GC/MS	Gas chromatography-mass spectrometry
SEC	Secretion system
CSD	Citrus stubborn disease
RS	Raman spectroscopy
ssDNA	Single-stranded DNA
dsDNA	double-stranded DNA
SPR	Surface plasmon resonance
QD	Quantum dots
MBs	Molecular beacons
QCM	Quartz crystal microbalances
sAMPs	Synthetic antimicrobial peptides
HRP	Horseradish peroxidase
ECEIA	Electrochemical enzyme-linked immunoassay
LFIA	Lateral flow immunoassay
FSNP	Fluorescent silica nanoparticle
FRET	Fluorescence resonance energy transfer
LSPR	Localized surface-plasmon resonance
GPS	Global positioning system
IMS	Immunomagnetic separation
LOD	Limit of detection
VBNC	Viable but non-culturable
SAW	Surface acoustic wave
IC-PCR	Immune-capture PCR
FTIR	Fourier transform infrared
DAS-ELISA	Double antibody sandwich-enzyme-linked immunosorbent assay
TP-ELISA	Tissue print-enzyme-linked immunosorbent assay

1. Introduction

Plant protection plays a significant role in disease, food quality, and agricultural production (Strange and Scott, 2005). Pathogens such as bacteria, fungi, oomycetes, nematodes, viruses, and viroids are persistent problems in sustainable agricultural production, causing substantial food losses (Savary et al., 2012). The most important challenges in foodborne diseases are bacterial infections (Alocilja and Radke, 2003). There are two major groups of pathogenic bacteria, gram-negative (accounting for approximately 95% of bacteria) and gram-positive (accounting for less than 5% of bacteria), which mainly differ in their cell structure and walls (Cui et al., 2020). It was projected that the consumption of nutrient food will continue to increase over the next 40 years due to the continued growth of the global population, indicating that an increase in food production of over 70% will be needed by 2050 (Rayfuse and Weisfelt, 2012). There are several pathogenic bacteria causing foodborne diseases, including *Escherichia coli, Salmonella enterica* (Sayad et al., 2016), *Staphylococcus aureus* (Rubab et al., 2018), *Campylobacter jejuni, Listeria monocytogenes,* and *Bacillus* spp. These bacterial pathogens cause serious health problems worldwide (Ali et al., 2021; Kumar et al., 2020).

Molecular-based assays have been well-established for plant infection diagnosis and plant disease detection using deoxyribonucleic acid (DNA)-based techniques, mostly involving polymerase chain reaction (PCR), including immune-capture PCR (IC-PCR), quantitative (qPCR), nested (PCR), and real-time (RT-PCR), along with DNA hybridization-based detection (Fig. 1). These methods have all proven to be sensitive and specific for genetic identification of fungi, viruses, and bacteria (Coy et al., 2014; Lin et al., 2018). In addition to DNA-based techniques, several other methods have also been developed for detecting plant diseases. Pathogen proteins and nucleic acids can be extracted from infected plant materials with suspected disease based on visual symptoms to confirm pathogen infiltration/progression (López et al., 2009). Furthermore, there are indirect methods of detection based on the examination of volatile organic compounds (VOCs) that plants trigger as defense mechanisms in response to pathogen attack (Scala et al., 2013). Many recent reviews have described the detailed techniques for identifying VOCs in plants for infection disease detection (Fang and Ramasamy, 2015; Martinelli et al., 2013). Serological assays, also known as immunoassays, include direct dot-blot immunoassay (DTBIA), lateral flow devices (LF), and enzyme-linked immunosorbent assay (ELISA), have been used to detect plant pathogen infection antigens (Ding et al., 2016). Moreover, new, innovative, and emerging biosensor techniques

are now widely used as diagnostic techniques for agriculture fields (plant and nurseries), environment, and clinical and plant bacterial pathogens (Khater et al., 2017; Rani et al., 2019). A biosensor system includes biological recognition and transducer active receptors such as antibodies, enzymes, and DNA probes, as well as phage-based biosensors that enable the diagnosis of an analyte according to monitoring particular interactions (Sadanandom and Napier, 2010). Biosensors represent the end products of a fast-growing field integrating engineering and computer or digital science to meet the crucial demand in several fields where its application is needed.

One of the most important and severe plant diseases that represents a global threat is citrus greening or Huanglongbing (HLB) disease, which is also known as yellow shoot disease, in China (Bove et al., 2006). In 1919, HLB disease was first reported in the Chaozhou district of Guangdong province in China, and has since spread to all citrus-producing areas of the world (Wang et al., 2017). The citrus economy is currently badly damaged by HLB disease, which results in the production of poor-quality fruits, leading to enormous losses of billions of dollars every year worldwide (Wang et al., 2017). HLB reduces fruit production by approximately 30%–100% in citrus groves, with several billion dollars of losses to the citrus industry of Florida alone experienced since 2005 (Bassanezi and Montesino, 2011). To date, no well-known resistant commercial citrus cultivars have been found (Albrecht et al., 2016). In the main citrus cultivated areas, including Asia and the USA, the accepted causal agent of HLB is a microscopic gram-negative, phloem-limited fastidious α-proteobacteria, for which three species have been recognized: '*Candidatus* Liberibacter asiaticus' from China (Canales et al., 2016), '*Candidatus* Liberibacter africanus' from Africa (Roberts et al., 2015), and '*Candidatus* Liberibacter americanus' from the USA (Gottwald, 2014). *Ca.* L. asiaticus is mainly spread by two types of citrus psyllids, the African citrus psyllid *Trioza erytreae* (Del Guercio) and the Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama (Bove et al., 2006), through citrus juice sucking, enabling bacterial cells to colonize the phloem cells. Diseased plants show leaf mottling, deformed and discolored fruits, asymmetrical chlorosis, and premature mortality (Nehela and Killiny, 2020a). *Ca.* Liberibacter spp, their vectors, and hosts are listed in Table 1.

This review provides a summary of the state-of-the-art detection techniques of the unculturable bacteria of *Ca.* L. asiaticus that have been implemented worldwide in food and agriculture science. The early quarantine and detection of bacterial diseases are important for reducing the spread and destruction of bacterial diseases internationally and locally, and these measures also reduce the cost-effectiveness and impact of false-positive detections. In addition, we extensively review the natural potential of different biosensors for plant bacterial detection. The specificity of biosensors can be greatly improved by using antibodies, DNA/RNA, enzymes, and specific recognition elements. In particular, we focused on immune sensors for the development of highly sensitive and selective diagnosis methods to detect unculturable bacteria. For example, an innovative electrical nanobiosensor detects a secreted protein of *Ca.* L. asiaticus as a biomarker. The antigen is detected based on the change in electrical conductivity resulting from the bridging of a nanogap by trapping of the nanoparticle. This nanosensor is expected to address the serious and urgent need of the multi-billion-dollar citrus industry by providing fast, simple, and cost-effective methods for detecting HLB disease.

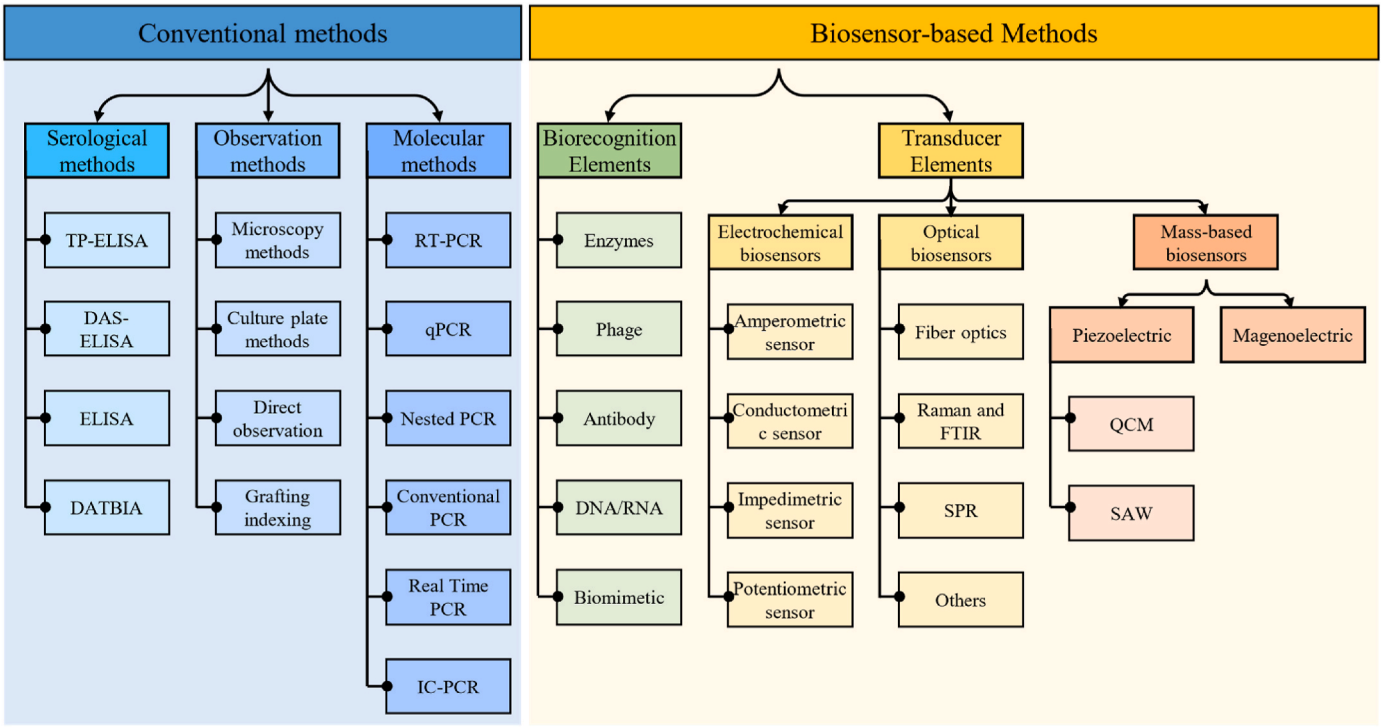

Fig. 1. Diagrammatic representation of conventional and biosensor-based methods for pathogen detection. Different conventional detection methods such as serological, direct observation, and molecular based methods have been established for the plant pathogenic bacteria. These methods are sensitive and specific for genetic identification of bacteria. New, innovative, and emerging biosensor techniques are now widely used as effective diagnostic techniques for clinical and plant bacterial pathogens in agricultural fields (plant and nurseries) and environment. A biosensor system includes biological recognition and transducer active receptors based on sensors used in plant-bacteria diagnosis. ELISA: Enzyme-linked immunosorbent assay, TP-ELISA: Tissue print-enzyme-linked immunosorbent assay, DAS-ELISA: Double antibody sandwich-enzyme-linked immunosorbent assay, DTBIA: Direct tissue blot immunoassay, PCR: Polymerase chain reaction, RT-PCR: Reverse transcription PCR, qPCR: Quantitative, IC-PCR: Immune-capture PCR, DNA: Deoxyribonucleic acid, RNA: Ribonucleic acid, SPR: Surface plasmon resonance, FTIR: Fourier transform infrared, QCM: Quartz crystal microbalances, SAW: Surface acoustic wave. The figure was created with smart draw software (https://www.smartdraw.com/).

Table 1
Different species of '*Candidatus* Liberibacter' and their vectors, distributions, and hosts associated with plants.

Bacterial pathogen	Insect vector	Disease	Distribution	Host	Reference
'*Ca.* L. asiaticus'	Asian citrus psyllid (*Diaphorina citri*)	Huanglongbing	Asia, Jamaica, Florida, China	Citrus, tobacco, *Cleome rutidosperma, Pisonia aculeate, Trichostigma octandrum*	(Duan et al., 2009; Katoh et al., 2014; Lin et al., 2013; Wu et al., 2015b, 2015c; Zheng. et al., 2014; Z. Zheng. et al., 2014a; Zheng et al., 2016),
'*Ca.* L. americanus'	*Diaphorina citri*	Huanglongbing	America, Brazil, Cuba	Citrus, tobacco	(Bove et al., 2006; Wulff et al., 2014)
'*Ca.* L. africanus'	African citrus psyllid (*Trioza erytreae*)	Huanglongbing	Africa: South Africa, Zimbabwe, Swaziland, Malawi, Burundi, Tanzania, Kenya, Somalia, Ethiopia, Cameroon, Nigeria	Citrus, tobacco	(Bove et al., 2006; Hong et al., 2015)
'*Ca.* L. europaeus'	Psyllid (*Cacopsylla pyri*)	Asymptomatic	Hungary, Italy	Pear, apple	(Raddadi et al., 2011) (Raddadi et al., 2011)
'*Ca.* L. psyllaurous'	Psyllid (*Bactericera cockerelli*)	Zebra chip disease	America, California, New Zealand	Solanaceae family crops	Hansen et al. (2008)
'*Ca.* L. solanacearum'	Psyllid (*Bactericera cockerelli*)	Zebra chip disease	America and New Zealand	Potato, tomato, potato, pepper, citrus	(Lin et al., 2011; Thompson et al., 2015; Wu et al., 2015a; Z Zheng. et al., 2014a)
Ca. *L. crescens*	unknown	Unknown	*Puerto Rico*	Mountain papaya (Caricastipulata × C. pubescens)	Leonard et al. (2012)
Ca. L. europaeus	*Broom psyllid (Arytainilla spartiophila)*	*Stunted growth of shoots, shortened internodes, leaf dwarfing, and leaf tip chlorosis*	*New Zealand, Australia*	*Scotch broom*	Thompson et al. (2015)
'*Ca.* L. asiaticus'	Unknown	Huanglongbing or yellow shoot disease	China	Navel orange	Yanling et al. (2019)

2. Conventional methods to detect phytopathogenic bacteria

2.1. Detection of HLB phytopathogenic unculturable bacteria

In the early stage, the identification of HLB bacteria in the field typically relies on observing visual symptoms on the leaves, stems, and fruits, as shown in Fig. 2, including asymmetrical chlorosis, blotchy mottle yellow shoots, and a reduction in fruit size with a lopsided shape (Etxeberria et al., 2009). Although visual examination is a more practical technique for identifying HLB in the field, this method is associated with more than 30% detection errors (Manhas et al., 2011), and identification might be worsened by abiotic and biotic factors or a mixture of disease with nutritional deficiencies (Lin et al., 2010). Citrus HLB disease symptoms can be confused with similar diseases such as *Citrus tristeza virus* (CTV), nutritional deficiencies, and citrus blight, which are difficult to discriminate from each other (Shokrollah et al., 2011). Several techniques have been adopted for identifying plant pathogens, including immunoassays and DNA-based techniques. Pathogen nucleic acids and proteins can be extracted from infected plant materials based on visual symptoms to confirm pathogen infiltration/progression (López et al., 2009).

Additionally, some promising indirect approaches have also been developed, such as the identification of VOCs, which are released by plants as a defensive strategy against pathogen attack (Scala et al., 2013). The presence of both symptomatic and asymptomatic trees remain challenging for the accurate detection of pathogens. HLB disease diagnosis can also be made by bio-indexing with a plant, such as *Citrus sinensis* L, sweet orange mandarin (*Citrus reticulata)* Blanco, and those of the non-rutaceous family, such as *C. roseus*. Additional advanced techniques have been established to identify and detect HLB bacteria, including serology, PCR, DNA probes, electron microscopy, and ELISA (Ding et al., 2005; do Carmo Teixeira et al., 2005), and qPCR is used for the confirmation and identification of citrus greening (Kogenaru et al., 2014). Loop-mediated isothermal amplification (LAMP) (Keremane et al., 2015), lateral flow dipstick assay (LFD) (Rigano et al., 2014), laser-induced breakdown spectroscopy (LIBS), and X-ray-fluorescence (XRF) are effective chemometric strategies used to monitor orchards affected by HLB (Manhas and Pereira, 2010).

Infrared spectroscopy is also used to diagnose citrus plants, and mid-infrared spectroscopy is used to treat HLB infections. However, with the exception of LIBS technology, none of these methods can be used for early disease diagnosis. Early detection methods and isolation of *Ca* L. asiaticus-infected trees are more important management methods that can be used to prevent HLB pathogens from invading HLB-free citrus-producing areas (Kogenaru et al., 2014). The limitations and benefits of recent detection and diagnostic methods for HLB disease worldwide (Table 2) must be recognized because early detection of the presence and mutation of '*Ca.* L. asiaticus' is important for reducing the spread of the disease in local and international trade, as the damage is very serious. This can also help to reduce the economic impact of false-positive detections.

2.2. Biological indexing or indicator plants

In field HLB diagnosis, symptoms are typically difficult to detect because specific symptoms are not known (Bove et al., 2006). The biological indexing indicator plants Ponkan mandarin and sweet orange can provide further confirmation tests of citrus greening. Although the qPCR method is currently the first choice for the detection of citrus greening disease in host plants, biological methods have previously been reported for more accuracy. Biological indexing methods may show different results because of the low percentage of graft transmission of HLB in the host. The seedling indicators at different temperature can be used to distinguish African HLB (20–25 °C) and Asian HLB (25–32 °C), and the plants show different symptoms such as mottle and asymptomatic and chlorosis. In addition, the plant shoots show reduced growth and more

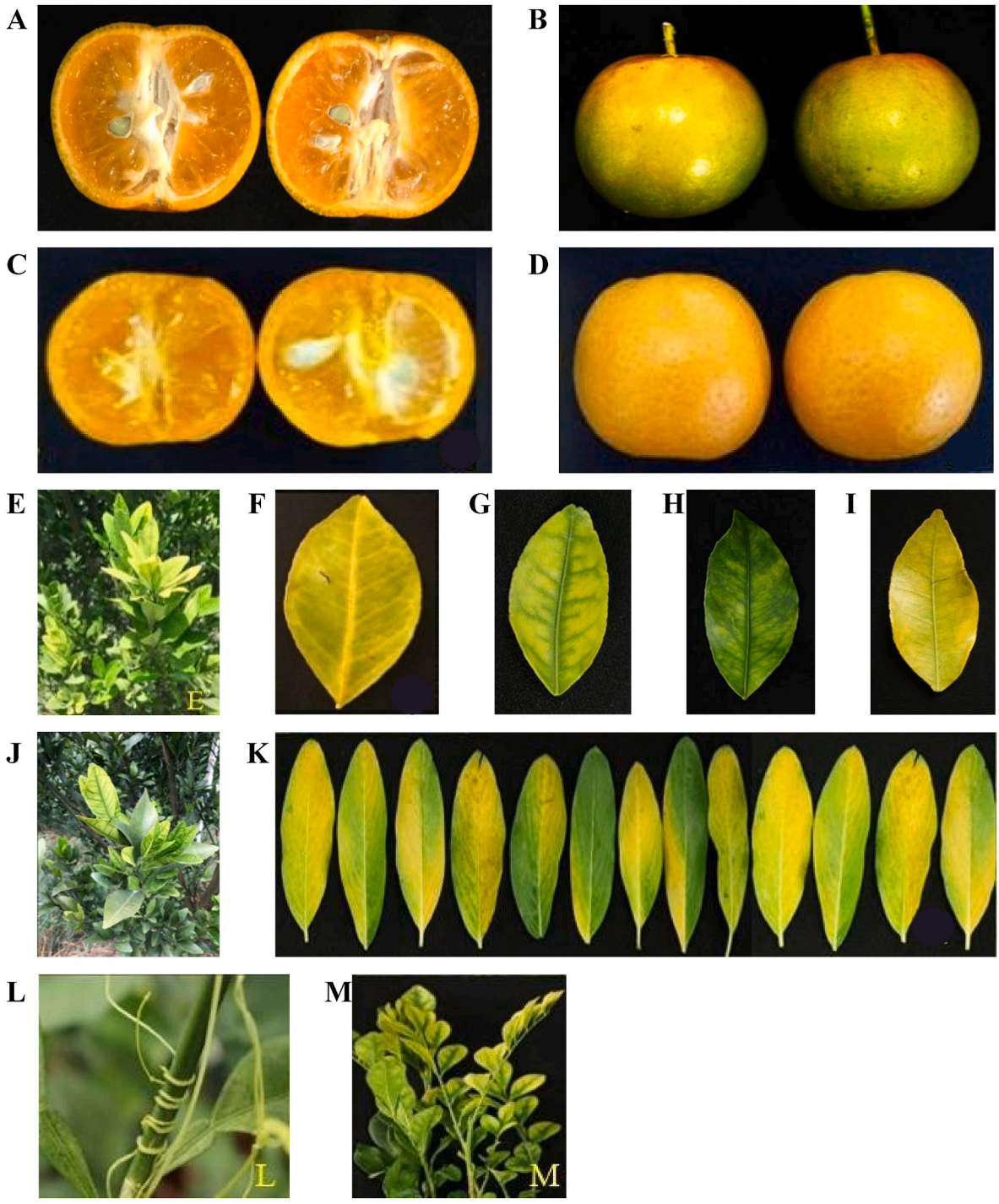

Fig. 2. HLB manifests result in a series of different yet associated symptoms that contain citrus responses to the bacterial infection. (A) Showing a longitudinal view of HLB-infected fruit, (B) showing greenish HLB-infected fruit (C) Longitudinal view of healthy fruit, (D) showing Healthy fruit of sweet orange (EFI) Showing the yellowing of shoot and leaves on *Citrus reticulata* (GH) asymmetrically chlorosis on leaves on citrus (J) Indicating inter-venial chlorosis like symptoms shoot on *Citrus sinensis* (K) Showing the yellowing of leaves on periwinkle plants (L) Displaying dodder connected with citrus HLB plants (M) showing zinc like symptoms on orange jessamine (*Murraya Paniculata*). Photographed by Qurban Ali from citrus greenhouse, Huazhong Agricultural University, Wuhan, China. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

chlorosis along with smaller leaves compared with those of controls and symptoms appears 8–12 weeks after inoculation. Plant materials can be preserved for numerous weeks and days for biological indexing, serological tests, and electronic microscopy, and can be preserved for several months until decay for molecular techniques (Li et al., 2008, 2009). Citrus psyllids vector samples can be preserved in 70% ethanol at very low temperatures for more than one year for real-time and conventional PCR (Li et al., 2008). Recent graft transmission of HLB disease and expression on specific germplasms of citrus are widely used in the commercial and local citrus industries, as well as under greenhouse conditions. However, with certain HLB disease vectors, symptom expression may not be completely successful through the biological indexing method, and the diseased plants may not show symptoms for a long time, suggesting the need for an alternate method (Folimonova

Table 2
Comparison of recent and prospective techniques for the detection of HLB bacteria.

Detection techniques	Methodologies	Benefits/time	Limitations	References
Molecular technique	PCR- based	Low concentrations of pathogen detection, qualitative, and rapid detection path	Time-consuming and expensive	(Hawkin et al., 2011; Khater et al., 2017)
	qPCR	Sensitive and quick detection technique	Time-consuming and expensive	Morgan et al. (2012)
	RT-qPCR	Capable of detecting the target with high specificity and sensitivity	HLB can be detected at later stages of infection	Kim and Wang (2009)
Microscopic technology	LM and TEM	Molecular techniques that detect different parts of infected plants (e.g., petioles, bark, leaves, and roots)	Time-consuming techniques, and cannot differentiate between '*Ca.* L. africanus', '*Ca.* L. americanus', and '*Ca.* L. asiaticus'	(Bove et al., 2006; Cevallos-cevallos et al., 2009)
Spectroscopy and imaging techniques	Visible and near-infrared spectroscopy	Lower costs of equipment and fast detection techniques	Detection of diseased hosts and leaf sampling is laborious or time-consuming.	(Alexander et al., 2014)
Isothermal amplification	LAMP with LFD	Can use a nylon membrane for determination of low copies of the bacterial genome instead of gel electrophoresis	Reduced suitability in the field condition	(Rani et al., 2019; Rigano et al., 2014)
Immunological techniques	ELISA	Low cost, visual symptoms can be used for detection	Low sensitivity for plant bacteria	Ding et al. (2016)
	FISH	High sensitivity	Autofluorescence	Kliot et al. (2014)
	DTBIA	Rapid, simple, practical, and easy to use under diverse field conditions	Unknown	Djelouah et al. (2014)

et al., 2009).

2.3. Microscopic techniques

Transmission electron microscopy (TEM) has been used to examine and detect disease-affected tissues by a gram-negative pathogenic bacterium (Folimonova and Achor, 2010), which was the first laboratory technique used in the 1970s for the confirmation and identification of citrus greening (Bove et al., 2006). Citrus greening symptoms are very complicated to categorize within citrus trees. Blotchy mottle, yellow shoot, small fruits, and lopsided fruits are difficult to identify before symptoms appear because none of the symptoms occurs in same trees (Folimonova and Achor, 2010). In 2009, TEM and light microscopy (LM) were used to detect HLB bacteria from the stems, petioles, bark, roots, and leaves of infected sweet orange trees (Cevallos-cevallos et al., 2009).

Microscopic methods have shown similar results among studies. An analysis of disease-infected tissue using TEM showed that the pathogenic bacteria have a gram-negative cell wall and are located entirely inside the sieve tube of the infected citrus tree (Folimonova and Achor, 2010). For TEM detection, the midribs of the blotchy mottle symptoms of citrus leaves were used. However, these techniques are laborious, time-consuming, and expensive and cannot effectively differentiate between the Asian, American, and African forms of '*Ca.* Liberibacter spp.' (Bove et al., 2006). These characteristics of bacteria have been used to detect HLB disease using electron microscopy, which is the only reliable detection method that has emerged in recent years based on the advancement of detection technology with PCR and DNA hybridization.

2.4. Serological assays

Serological assays are broadly applied in plant disease detection owing to their low cost and great efficiency but have not yet been widely used for detection of the HLB pathogen '*Ca.* L. asiaticus' because this bacterium cannot grow on Petri plates *in vitro* to produce antibodies against '*Ca.* L. asiaticus' cells (Duan et al., 2009) Currently, the OmpA protein is effectively used to diagnose '*Ca.* L. asiaticus' (Ding et al., 2016). OmpA protein is the main outer membrane protein of gram-negative bacteria, including '*Ca.* Liberibacter spp.' *Anti*-OmpA antibodies to '*Ca.* L. asiaticus' cells can be detected in phloem tissues using a simple tissue blot assay (Ding et al., 2017). Furthermore, several immunological methods have been developed that can be used against different plant pathogens. For example, DTBIA and ELISA are widely used to detect pathogens, as antigen-based, rapid, simple, practical, and easy to use methods under diverse field conditions (Ali et al., 2021). New methodologies have been established derived from serological assays for the detection of plant pathogens (Sharma and Sharma, 2016). Antibody-based nanosensors can be applied for the rapid detection and development of point-of-care devices for HLB diagnostics.

2.5. Molecular techniques

In recent years, molecular techniques have been established for the identification of plant pathogens (López et al., 2009). Molecular techniques are sensitive, and even a small quantity of bacteria can be easily identified in the sample. PCR, qPCR, ELISA, and other molecular techniques, including fluorescence *in situ* hybridization (FISH), immunofluorescence (IF), and DNA microarray, are all commonly used molecular techniques for the detection of plant pathogens (Sankaran et al., 2010). The PCR method was first used to detect HLB bacteria based on its 16 S rDNA sequence (Jagoueix et al., 1996). Time-consuming enzyme digestion of 1160-base PCR products with *Xbal* was needed to distinguish between two '*Ca.* Liberibacter spp.' Subsequently, primers targeting the nusG-rplK operon region (A2/J5 and MHO353/MHO354) were developed together with some other primer sets based on conserved genes (Fujikawa and Iwanami, 2012). '*Ca.* L. asiaticus' was sequenced from the *nusG–rplKAJL–rpoBC* gene clusters of '*Ca.* L. africanus and '*Ca.* L. asiaticus' in São Paulo, Brazil (Teixeira et al., 2008) Microarrays have been used for plant bacterial disease studies such as bacterial spot and bacterial blight (Li et al., 2006), as well as for fungal and viral diseases (Albrecht and Bowman, 2008). Moreover, dot hybridization with DNA probes labeled with biotinylated nucleotides was utilized as an efficient method for the identification of '*Ca.* L. asiaticus' in citrus hosts, such as sweet oranges, mandarins, and pumelo (Hung et al., 2011). This probe might respond to different '*Ca.* L. asiaticus' strains, but not those from South Africa (Hung et al., 2011). Compared with traditional PCR, qPCR is a more sensitive and fast detection technology. *Taq*man probe qPCR was first developed in 2006 to accurately detect HLB bacteria (Li et al., 2006), and compared with nested PCR, its detection sensitivity is increased by 10 times, which is approximately 100–1000 times that of traditional PCR (Morgan et al., 2012).

LAMP was the first DNA amplification technique applied to the detection of HLB bacteria. This technique was combined with an LFD device for visual assessment of the resulting amplicons, removing the need for gel electrophoresis (Rigano et al., 2014). LAMP is based on the source of auto-cycling thread discoloration from DNA fusion of DNA polymerase, and is used to detect specific DNA sequences (Crippa et al., 2012). This method uses four to six specific primers that distinguish between six and eight fragments of the target DNA and provides extraordinary specificity (Nagamine et al., 2002). Amplification can be performed using simple and low-cost equipment such as a water bath at temperatures between 60 °C and 65 °C (Nagamine et al., 2002). LAMP products can be detected by gel electrophoresis, which reduces their

applicability in field applications (Rigano et al., 2014). Other very recently developed methods (e.g., HLB-RPA-LFA) have shown great potential. The recombinase polymerase-based LAMP method combined with the LFD method was developed as a sensitive, fast, reliable, and cheap diagnostic tool for detection of '*Ca.* L. asiaticus' for farmers, mobile plant pathology, and quarantine programs (Ghosh et al., 2018).

Molecular techniques, including PCR, microarray technology, dot hybridization, qPCR, and LAMP, are laboratory-based techniques, as shown in Fig. 3, which can specifically identify citrus greening; however, a broadcast and sensing system is needed for accurate and reliable detection of HLB bacteria under field conditions and real-time conditions. Although molecular techniques are time-consuming and very expensive (Hawkin et al., 2011), a pre-screening method that can detect the diseased host will decrease the time required for investigation. This can also decrease the number of PCR testing samples, which reduces the overall disease control expenses and enable more useful detection of plant pathogens.

2.6. Spectroscopic and imaging techniques

The most accurate HLB diagnosis involves PCR, but the identification of infected trees and sampling of leaves is time-consuming. Moreover, the average accuracy achieved in visually inspecting and identifying infected trees by scouts is reported to be between 47% and 59% (Futch et al., 2009). Electromagnetic spectra have been applied to detect physiological stress in infected plants, which show irregular spectral signatures as compared to healthy plants in particular ranges; thus, electromagnetic spectra techniques can be applied as a marker for plant stress (Sankaran et al., 2010). Spectra in the reflectance from the tree canopy in the visible detection range and ultraviolet range have been measured for disease detection in different varieties of crops since spectroscopy techniques are quick and easy tools that can be used for crop evaluation in the field in real time (Sankaran et al., 2010). For example, spectral reflectance has been used to identify the apple scab (*Venturia inaequalis)* (Delalieux et al., 2007).

Visible near-infrared spectral reflectance was applied to citrus trees for the detection of HLB, demonstrating approximately 92% accuracy of spectral reflectance data in the wavelength range of 350–2500 nm (Sankaran and Ehsani, 2011). Inexpensive visual sensors were evaluated for their ability to distinguish infected hosts from healthy hosts (Mishra et al., 2011). The unmanned aerial vehicle (UAV) is an innovative method that uses high-resolution above-ground imaging for inexpensive

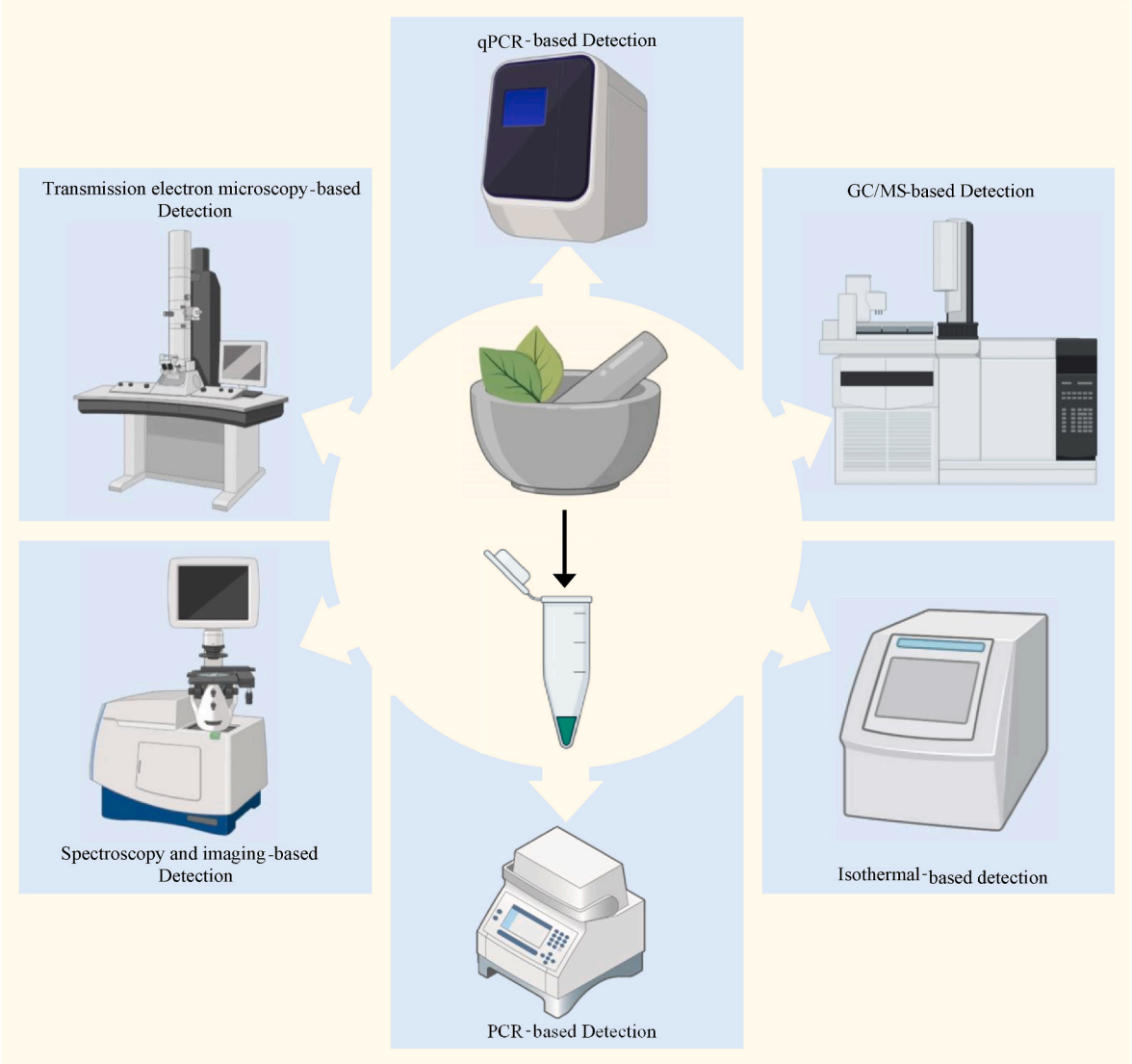

Fig. 3. Diagram showing the different HLB detection methods (through Polymerase chain reaction (PCR), Quantitative (qPCR), Loop-mediated isothermal amplification (LAMP) Gas chromatography-mass spectrometry (GC/MS), Transmission electron microscopy (TEM). The figure was created with BioRender (https://biorender.com).

citrus greening detection. The UAV is attached to a multi-band sensor that can acquire airborne images at different resolutions by adjusting the altitude (Garcia-ruiz et al., 2013). In addition, studies have been conducted on a smaller scale to detect HLB according to fastidious symptoms such as a starch deposit in the citrus leaves; narrow-banding imaging and polarizing filters can be used to identify the deposited starch in infected citrus leaves. Imaging sensors show visibly stressed starch deposits in citrus greening disease leaves and could differentiate from visual symptoms related to zinc deficiency (Pourreza et al., 2015).

A disease diagnosis method was also developed based on the biochemical analysis of unrestricted VOCs derived from diseased plants. This method is based on the concept of identifying biomarker "fingerprints" associated with a particular causal pathogen improved by using systematic approaches such as gas chromatography-mass spectrometry (GC/MS). The disease detection technique based on VOC identification exhibited excellent accuracy of 90% over the whole year and may be increased to 100% under favorable analysis situations, such as for detecting the very early period of disease that other techniques cannot handle efficiently (Alexander et al., 2014). Premature disease detection based on VOCs is superior to visual infection and established DNA-based methods such as real-time PCR (RT-PCR). Advanced methods for the rapid detection of HLB, such as spectroscopic methods, have been improving day by day, with advantages of low cost and fast detection to minimize false detection results (Alexander et al., 2014).

2.7. 'Ca. L. Asiaticus' secreted proteins as detection markers

'*Ca.* L. asiaticus' may have a protein secretion system different from the typical type I, which is important for different cellular processes (Green and Mecsas, 2016). In particular, secreted proteins play an important role in the pathogenicity of HLB (A. Sugio et al., 2011). Whole-genome sequence analysis of '*Ca* Liberibacter spp.' pathogens revealed that the SEC secretion system is common (Duan et al., 2009), which secretes proteins with *N*-terminal signal peptides from bacterial cells to the external environment. Bioinformatics predictions and studies based on *E. coli* alkaline phosphatase (PhoA) fusions have successfully predicted 86 proteins with '*Ca.* L. asiaticus' functional SEC-dependent secretion signals (Prasad et al., 2016). These secreted proteins show different expression levels in the host citrus and the vector psyllid, suggesting that they may act as "effectors" that manipulate host processes (Yan et al., 2013).

Here, we discuss different markers used for detecting HLB disease. The SEC transmission effector (SDE) is the well-studied in phloem cells transmitted by insects colonized in plant plastids and bacterial pathogens (MacLean et al., 2011). Aster yellows phytoplasma (witches broom) strain is expected to secrete approximately 56 SDEs (Bai et al., 2009). Related to '*Ca.* L. asiaticus', plant plasma cells have limited movement and restricted cells in the phloem of infected plants, and some SDEs have been shown to transport from the root to the phloem of budding meristems through the root system in plants (Sugio et al., 2011).

Moreover, SDEs commonly play an important role as detection markers with high specificity. Previous studies used similar techniques for antibody-based recognition techniques for the bacterial pathogen *Spiro plasma citri*, which causes citrus stubborn disease (CSD) (Shi et al., 2014). The SDEs produced by this antibody were able to detect CSD disease from infected trees for which PCR showed negative results (Shi et al., 2014). Successful binding of polyclonal antibodies to proteins has been observed, and a serological detection method that can effectively detect '*Ca.* L. asiaticus' infection has also been established (Pagliaccia et al., 2017). In recent years, '*Ca.* L. asiaticus' secreted proteins have been developed as detection markers for the high-throughput identification of HLB disease.

2.8. Plant VOCs as detection markers

VOCs play an important role in protecting host plants against '*Ca.* L. asiaticus' pathogens and their vector ACP (Hijaz et al., 2016). HLB disease symptoms increase due to high accumulation of starch in the leaf tissues; although this symptom can be used as a detection tool, nutritional deficiencies and viral infection cause the same effects (Nehela and Killiny, 2020b). Specific genes related to starch and carbohydrate metabolism are significantly altered in the plant after HLB infestation (Albrecht and Bowman, 2008). Metabolomics is a promising area of analytical chemistry that focuses on the identification of various metabolites (Killiny et al., 2018; Killiny and Nehela, 2017; Nehela et al., 2016, 2018; Nehela and Killiny, 2018, 2019, 2020a, 2020b). In general, pharmaceutical applications of metabolomics have led to the development of major instruments in food science and agricultural science (Brennan et al., 2018), and has helped to classify metabolic changes in plants due to biotic and abiotic factors, including biological stress such as food deprivation and infections (Peluffo et al., 2010). Different metabolic devices can classify different varieties of citrus, including those infected by HLB (Cevallos-cevallos et al., 2011).

VOCs are produced by the oil glands present in most citrus plant parts. These host plant volatiles are generally easily stimulated by abiotic and biotic stresses (Arimura et al., 2009). The movement of the pathogen within the tissues is suppressed by the antimicrobial activity of VOCs at the beginning of infection (Maffei, 2010). The family of citrus plants is the key host plant for ACP. Orange jasmine (*Murraya paniculata*) is the most likely host of ACP. However, hardy orange (*Poncirus trifoliate*) is also a host of ACP (Halbert, 2004). Among different citrus varieties such as grapefruit (*Citrus paradise*), sour orange (*M. paniculata),* and rough lemon (*Citrus jambhiri* Lush), grapefruit was found to be the preferred host of ACP. *P. trifoliata* has been shown to be more tolerant of ACP than lemon (*Citrus macrophylla)* based on antibiosis and antixenosis resistance mechanisms (Hall et al., 2013). Further, the compounds citronellal, undecanal, D-limonene, and b-phellandrene in sweet orange (*Citrus sinensis* (L.) were induced under '*Ca.* L. asiaticus' infection. Therefore, VOCs that originate in the citrus plant plays a vital role in battling '*Ca.* L. asiaticus' infection and ACP (Hijaz et al., 2013).

Citrus cultivars with tolerance against '*Ca.* L. asiaticus' pathogens have been developed in greenhouse controlled environment studies (Folimonova et al., 2009). Several antibacterial compounds in the seeds and fruits of *P. trifoliata* have been reported, suggesting that similar antibacterial compounds may exist in the phloem of '*Ca.* L. asiaticus'-tolerant citrus varieties (Albrecht and Bowman, 2011). '*Ca.* L. asiaticus'-tolerant cultivars produce undecanal, geranial, citronellal, and neral, which can regulate the movement of '*Ca.* L. asiaticus' in phloem tissues (Hijaz et al., 2016). Pelargonaldehyde, decanal, glutaraldehydes, benzaldehyde, and formaldehyde are known to have antimicrobial activities (Park et al., 2012). Many bacterial pathogens such as *Escherichia coli, Acinetobacter calcoaceticus, Enterococcus faecalis, Citrobacter freundii, Bacillus subtilis, Alcaligenes faecalis, Brochothrix thermosphacta, Beneckeanat riegens,* and *Clostridium sporogenes* were moderately inhibited by neural and geranial oils, although few pathogens are actively inhibited by citronellal (Ali et al., 2021). Compounds found in '*Ca.* L. asiaticus'-tolerant citrus varieties such as sesquiterpenes (germacrene D, caryophyllene, geranyl acetate, g-element, and b-elements) exhibit antibacterial effects against '*Ca.* L. asiaticus' (Hijaz et al., 2016).

The above findings suggest that VOCs can play an important role in the detection of '*Ca.* L. asiaticus' and showed antibacterial activities against the pathogen. However, more studies on VOCs are needed to understand the metabolic activities in phloem tissues and the metabolic profile of the '*Ca.* L. asiaticus' bacterium.

2.9. Raman spectroscopy (RS)-based detection technique

RS is a non-invasive, label-free, non-destructive spectroscopic method that provides knowledge about the biochemical structure of analyzed samples. RS is mostly used in food analysis chemistry, forensics, material science, and electrochemistry (Zeng et al., 2016). This

method has the capability to detect variations in protein structure and secondary metabolites (Kurouski et al., 2012), gunshot residues (Bueno and Lednev, 2013), and clarify the chemical composition and source of body fluids (Virkler and Lednev, 2009). Although RS is a commonly known and widely used lab-based techniques, several portable RS devices have also been developed in recent years; however, they have not yet been tested in field conditions. At a basic level, conventional diagnostic methods mainly detect the titer of a pathogen. RS diagnostic methods are based on the detection of pathogens that promote changes in host plant molecules that are highly specific to a single disease or condition. These structural changes are reproduced as shifts or consistent changes in the specific Raman bands that can be assigned to these molecules (Richter et al., 2020). When pathogen infection is very low, RS is a very useful and sensitive technique for capturing symptoms of early disease. The RS approach was recently used for detection of fungal pathogens (Egging et al., 2018) as well as to detect insect larvae (Sanchez et al., 2019). Recently, it was established that RS can be applied to detect unculturable HLB bacteria during the primary and late periods of disease progression (Sanchez et al., 2019).

3. Detection of phytopathogenic bacteria using portable biosensors

An extensive variety of biosensors have emerged, such as innovative and portable biosensors used for different detection targets in the environment, food analysis, and clinical laboratories. Plant pathogen biosensing techniques are used on various biological receptors such as antibodies, DNA probes, phages, enzyme point-of-care (POC) testing, and robotic and cell phone-based devices (Ali et al., 2021; Rani et al., 2019). Biosensor systems or techniques and their limitations and benefits are listed in Table 3.

3.1. DNA/RNA-based biosensors systems

A newly developed biosensor technique is used for pathogen detection through DNA-and ribonucleic acid RNA-based nucleic acid fragments as elements (Feng et al., 2019). The detection of diseases before any viewable symptoms can be achieved using DNA-based biosensors. Specific DNA sequences have been used for the identification of organisms. DNA-based biosensors facilitate the rapid and cost-effective analysis of infectious diseases (Chen et al., 2020; Kaisti et al., 2019). Single-stranded DNA (ssDNA) probes are used to observe hybridization between DNA probes and complementary DNA analytes with electroactive indicators on the electrodes. In addition to DNA, other molecules such as proteins or toxins have been detected using single-nanochip technology (de la Escosura-Muñiz, 2016). DNA-based biosensors include piezoelectric, optical, and electrochemical DNA biosensors. In optical DNA biosensors, changes in physicochemical properties (including temperature, mass, electrical, and optical properties) are used to detect DNA analytes based on the role of double-stranded DNA (dsDNA) (de la Escosura-Muñiz, 2016). Optical DNA-based biosensors can be further classified into surface plasmon resonance (SPR), quantum

Table 3
Different biosensor techniques and their limitations and benefits in plant pathogen detection.

Biosensor detection techniques	Methodologies	Benefits/time	Limitation	References
Antibody-based techniques	Volta-metric enzyme-based detection	Detects plant pathogens with higher selectivity and sensitivity by enzyme immunoassay coupled with electrochemical detection	Low availability of enzyme-conjugated antibodies	Paternolli et al. (2004)
	Quartz crystal microbalance-based approaches (QCM)	These immuno-sensors are widely used for detecting foodborne pathogens and they are highly sensitive and label-free.	Similar to ELISA, which limits the recording detection	(Bragazzi et al., 2015; Masdor et al., 2019; Noi et al., 2019)
	Electrochemical impedance spectroscopy (EIS)-based detection	Effectively trace reactions with high sensitivity; these biosensors are generally label-free	The low selectivity and sensitivity in a real complex sample is the major problem limiting commercial usage	(Daniels and Pourmand, 2007)
	Electrochemical biosensors	Cost-effective, sensitive, label free, and rapid detection	Limited coping abilities for complex clinical samples, low stability, and reproducibility	Ali et al. (2021)
	Ampero-metric	Cost-effective fabrication and high sensitivity detection	Fouling agents and interferents in the sample matrix reduce signal strength.	Muniandy et al. (2019)
	Fluorescent approaches	Highly sensitive and can detect multiple pathogens from a single assay	Require fluorescent readers; the complexity of this assay further limits is application	Kadadou et al. (2020)
	Optical biosensors	Specific, more sensitive, and rapid diagnostic	High level of sophistication and a long list of pretreatment stages	(Ying Chen et al., 2018)
	Lateral flow immunoassays (LFIA)	One of the most popular diagnostic tools, which is fast, stable, and provides targeted analysis. Widely used in plant disease diagnostics, environmental analysis, and food safety	Its sensitivity is improved by using fluorescent tags as compared with previous versions; however, it does not support visual detection (which is a requisite of a fluorescence reader)	Parolo et al. (2013)
	Surface plasmon resonance (SPR)	Follows the bioaffinity reactions, measures selectively, and is a label-free approach.	The sensor surface causes non-specific adsorptions, which should be controlled carefully.	Mudgal et al. (2020)
DNA- based biosensors	DNA hybridization voltammetric detection	Low-cost analysis	Lower sensitivity in complex real samples; in addition, other phytopathogens are not characterized by this approach	Lillis et al. (2006)
	DNA microarrays via fluorescent approach	Flexibility in rapid DNA hybridization and DNA probe designing, and requires very little sample.	The complexity of this system together with the requirement for fluorescent reader	Zeng et al. (2013).
	AuNPs aggregation-based DNA analysis	Can detect pathogens at an early disease (asymptomatic) stage	The isothermal DNA detecting amplicons are as small as 0.5 ng/μl	Vaseghi et al. (2013a)
New emerging sensors	Nanomaterial sensors	Easy to fabricate, stable, and real-time detection	Lower target efficiency than DNA/RNA-based biosensors, enzymes, and antibodies.	(Fang et al., 2014; Madufor et al., 2018)
	Mass-based methods	Cost-effective than other techniques, easy to use, have capabilities to diagnose in real time and provide label free detection	Sensitivity and specificity are very low, needed long term incubation period and difficult to regenerate the crystal surface	Alahi and Mukhopadhyay (2017)
	Nanomaterial sensors	Easy to use and friendly measurement and used in real time	There are concerns about the nanomaterials' toxicity, and it is likely that the sensor may not be regenerated	Sharifi et al. (2020)
	Aptamers-based detection system	Target site detection with increased specificity and sensitivity	SELEX is time-consuming laborious, expensive, and inefficient	(Arachchillaya, 2018; Khedri et al., 2018)

dots (QD), and molecular beacons (MBs). In contrast to the optical type, piezoelectric DNA biosensors use a quartz crystal to detect analytes. Strip-type DNA biosensors can also be used to detect DNA hybridization using a nanoparticle-based colorimeter, as shown in Fig. 4. Electrochemical DNA-based biosensors are used for the sequence-specific detection of analyte DNA. In amperometric electrochemical DNA biosensors, current fluctuations with a constant applied potential can be used to detect DNA hybridization (Yuhan Chen et al., 2018). Bacterial pathogens are detectable by DNA-based biosensors because of their unique nucleic acid sequences, which can be specifically hybridized with a complementary DNA probe. This is different from the principle of antibody-based biosensors, where hydrophobic, ionic, and hydrogen bonds play a role in the stabilization of antigen-antibody complexes.

The use of other plant pathogenic bacteria such as *Xanthomonas axonopodis* pv. *citri, P. viridiflava, X. alfalfa* subsp. *citrumelonis, Pseudomonas fluorescens, Pectobacterium cartovortum* subsp. *cartovorum,* and *Pseudomonas* genus confirmed the principle recently used to detect stems in citrus *P. syringae*. When the target DNA of *P. syringae* was analyzed, the results showed that the target DNA of *P. syringae* was as low as 15 ng/ml, demonstrating that this method was highly sensitive and specific (Vaseghi et al., 2013b). A gold nanoparticle (AuNP)-labeled DNA probe has also been used, such as in detection of the competitive DNA hybrid bacterial melon disease pathogen *Acidovorax avenae* subsp. *citrulli* (Zhao et al., 2011). Gold-labeled DNA band sensors can be used against five other plant bacterial pathogens: state *Lavibacter michiganensis, X. campestris, P. syringae, Acidovorax avenae,* and *Erwinia carotovora* (Zhao et al., 2011). Aptasensors combined with other devices such as nanomaterials are good candidates for plant pathogen detection (Kim et al., 2016).

3.2. Antibody-based biosensors

In this era, antibody-based biosensors are essential for the rapid quantitative analysis of foodborne bacterial pathogens. These biosensors do well in the enhancement of sensitivity, real-time detection, fast detection, and feasibility for qualification, as shown in Fig. 4. The plant pathogen detection field has been altered by development of these biosensors (Khater et al., 2017). Because of these biosensors, we can now perceive the pathogens in water, seeds, and air in different phases, including greenhouses, fields, and during post-harvest storage (Skottrup et al., 2008). Pathogenic microorganisms can be detected by small detecting analytes such as proteins, nucleic acids, and cantilever-based sensors (Nayak et al., 2009).

The connection of a specific antibody to a specific transducer into a specific event. A specific binding change of the antibody on the biosensor to an antigen, such as a pathogen of interest, is a necessary

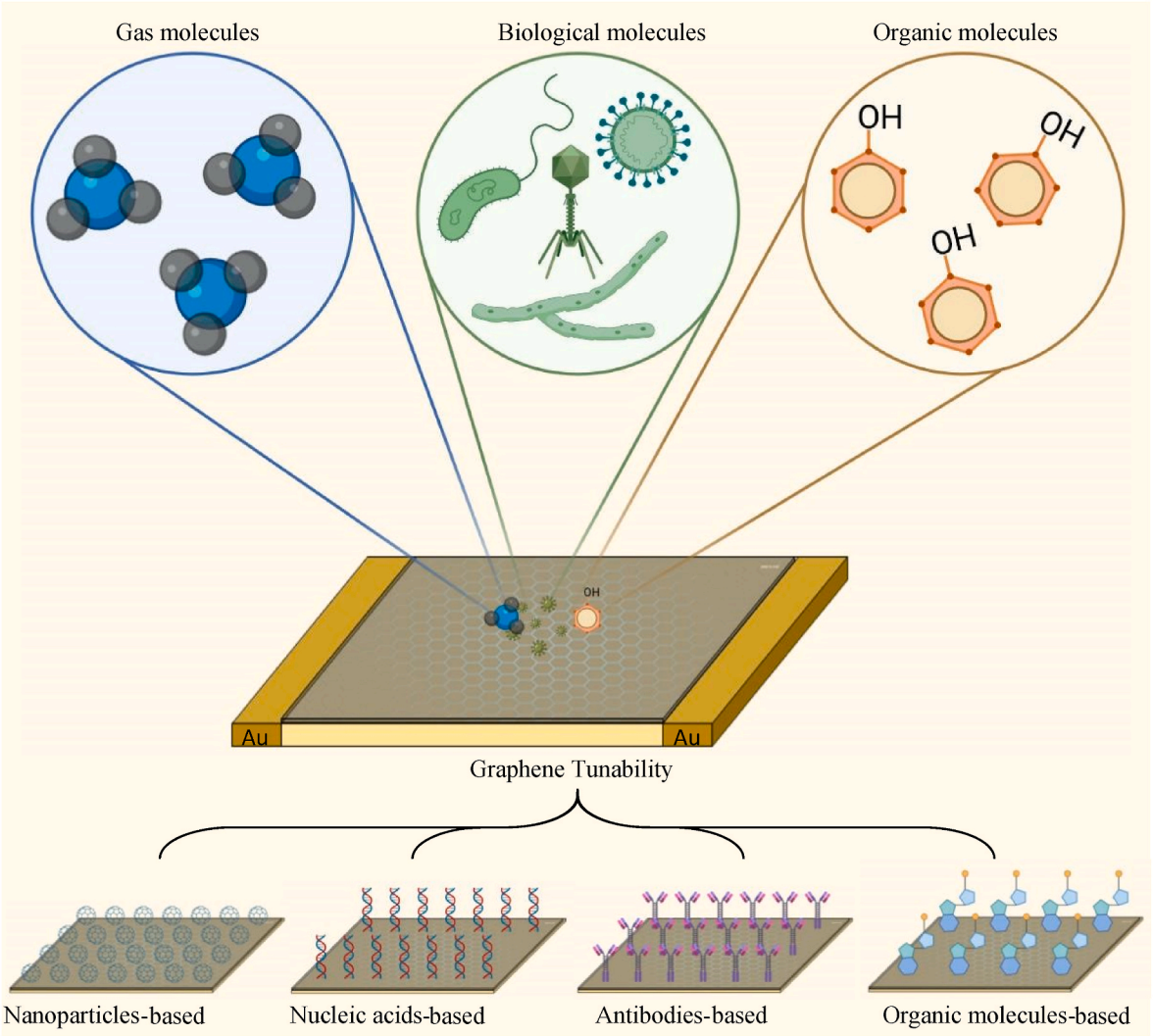

Fig. 4. Schematic diagrams of antibody-based biosensors and DNA/RNA-based biosensors for analyte detection. Specific combinations of analytes and immobilized antibodies DNA/RNA probes produce physical and chemical changes, such as mass, temperature, optical properties, or electrical potential. The change can be converted into a measurable signal for detection. The figure was created with BioRender (https://biorender.com).

step in establishing antibody-based immunosensors (Su et al., 2019). Different types of electrochemical sensors are used in most antibody-based biosensors, including impedimetry, potentiometry, and amperometry. Impedimetric biosensors are commonly used for biomass detection through microbial metabolism, based on microbial metabolism redox reactions (Byrne et al., 2009). In contrast, potential analysis biosensors convert the biometrics of an analyte into a voltage signal (Nouraei and Martin, 2006). The new electronic message generated from a particular binding event is used in amperometric biosensing (Palchetti and Mascini, 2008). In conducting metric biosensors, the biological indicator is transformed into an electrical signal using a conductive polymer such as polyaniline and polypyrrole (Sadanandom and Napier, 2010).

Some non-electrochemical sensors have also been reported, such as cantilever-based sensors, SPR, and quartz crystal microbalances (QCM). The QCM-based sensor detects the change in mass per unit area of a QCM crystal by measuring the frequency change of the quartz crystal resonator. QCM crystals are usually used as adaptive antibodies. Cantilever-based sensors are used to measure changes in the resonance frequency during the fusion of an analyte with the sensor surface (Skottrup et al., 2008). In SPR-based sensors, the analyte attaches to the metal surface, which helps to measure changes in the refractive index (Zeng et al., 2013). An antibody biosensor is susceptible and easily denatured, which requires specific environmental conditions such as temperature and pH. An antibody-based sensor will also be compromised during storage due to deterioration of the antibody over time (Byrne et al., 2009). Recently, aptamers, antibodies, and DNA-probes have been well matched with different detection devices such as SPR, chemiluminescence, electrochemical, fluorometric, optical, colorimetric, and magnetic devices (Nunes Pauli et al., 2015). The use of different nanomaterials in aptasensors is expected to improve the specificity and sensitivity of these devices (Khedri et al., 2018). However, these biosensors are not yet widely used for plant-bacteria detection. The adaptable nature of aptamer sensors and their potential for use in plant-bacteria diagnostics is in an early stage of development (Ali et al., 2021).

3.3. Bacteriophage-based biosensors

Bacteriophages are viruses composed of protein capsids that encapsulate DNA or RNA genomes. Bacteriophages infect and replicate within the bacteria, and lyses the bacterial host cell to propagate. Bacteriophages have been widely studied and used in phage therapy to treat bacterial infections in human diseases as well as for plant disease control. In addition to phage therapy, bacteriophages are emerging as a promising alternative for pathogen detection because of their high sensitivity, selectivity, low cost, and high thermostability (Grath et al., 2007). Immunosensor-based detection methods have been used to detect and identify *P. cannabina* pv. *alisalensis* from cultures, diseased plants (Schofield et al., 2013), and viruses (Zhang and Miller, 2019). Recently, bacteriophages have been shown to be effective in controlling plant pathogens and bacteria infecting tomatoes and potatoes (*Dickeya solani)* (Adriaenssens et al., 2012). Furthermore, the bacteriophage *P. cannabina* pv. *alisalensi* combined with additional plant pathogenic bacteria has been described and studied, such as bacterial canker of kiwifruits caused by *P. syringae* pv. *actinidia* and *Ralstonia solanacearum*, and many soil-borne bacteria causing bacterial wilt in other cereal crops (Askora et al., 2009).

Compared with recently developed antibody-based biosensors, bacteriophage-based sensors are more suitable and effective for detecting plant bacterial pathogens at different temperatures and for a longer time. Bacteriophages can separate live and dead bacteria pathogens, which influence the false-positive results during detection (Tlili et al., 2013). Recently developed site-specially oriented synthetic antimicrobial peptides (sAMPs) use novel recognition agents for the detection of pathogenic bacteria (Liu et al., 2016). Thus, bacteriophages provide the possibility of construction of a bacteriophage sensor for plant-bacteria detection (Ertürk and Lood, 2018).

3.4. Enzymatic electrochemical biosensors systems

Due to the high specificity of an enzyme for an analyte, enzyme as bio-recognition elements can perform extremely accurate detection of a target analyte. A specific enzyme for a target analyte is immobilized on a nanomaterial-modified electrode. In the amperometric method, a bio-electrocatalytic reaction occurs that generates an electrical signal that can be used to quantitatively detect analytes (Ronkainen et al., 2010). A rapid biosensing methodology was adopted for the detection of plant pathogens, food quality, and environmental monitoring (Ying Chen et al., 2018). Enzymatic biosensors can be used for plant pathogen detection if the target sample is collected in liquid form with a novel electrochemical method (Feng et al., 2019). Previous studies have demonstrated the detection of methyl salicylate using a dual enzyme system (Feng et al., 2014). Plant pathogens can be targeted and sensed through recently developed phage-based DNA and electrochemical biosensors (Wang et al., 2016). The occurrence of *P. cannabina* pv. *alisalensis* was determined using bioluminescent-phage-based technology (Schofield et al., 2013). The sensitivity of an immunological assay can be enhanced using AuNPs. AuNP tags loaded with horseradish peroxidase (HRP)-labeled antibodies were used for the first time in an electrochemical enzyme-linked immunoassay (ECEIA) to detect the phytopathogenic bacterium *Pantoea stewartii* subsp. *stewartii* (Zhao et al., 2014).

A lateral flow immunoassay (LFIA) was performed in 2015 to detect *P. stewartii* subsp. *stewartii* extracted from corn seed samples (Fang and Ramasamy, 2015). LFIA is performed in the presence of other phytopathogenic bacteria, including *X. oryzae, P. syringae,* and *Burkholderia glumae.* LF test strips were used to analyze various plant diseases (Zhao et al., 2014), and a DNA hybridization format was proposed for detecting cucurbit bacterial diseases caused by *Acidovorax avenae* subsp. *citrulli*. The test strip was used to test different plant bacterial pathogens such as *X. campestris, Clavibacter michiganensis, P. syringae, Erwinia carotovora,* and *Acidovorax avenae*. Vaseghi et al. (2013b) recently reported an aggregation-based test with AuNPs for the detection of *P. syringae* (Vaseghi et al., 2013b). In addition, the technology has been tested on *P. viridiflava, P. fluoresce, Pectobacterium cartovortum* subsp. *cartovorum, X. alfalfa* subsp. *citrumelonis,* and *X. axonopodis* pv. *citri.* Other citrus bacteria such as the genera *Pseudomonas* and *Enterobacteriaceae* can be detected by electrochemical DNA-based biosensors (Zhang et al., 2019). In the past few years, viruses and bacteria that infect animals and humans have been detected using microchips (Yang et al., 2019).

3.5. Biosensor platforms based on nanomaterials

The innovation of nanotechnology has enabled the fabrication of different nanostructures and nanoparticles despite some technical hurdles. Nanoparticles have shown remarkable visual and electronic properties using different types of materials for sensing and electronics (Alhamoud et al., 2019) (Fig. 5). For biosensing applications, the limit of detection and the overall performance of a biosensor can be greatly improved by using nanomaterials for their construction (Lv et al., 2018). Several types of nanostructures have been established as platforms for controlling bio-recognition elements to fabricate biosensors. Immobilizing enzymes, DNA, and antibodies can be attained using several methods, including biomolecular adsorption. Nanoparticles are helpful for biosensor creation, including metal and metal oxide nanomaterials, such as QDs and carbon nanoparticles (Sharifi et al., 2020). Nanomaterials are used with other natural and synthetic materials, including antibodies and microfluidic chips, for the diagnosis of bacterial spot disease caused by *X. axonopodis*. Biosensor techniques play a key role in the rapid detection of *E. coli* using gold nanomaterials (Lee et al., 2020; Zheng et al., 2019).

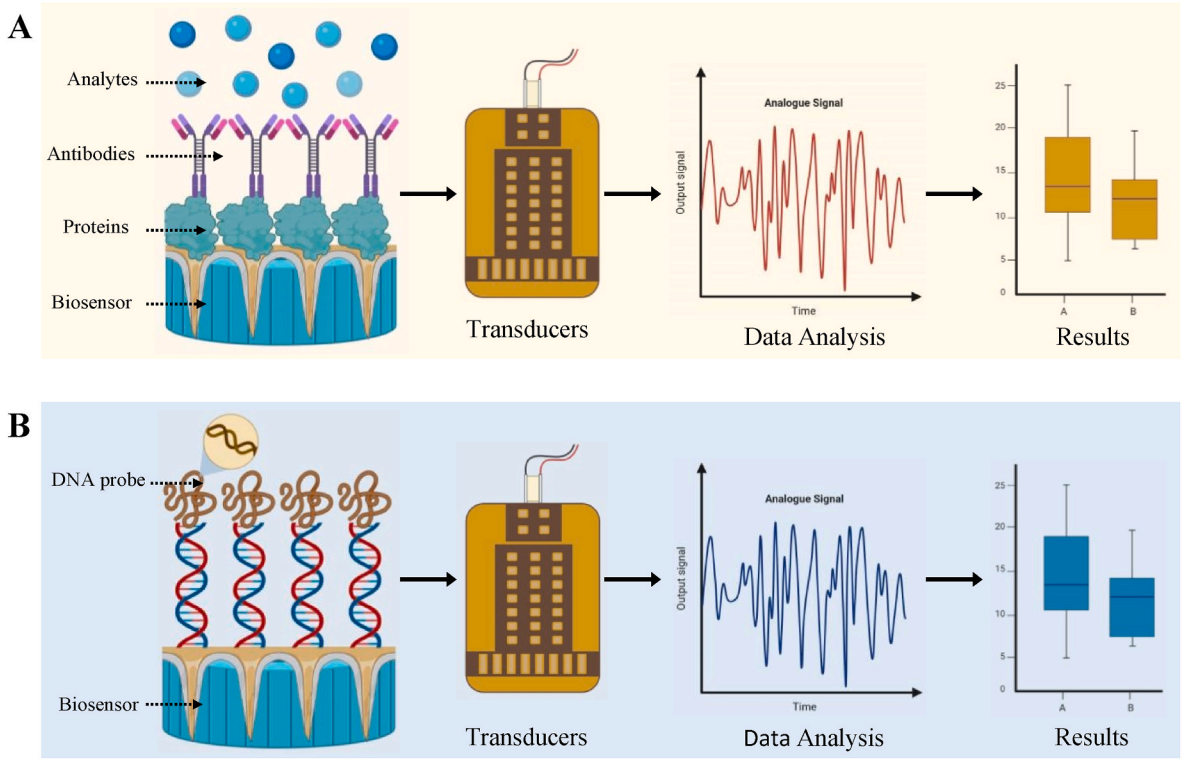

Fig. 5. The illustration representing biosensors based detection system by using (A) nanoparticles, DNA/RNA, antibody and organic molecules. (B) "Nanoparticles based", "DNA/RNA based", "antibody-based" and "organic molecules and Gas chromatography-mass spectrometry GC/MC". The figure was created with BioRender (https://biorender.com).

In addition to single-probe sensors, nanochip technology based on microarrays with fluorescent oligonucleotide probes has been described for the detection of single nucleotides in bacteria and viruses with high specificity and sensitivity according to identification of DNA hybridization mutations (Wang et al., 2011). Antibodies that incorporate fluorescent silica nanoparticle (FSNP) technology as biomarkers have been studied as probes that can effectively detect bacterial spot disease caused by *X. axonopodis* pv. *vesicatoria* in the Solanaceae family of plants (Yao et al., 2009). Moreover, QDs are used for biosensor fabrication for disease detection (Devi et al., 2019). Owing to their unique and beneficial visual optical properties, they have been applied for disease diagnosis using fluorescence resonance energy transfer (FRET) devices (Algar and Russ, 2008). Several QD-FRET-based biosensors have been developed for plant disease detection, including *Candidatus* phytoplasma aurantifolia, the causal agent of witches' broom disease of lime. Immune-sensor techniques showed great sensitivity and specificity in the detection of *Ca.* P. aurantifolia disease (Rad et al., 2012). Gold nanomaterials are widely used because of their high electron conductivity and electro-activity for electron transfer (Cao et al., 2011). Recently, electrochemical sensors based on nanomaterials have been described for plant disease detection (Umasankar and Ramasamy, 2013). The application of AuNP-based biosensors with an improved electrode was described for the electrochemical detection of DNA and methyl salicylate, the main VOCs released by plants during infections (Blair and Corrigan, 2019). Recently, a localized surface-plasmon resonance (LSPR)-based optical sensor with AuNPs and bovine serum albumin has been used in the detection of foodborne, water, and soil pathogens (Sadani et al., 2019; Zhou et al., 2019).

3.6. POC testing

Ideally, pathogen detection must be made at the point of care POC, so that detection can start directly and it does not require highly trained staff or availability of a laboratory (Kou et al., 2020). POC-based systems might be used to improve plant disease detection depending on the availability of resources (Scala et al., 2018). A POC method is a fast and specific system that can perform detection at any site where it is required. These devices provide clear results, are easy to use, and provide quantitative results (Scala et al., 2018). The use of POC at the target site along with mapping the data to describe positions via the global positioning system (GPS) would not require the farmer to perform a target site application of pesticides or bactericides, thereby decreasing and optimizing the use of agrochemicals (West and Heard, 2014). In 2017, the sale of POC systems reached an expected $US 23.71 billion, which is projected to increase to $US 38.13 billion by 2022 (https://www.marketsandmarkets.com/PressReleases/point-of-care-diagnostic.asp). POC systems are easy to use and have the ability to detect plant pathogenic bacteria in the early stage of plants (nurseries) with high specificity, sensitivity, portability, and robustness (Rani et al., 2019).

3.7. Robotics and cell phone-based systems

The first image-based detection technique named "Robo Kisan," which was used through robotic devices in India in 2010, was developed for the detection of downy mildew grapes (*Plasmopara viticola*) (Srivastava and Sharma, 2010). Similar image-based detection techniques are used for the diagnosis of plant pathogens, such as apple (*Malus domestica*), citrus (*Citrus* spp.), and pomegranate (*Punica granatum*) (Kadam, 2014). Robotic devices alone, or in combination with other bio-nanosensors, exhibit high potential for progress as automatic high-throughput detection devices at the POC in the early stages of plants (Awate et al., 2016). Cell phone-based detection of plant disease was developed in 2016, covering 54,306 pictures of infected and healthy plants for 26 diseases in several crops. This typical model achieved 99.35% accuracy in identifying diseased plants (Mohanty et al., 2016). Data from the plant pathogen Nikos Petrellis version 2.3 app showed that 90% of people recognize grape disease (Petrellis, 2017). The Google app Plantix, developed by German-based AgTech, includes more than

5000 images of plants, and it can detect more than 60 different plant diseases (http://www.fao.org/eagriculture/news/plantix-app-detect-and-cure-your-plant-diseases) (Neumann et al., 2016).

Another cell phone-based system was developed for the detection of plant pathogens such as *Blumeria graminis, Puccinia triticina, Septoria tritici, Cercospora beticola,* and *Uromyces betae* in sugarcane and wheat crops (Neumann et al., 2016). However, these techniques require further improvement and optimization before their use in plant pathogen detection will replace older techniques such as molecular and serological methods, particularly in terms of sensitivity and specificity. However, these applications are suitable for a large number of pathogens, revealing their ability to summarize a large number of infected and healthy characters in plants (Vashist et al., 2015). Cell phone-based techniques are recommended for the detection of plant-pathogen bacteria under field conditions.

These techniques are already used in the detection of other plant pathogens such as fungi, and scientists should consider applying similar or newly developed techniques for the detection of plant bacteria. Robotics and cell phone-based systems have the ability to categorize large amounts (healthy and unhealthy) of plant traits and are recommended for future detection of plant bacteria under field conditions.

3.8. Biorecognition and transduction

There are numerous effective biorecognition–transduction schemes for developing pathogen biosensors, from simple detectors (such as colorimetric assays and disposable sensor strips) to complex arrays (multiplexing biochips with microfluidics and dielectrophoresis). Regardless of the complexity of the device, the working scheme for a biosensor involves a three-step process: biorecognition, transduction, and signal acquisition. In the biorecognition step (Fig. 1), a molecular interaction between the target and a macromolecular structure on the sensor results in highly specific binding. In the transduction step, the selective binding of the target produces a change in the energy state of the system. Transduction can be inherent (such as a change in impedance due to antibody–antigen binding in immunoassays) or engineered [cascade reactions involving FRET as a function of nucleotide binding in PCR or addition of exogenous reagents]. Although covered in this review, inclusion of nanomaterials such as nanocarbon (e.g., nanotubes and nano-sheets), nanometals (gold, platinum, metal oxides), and many other structures has been shown to significantly improve transduction, and in some cases even biorecognition. The three most common classes of transduction are changes in mass, electrochemical, or optical properties, although other transduction processes are possible (such as magneto-elastic transduction). In the acquisition step, the change in the energy state is measured and the output is correlated to the presence of the analyte. Acquisition also includes *post hoc* analyses such as data filtering, statistics, machine learning, and data visualization. Among the transduction/acquisition approaches, plasmonic ELISA is emerging as an ultrasensitive method for the recognition of pathogens and macromolecule markers of pathogen contamination in food (Chen et al., 2015). Other common approaches include antibody immobilization in microfibers to enlarge the binding area or immunomagnetic separation (IMS) for specific concentrations of bacteria from complex matrices (Sturbaum et al., 2002).

4. Challenges, limitations, and future directions

The field of nano-biosensors has made exponential progress in the last decade, with advanced sensor modalities and hybrid nanomaterials helping to improve the lowest limit of detection (LOD), response time, and sensitivity. However, there are challenges with the aforementioned technologies. The biggest challenges are selectivity over non-target compounds, detection of viable cells, and lack of international standardization for technology research and development. With improvements in these areas, monitoring technology will likely significantly improve as social–technological components converge, as discussed below.

4.1. Selectivity

Fate and particulates can interfere with antibody–antigen interactions, and compounds, including carbohydrates, polyphenolics, sodium chloride, sucrose, or lysine, can alter DNA polymerase activity during PCR cycling (Dwivedi and Jaykus, 2011). Matrix effects have been reported for many food products, including apple juice and cucumbers (Wang et al., 2013). To resolve poor performance due to matrix effects, many methods require pre-concentration steps such as centrifugation, filtration, IMS, magnetic nanoparticle separation, and microfluidic sorting, as discussed previously (Vanegas et al., 2017). Although rapid techniques are typically not as selective as molecular methods such as PCR (Table 2), use of a pre-concentration step resolves this problem and has secondary benefits that are similar to enrichment, including: (i) dilution of inhibitors from food samples, (ii) discrimination of cell viability, and (iii) revival of stressed cells that would otherwise not be detected as viable (Valderrama et al., 2016). The recovery of pre-concentration methods varies from as low as 10% to as high as 99% (Alocilja et al., 2016; Valderrama et al., 2016).

The challenge with this approach is to maintain costs and throughput that are competitive with standard techniques without additional costs. For rapid techniques to have a clearly defined role in the mainstream market, the method should not require more than 1 h (total time) to produce a result, and ideally cost less than US $5 per sample (with the benefit exponentially increasing as the analysis cost decreases) (Valderrama et al., 2016). In addition to pre-concentration, consideration of a secondary validation step is a critical aspect, as most positive results are regarded as “presumptive positive” until validated by standard culture-based methods.

4.2. Detection of cell viability

Among culture-independent methods, one of the most important performance characteristics for next-generation methods is the ability to detect bacterial cell viability. Although traditional culture-based methods discern viability, the method underestimates diversity as only a small percentage of microbes can be cultured in a Petri dish (Ramamurthy et al., 2014). By contrast, molecular methods such as PCR-based methods (Keer and Birch, 2003) or metagenomics methods capture species diversity, whereas assessing viability is complex and requires repetitive analysis from subsample populations, producing limited information on the viability of a specific sample (in other words, there are problems with cross-sectional viability determination) (Cangelosi et al., 2010). Furthermore, differentiating between viable cells and free DNA fragments using PCR or metagenomic analysis is challenging. Among the methodologies that avoid some of these inherent problems, most techniques focus on cell membrane permeability as a marker of cell viability. This type of assay assumes that lysis is the most dominant outcome following cell death. Examples of cell permeability analytical techniques include cell live/dead labeling assays (Nocker and Camper, 2009).

Cell labeling uses a combination of a membrane-impermeable stain (such as propidium iodide) and a membrane-permeable strain (such as SYTO9) for analysis by fluorescence microscopy and/or flow cytometry. However, live/dead stains are known to have major issues with false positives (Stiefel et al., 2015). The PCR method also uses the live/dead tagging concept, but the detection mechanism is based on the inhibition of PCR amplification by cell impairment and photo activated reagents (such as propidium monoazide). Molecular viability testing is also a PCR-based method that uses RT-qPCR to detect the production of a species-specific macromolecule in response to exogenous nutrients. None of the biorecognition–transduction pathways covered in this review provide direct information on cell viability. However, cell labeling

can be easily combined with many devices to determine viability (Li et al., 2016).

4.3. Standardization

Although many pathogen biosensors in the published literature have merit (and likely a niche market), a system-level comparison (i.e., trade study) within the framework of food safety and agriculture productivity is nearly impossible due to a lack of standardization. For example, many industrial analyses use the term "sensitivity" to describe the probability of a test to detect a true positive and the term "specificity" to describe the probability of detecting a false negative (Valderrama et al., 2016). Engineers working in technology development use the term "sensitivity" to denote the relationship between device input and output, where the term "LOD," used herein, denotes the true positive value. For example, response time should be calculated as the total time required for analysis from the moment a product is taken off the line to the time when the data are analyzed, or a similar appropriate metric based on the application, including any time required for incubation, pre-concentration, magnetic separation, filtering/centrifuging, or microfluidic sorting, as these steps considerably add to the analysis time.

Although culture-based techniques are often scrutinized as being "slow" (typically at least 1 day), many biosensors and other techniques require hours or even days for pre-concentration in addition to sample preparation that is typically not included in the reported "response time," resulting in a biased comparison. It is also an unfair comparison to directly compare a biosensor (which cannot distinguish viability) to a culture-based method that can report viable cell numbers. Within culture-based methods, there are specific technical issues that further convolute a direct comparison, such as a metabolic state known as viable but non-culturable VBNC, where pathogens are underestimated and food products may be released under a false negative test result (Li et al., 2016). Thus, a direct comparison of "apples to oranges" does not advance the field and perpetuates the problem rather than alleviating it. For a typical design, the response time is reduced at the expense of higher LODs and lower sensitivity, which is an important trade-off when considering safety monitoring at the system level.

Response time is not the only performance characteristic that requires standardization. The calculation of LOD is not standardized, and some biosensor papers report LOD values that have not been replicated, or results that were obtained with a low confidence interval, both of which are not acceptable for meeting industry standards. Selectivity is another major issue, and future reports of new techniques should include the results of validation studies that confirm strain/serotype specificity with a culture-based method, standardized/certified kit, or other approved standard methodology. For example, ELISAs (Lee et al., 2015) can be used to validate biosensors together with replicates and controls that align with industry standards in live culture-based techniques. For this situation to become a reality, a standard nomenclature (at the commercial scale) and performance characterization must be used in the field of nanosensors/biosensors.

5. Conclusion

In this review, we show that there is a dire need to develop quick, innovative, and accurate detection methods for plant bacterial pathogens. The older detection techniques are laborious and time-consuming, and the analysis and data extraction are highly sensitive and costly, requiring heavy bioinformatics tools and computational biology. However, to date, no initiative has been taken to develop a portable DNA sequencing biosensor methodology that is easy, simple, and highly accurate for detecting plant bacterial pathogens. For direct disease detection, PCR seems to be the most sensitive and accurate method; however, this approach requires specific primers to amplify targeted DNA for detecting various pathogens. Moreover, the high cost and uneven polymerase activity limit its application and introduce uncertainty in the results. Other PCR-based detection methods such as RT-PCR have been used for on-field detection. Although FISH, IF, and GC/MS provide excellent sensitivity, their application is limited due to difficult sample preparation and expertise for data analysis, which requires a trained person. In addition, we provided an overview on affinity biosensors, which also remain in the laboratory, and require deterioration of the DNA/RNA probe and antibody system. DNA-based biosensors can be used for on-field testing. Nanomaterial-based biosensors such as metallic nanoparticles, nanofabrication, and nanochannels have been established and show great sensitivity. Bacteriophage phage-based biosensors can be used to detect live bacterial pathogens and host bacterial pathogens with the discovery of more bacteriophages. The specificity and sensitivity of biosensors can be greatly increased by the use of antibodies, DNA/RNA, and enzymes. We also highlighted emerging techniques such as cell phones, POC devices, robotic devices, and aptamer systems.

Recent techniques for HLB detection, which are based on disease symptoms and nucleic acid assays, are not only inaccurate but also unsuitable for field examinations due to variable dormant periods and the irregular distribution of pathogens in crop plants. Immuno-sensors can offer highly sensitive and selective, cost-effective, accurate, fast, and quantitative diagnosis methods to detect '*Ca.* L. asiaticus'-secreted proteins, which act as HLB biomarkers, as these proteins are systematically spread in infected citrus trees, thus providing a direct, innovative, and reliable detection method of HLB. An innovative electrical nanobiosensor has been developed to detect the '*Ca.* L. asiaticus' antigen as a biomarker based on the change in electrical conductivity resulting from bridging of a nanogap by trapping of the nanoparticle. This nanosensor will address the serious and urgent need of the multi-billion-dollar citrus industry by providing fast, simple, and cost-effective methods for detecting HLB disease. The current breakthrough for disease diagnosis is found in spectroscopic imaging techniques and volatile organic metabolites as biomarkers; however, these technologies are constantly evolving and new inputs can be expected in the field of diagnostics. These strategies can also permit the reduction of the massive application of chemicals, meet farmer requirements, localize sprayings, and enable performing preventive applications in a timely manner to reduce costs and pathogen damage.

This review shows that these current and advanced bacterial disease detection technologies have great potential for detecting plant bacterial diseases. Early identification and isolation are critical for reducing the spread and destruction of bacterial diseases and minimizing the economic impact of potential false-positive detection.

Author Contributions

Q.A,**H.M** and **L.Z**. planned and designed this review manuscript. **Q. A**. **H.Z**, and **M.J.R**. wrote this review paper. **M.H.S**, **M.A**, and **Y.N**. help to draw the figures. **M.A.S**, **A.M.A**, and **K.A.K**. helped to improve the manuscript writing. **Q. A**, **A.H**, **S.A** and**K·Y**, contributed to the critically revising of the manuscript. All the authors have reviewed, edited, and approved the manuscript before submission.

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Declaration of competing interest

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References

Adriaenssens, E.M., Vaerenbergh, J. Van, Vandenheuvel, D., Dunon, V., Ceyssens, J., Proft, M. De, Kropinski, A.M., Noben, J., Maes, M., 2012. T4-Related Bacteriophage LIMEstone Isolates for the Control of Soft Rot on Potato Caused by ' Dickeya Solani ' 7. https://doi.org/10.1371/journal.pone.0033227.

Alahi, M.E.E., Mukhopadhyay, S.C., 2017. Detection methodologies for pathogen and toxins: a review. Sensors 17, 1–20. https://doi.org/10.3390/s17081885.

Albrecht, U., Bowman, K.D., 2011. Tolerance of the trifoliate citrus hybrid US-897 (Citrus reticulate Blanco × poncirus trifoliata L. Raf.) to huanglongbing. Hortscience 46, 16–22. https://doi.org/10.21273/HORTSCI.46.1.16.

Albrecht, U., Bowman, K.D., 2008. Plant Science Gene expression in Citrus sinensis (L) Osbeck following infection with the bacterial pathogen Candidatus Liberibacter asiaticus causing. Huanglongbing in Florida 175, 291–306. https://doi.org/10.1016/j.plantsci.2008.05.001.

Albrecht, U., Fiehn, O., Bowman, K.D., 2016. Metabolic variations in different citrus rootstock cultivars associated with different responses to Huanglongbing. Plant Physiol. Biochem. 107, 33–44. https://doi.org/10.1016/j.plaphy.2016.05.030.

Alexander, A., Pasamontes, A., Peirano, D.J., Zhao, W., Dandekar, A.M., Fiehn, O., Ehsani, R., Davis, C.E., 2014. Detection of huanglongbing disease using differential mobility spectrometry. Anal. Chem. 86, 2481–2488. https://doi.org/10.1021/ac403469y.

Algar, W., Russ, K.J.U., 2008. Quantum dots as donors in fluorescence resonance energy transfer for the bioanalysis of nucleic acids, proteins, and other biological molecules. Anal. Bioanal. Chem. 391, 1609–1618. https://doi.org/10.1007/s00216-007-1703-3.

Alhamoud, Y., Yang, D., Fiati Kenston, S.S., Liu, G., Liu, L., Zhou, H., Ahmed, F., Zhao, J., 2019. Advances in biosensors for the detection of ochratoxin A: bio-receptors, nanomaterials, and their applications. Biosens. Bioelectron. 141, 111418. https://doi.org/10.1016/j.bios.2019.111418.

Ali, Q., Ahmar, S., Sohail, M.A., Kamran, M., Ali, M., Saleem, M.H., Rizwan, M., Ahmed, A.M., Mora-Poblete, F., do Amaral Júnior, A.T., Mubeen, M., Ali, S., 2021. Research advances and applications of biosensing technology for the diagnosis of pathogens in sustainable agriculture. Environ. Sci. Pollut. Res. 28, 9002–9019. https://doi.org/10.1007/s11356-021-12419-6.

Alocilja, E.C., Jain, P., Pryg, K., 2016. Immunosensor for rapid extraction/detection of enteric pathogens. Technology 4, 194–200.

Alocilja, E.C., Radke, S.M., 2003. Market analysis of biosensors for food safety. Biosens. Bioelectron. 18, 841–846. https://doi.org/10.1016/S0956-5663(03)00009-5.

Arachchillaya, B., 2018. Development and evaluation of a paper based biochemical sensor for realtime detection of food pathogen. Bachelor Proj 1–60. https://doi.org/10.13140/RG.2.2.29480.62726.

Arimura, G.I., Matsui, K., Takabayashi, J., 2009. Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. Plant Cell Physiol. 50, 911–923. https://doi.org/10.1093/pcp/pcp030.

Askora, A., Kawasaki, T., Usami, S., Fujie, M., Yamada, T., 2009. Host recognition and integration of fi lamentous phage φ RSM in the phytopathogen , Ralstonia solanacearum. Virology 384, 69–76. https://doi.org/10.1016/j.virol.2008.11.007.

Awate, A., Deshmankar, D., Amrutkar, G., Bagul, U., Sonavane, S., 2016. Fruit disease detection using color, texture analysis and ANN. In: Proc. 2015 Int. Conf. Green Comput. Internet Things. https://doi.org/10.1109/ICGCIoT.2015.7380603. ICGCIoT 2015 970–975.

Bai, X., Correa, V.R., Toruño, T.Y., Ammar, E., Kamoun, S., Hogenhout, S.A., 2009. AY-WB Phytoplasma Secretes a Protein that Targets Plant Cell Nuclei AY-WB Phytoplasma Secretes a Protein that Targets Plant Cell Nuclei. https://doi.org/10.1094/MPMI-22-1-0018.

Bassanezi, R.B., Montesino, L.H., 2011. Yield Loss Caused by Huanglongbing in Different Sweet Orange Cultivars in São Paulo, pp. 577–586. https://doi.org/10.1007/s10658-011-9779-1. Brazil.

Blair, E.O., Corrigan, D.K., 2019. A review of microfabricated electrochemical biosensors for DNA detection. Biosens. Bioelectron. 134, 57–67. https://doi.org/10.1016/j.bios.2019.03.055.

Bove, J.M., Genomique, D.R., Pathogene, P., Recherche, C. De, Bordeaux, I. De, Bourlaux, E., Cedex, O., Df, A., Pereira, A., Araraquara, P., 2006. Huanglongbing : a destructive, Newly-emergening, centruy. old disease of citrus 88, 7–37.

Bragazzi, N.L., Amicizia, D., Panatto, D., Tramalloni, D., Valle, I., Gasparini, R., 2015. Quartz-crystal microbalance (QCM) for public health : an overview of its applications. In: Advances in Protein Chemistry and Structural Biology, first ed. Elsevier Inc. https://doi.org/10.1016/bs.apcsb.2015.08.002.

Brennan, L., Roche, H.M., German, B., van Ommen, B., Gibney, M.J., Walsh, M., 2018. Metabolomics in human nutrition: opportunities and challenges. Am. J. Clin. Nutr. 82, 497–503. https://doi.org/10.1093/ajcn/82.3.497.

Bueno, J., Lednev, I.K., 2013. Advanced statistical analysis and discrimination of gunshot residue implementing combined Raman and FT-IR data. Anal. Methods 5, 6292–6296. https://doi.org/10.1039/c3ay40721g.

Byrne, B., Stack, E., Gilmartin, N., O'Kennedy, R., 2009. Antibody-based sensors: principles, problems and potential for detection of pathogens and associated toxins. Sensors 9, 4407–4445. https://doi.org/10.3390/s90604407.

Canales, E., Coll, Y., Hernández, I., Portieles, R., García, M.R., López, Y., Aranguren, M., Alonso, E., Delgado, R., Luis, M., 2016. 'Candidatus Liberibacter asiaticus', causal agent of citrus Huanglongbing, is reduced by treatment with Brassinosteroids. PLoS One 11, e0146223.

Cangelosi, G.A., Weigel, K.M., Lefthand-Begay, C., Meschke, J.S., 2010. Molecular detection of viable bacterial pathogens in water by ratiometric pre-rRNA analysis. Appl. Environ. Microbiol. 76, 960–962.

Cao, X., Ye, Y., Liu, S., 2011. Gold nanoparticle-based signal amplification for biosensing. Anal. Biochem. 417, 1–16. https://doi.org/10.1016/j.ab.2011.05.027.

Cevallos-cevallos, J.M., Etxeberria, E., Danyluk, M.D., Rodrick, G.E., 2009. Metabolomic analysis in food science : a review. Trends Food Sci. Technol. 20, 557–566. https://doi.org/10.1016/j.tifs.2009.07.002.

Cevallos-cevallos, J.M., García-torres, R., Etxeberria, E., Reyes-de-corcuera, J.I., 2011. GC-MS analysis of headspace and liquid extracts for metabolomic differentiation of citrus huanglongbing and zinc deficiency in leaves of ' valencia ' sweet orange from commercial groves, pp. 236–246. https://doi.org/10.1002/pca.1271.

Chen, Yuhan, Guo, S., Zhao, M., Zhang, P., Xin, Z., Tao, J., Bai, L., 2018. Amperometric DNA biosensor for Mycobacterium tuberculosis detection using flower-like carbon nanotubes-polyaniline nanohybrid and enzyme-assisted signal amplification strategy. Biosens. Bioelectron. 119, 215–220. https://doi.org/10.1016/j.bios.2018.08.023.

Chen, Y., Qian, C., Liu, C., Shen, H., Wang, Z., Ping, J., Wu, J., Chen, H., 2020. Nucleic acid amplification free biosensors for pathogen detection. Biosens. Bioelectron. 153, 112049. https://doi.org/10.1016/j.bios.2020.112049.

Chen, Y., Xianyu, Y., Wang, Y., Zhang, X., Cha, R., Sun, J., Jiang, X., 2015. One-step detection of pathogens and viruses: combining magnetic relaxation switching and magnetic separation. ACS Nano 9, 3184–3191.

Coy, M.R., Hoffmann, M., Kingdom Gibbard, H.N., Kuhns, E.H., Pelz-Stelinski, K.S., Stelinski, L.L., 2014. Nested-quantitative PCR approach with improved sensitivity for the detection of low titer levels of Candidatus Liberibacter asiaticus in the Asian citrus psyllid, Diaphorina citri Kuwayama. J. Microbiol. Methods 102, 15–22. https://doi.org/10.1016/j.mimet.2014.04.007.

Crippa, M., Bartolucci, G.B., Toffoletto, F., Marcer, G., 2012. Occupational Diseases Due to Allergic and Toxic Chemicals in Health Care Workers: Fitness for Work. Med. del Lav.

Cui, F., Ye, Y., Ping, J., Sun, X., 2020. Carbon dots: current advances in pathogenic bacteria monitoring and prospect applications. Biosens. Bioelectron. 112085. https://doi.org/10.1016/j.bios.2020.112085.

Daniels, J.S., Pourmand, N., 2007. Label-free impedance biosensors: opportunities and challenges. Electroanalysis 19, 1239–1257. https://doi.org/10.1002/elan.200603855.

de la Escosura-Muñiz, 2016. Nanochannels for electrical biosensing. TrAC Trends Anal. Chem. (Reference Ed.) 79, 134–150. https://doi.org/10.1016/j.trac.2015.12.003.

Delalieux, S., Aardt, J. Van, Keulemans, W., Schrevens, E., Coppin, P., 2007. Detection of biotic stress (Venturia inaequalis) in apple trees using hyperspectral data. Non-parametric statistical approaches and physiological implications 27, 130–143. https://doi.org/10.1016/j.eja.2007.02.005.

Devi, P., Saini, S., Kim, K.-H., 2019. The advanced role of carbon quantum dots in nanomedical applications. Biosens. Bioelectron 141 (111158). https://doi.org/10.1016/j.bios.2019.02.059.

Ding, F., Duan, Y., Yuan, Q., Shao, J., Hartung, J.S., 2016. Serological detection of â € Candidatus Liberibacter asiaticus' in citrus, and identification by GeLC-MS/MS of a chaperone protein responding to cellular pathogens. Sci. Rep. 6 https://doi.org/10.1038/srep29272.

Ding, F., Paul, C., Brlansky, R., Hartung, J.S., 2017. Immune tissue print and immune capture-PCR for diagnosis and detection of candidatus liberibacter asiaticus. Sci. Rep. 7, 1–9. https://doi.org/10.1038/srep46467.

Ding, F., Wang, G., Yi, G., Zhong, Y., Zeng, J., Zhou, B., 2005. Infection OF wampee and lemon BY the citrus huanglongbing pathogen (CANDIDATUSLIBERIBACTER asiaticus) IN China. J. Plant Pathol. 87, 207–212.

Djelouah, K., Frasheri, D., Valentini, F., D'Onghia, A.M., Digiaro, M., 2014. Direct tissue blot immunoassay for detection of Xylella fastidiosa in olive trees. Phytopathol. Mediterr. 559–564.

do Carmo Teixeira, D., Luc Danet, J., Eveillard, S., Cristina Martins, E., Cintra de Jesus Junior, W., Takao Yamamoto, P., Aparecido Lopes, S., Beozzo Bassanezi, R., Juliano Ayres, A., Saillard, C., Bové, J.M., 2005. Citrus huanglongbing in São Paulo State, Brazil: PCR detection of the 'Candidatus' Liberibacter species associated with the disease. Mol. Cell. Probes 19, 173–179. https://doi.org/10.1016/j.mcp.2004.11.002.

Duan, Y., Zhou, L., Hall, D.G., Li, W., Doddapaneni, H., Lin, H., Liu, L., Vahling, C.M., Gabriel, D.W., Williams, K.P., Dickerman, A., Sun, Y., Gottwald, T., Pierce, F., Pathology, P., 2009. Complete genome sequence of citrus huanglongbing bacterium. ' Candidatus Liberibacter asiaticus ' Obtained Through Metagenomics 22, 1011–1020.

Dwivedi, H.P., Jaykus, L.-A., 2011. Detection of pathogens in foods: the current state-of-the-art and future directions. Crit. Rev. Microbiol. 37, 40–63.

Egging, V., Nguyen, J., Kurouski, D., 2018. Detection and identification of fungal infections in intact wheat and sorghum grain using a hand-held Raman spectrometer. Anal. Chem. 90, 8616–8621. https://doi.org/10.1021/acs.analchem.8b01863.

Ertürk, G., Lood, R., 2018. Bacteriophages as biorecognition elements in capacitive biosensors: phage and host bacteria detection. Sensor. Actuator. B Chem. 258, 535–543. https://doi.org/10.1016/j.snb.2017.11.117.

Etxeberria, E., Gonzalez, P., Achor, D., Albrigo, G., 2009. Anatomical distribution of abnormally high levels of starch in HLB-affected Valencia orange trees. Physiol. Mol. Plant Pathol. 74, 76–83. https://doi.org/10.1016/j.pmpp.2009.09.004.

Fang, Y., Ramasamy, R.P., 2015. Current and prospective methods for plant disease detection. Biosensors 5, 537–561. https://doi.org/10.3390/bios5030537.

Fang, Y., Umasankar, Y., Ramasamy, R.P., 2014. Electrochemical detection of p-ethylguaiacol, a fungi infected fruit volatile using metal oxide nanoparticles. Analyst 139, 3804–3810. https://doi.org/10.1039/c4an00384e.

Feng, Y., Zhang, X., Su, L., Zhang, Y., He, F., 2019. A supersensitive MSPQC bacterium sensor based on 16S rRNA and "DNA-RNA switch. Biosens. Bioelectron. 138, 111302. https://doi.org/10.1016/j.bios.2019.05.007.

Feng, Z., Mao, Y., Xu, N., Zhang, B., Wei, P., Yang, D.-L., Wang, Z., Zhang, Z., Zheng, R., Yang, L., Zeng, L., Liu, X., Zhu, J.-K., 2014. Multigeneration analysis reveals the inheritance, specificity, and patterns of CRISPR/Cas-induced gene modifications in Arabidopsis. Proc. Natl. Acad. Sci. Unit. States Am. 111, 4632–4637. https://doi.org/10.1073/pnas.1400822111.

Folimonova, S.Y., Achor, D.S., 2010. Early events of citrus greening (huanglongbing) disease. Development at the Ultrastructural Level 100, 949–958.

Folimonova, S.Y., Robertson, C.J., Garnsey, S.M., Gowda, S., Dawson, W.O., 2009. Examination of the responses of different genotypes of citrus to huanglongbing (citrus greening) under different conditions. Phytopathology 99, 1346–1354. https://doi.org/10.1094/phyto-99-12-1346.

Fujikawa, T., Iwanami, T., 2012. Sensitive and robust detection of citrus greening (huanglongbing) bacterium " Candidatus Liberibacter asiaticus " by DNA ampli fi cation with new 16S rDNA- speci fi c primers. Mol. Cell. Probes 26, 194–197. https://doi.org/10.1016/j.mcp.2012.06.001.

Futch, S., Eingarten, S.H.W., Rey, M.I.K.E.I., 2009. Determining HLB infection levels using multiple survey methods in Florida citrus, pp. 152–157.

Garcia-ruiz, F., Sankaran, S., Mari, J., Suk, W., Rasmussen, J., Ehsani, R., 2013. Comparison of two aerial imaging platforms for identification of Huanglongbing-infected citrus trees. Comput. Electron. Agric. 91, 106–115. https://doi.org/10.1016/j.compag.2012.12.002.

Ghosh, D.K., Kokane, S.B., Kokane, A.D., Warghane, A.J., Motghare, M.R., Bhose, S., Sharma, A.K., Krishna Reddy, M., 2018. Development of a recombinase polymerase based isothermal amplification combined with lateral flow assay (HLB-RPA-LFA) for rapid detection of "Candidatus Liberibacter asiaticus. PLoS One 13. https://doi.org/10.1371/journal.pone.0208530.

Gottwald, T.R., 2014. Citrus Canker and Citrus Huanglongbing Citrus Canker and Citrus Huanglongbing, Two Exotic Bacterial Diseases Threatening the Citrus Industries. https://doi.org/10.1564/18dec09.

Grath, S.M., Fitzgerald, G.F., Sinderen, D. Van, 2007. Bacteriophages in Dairy Products : Pros and Cons, pp. 450–455. https://doi.org/10.1002/biot.200600227.

Green, E.R., Mecsas, J., 2016. Bacterial secretion systems – an overview CHAPTER SUMMARY. Microbiol. Spectr. 4, 1–32. https://doi.org/10.1128/microbiolspec.VMBF-0012-2015.

Halbert, S., 2004. Asian citrus psyllids (Sternorrhyncha: psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Fla. Entomol. 87, 330–353.

Hall, D.G., Richardson, M.L., Ammar, E.-D.D., Halbert, S.E., 2013. Asian citrus psyllid, Diaphorina citri, vector of citrus huanglongbing disease. Entomol. Exp. Appl. 146, 207–223. https://doi.org/10.1111/eea.12025.

Hansen, A.K., Trumble, J.T., Stouthamer, R., Paine, T.D., 2008. A new huanglongbing species , " candidatus liberibacter psyllaurous ," found to infect tomato and potato , is vectored by the psyllid bactericera cockerelli (sulc). ס 74, 5862–5865. https://doi.org/10.1128/AEM.01268-08.

Hawkin, G., Gottwald, T., Windham, W.R., Lawrence, K.C., Park, B., Hawkins, S.A., 2011. Detection of citrus huanglongbing by fourier transform infrared—attenuated total reflection spectroscopy. Appl. Spectrosc. 64, 100–103. https://doi.org/10.1366/000370210790572043.

Hijaz, F., El-Shesheny, I., Killiny, N., 2013. Herbivory by the insect Diaphorina citri induces greater change in citrus plant volatile profle than does infection by the bacterium, Candidatus Liberibacter asiaticus. Plant Signal. Behav. 8, 1–10. https://doi.org/10.4161/psb.25677.

Hijaz, F., Nehela, Y., Killiny, N., 2016. Possible role of plant volatiles in tolerance against huanglongbing in citrus. Plant Signal. Behav. 11, 1–12. https://doi.org/10.1080/15592324.2016.1138193.

Hong, L., Han, C., Read, David A., Lou, B., Civerolo, Edwin L., 2015. Complete genome sequence of "candidatus liberibacter africanus," a bacterium associated with citrus huanglongbing hong. Genome 3, 3–4. https://doi.org/10.13406/j.cnki.cyxb.2006.06.005.

Hung, T.-H., Wu, M.-L., Su, H.-J., 2011. Detection of fastidious bacteria causing citrus greening disease by nonradioactive DNA probes. Japanese J. Phytopathol. 65, 140–146. https://doi.org/10.3186/jjphytopath.65.140.

Jagoueix, S., Bové, J.M., Garnier, M., 1996. PCR detection of the two "Candidatus" liberobacter species associated with greening disease of citrus. Mol. Cell. Probes 10, 43–50. https://doi.org/10.1006/mcpr.1996.0006.

Kadadou, D., Tizani, L., Wadi, V.S., Banat, F., Alsafar, H., 2020. Since January 2020 Elsevier Has Created a COVID-19 Resource Centre with Free Information in English and Mandarin on the Novel Coronavirus COVID- 19. The COVID-19 resource centre is hosted on Elsevier Connect , the company ' s public news and information.

Kadam, V., 2014. D etection & C ontrol O F D owney M ildew D isease I N. Int. J. Adv. Eng. Technol. 7, 827–837.

Kaisti, M., Cheng, N., Shi, Q., Zhu, C., 2019. Engineering nanomaterials-based biosensors for food safety detection. Biosens. Bioelectron. 142, 122–128. https://doi.org/10.1016/j.bios.2019.03.042.

Katoh, H., Miyata, S.I., Inoue, H., Iwanami, T., 2014. Unique features of a Japanese "Candidatus Liberibacter asiaticus" strain revealed by whole genome sequencing. PLoS One 9. https://doi.org/10.1371/journal.pone.0106109.

Keer, J.T., Birch, L., 2003. Molecular methods for the assessment of bacterial viability. J. Microbiol. Methods 53, 175–183.

Keremane, M.L., Ramadugu, C., Rodriguez, E., Kubota, R., Shibata, S., Hall, D.G., Roose, M.L., Jenkins, D., Lee, R.F., 2015. A rapid fi eld detection system for citrus huanglongbing associated ' Candidatus Liberibacter asiaticus ' from the psyllid vector , Diaphorina citri Kuwayama and its implications in disease management. Crop Protect. 68, 41–48. https://doi.org/10.1016/j.cropro.2014.10.026.

Khater, M., de la Escosura-Muñiz, A., Merkoçi, A., 2017. Biosensors for plant pathogen detection. Biosens. Bioelectron. 93, 72–86. https://doi.org/10.1016/j.bios.2016.09.091.

Khedri, M., Ramezani, M., Rafatpanah, H., Abnous, K., 2018. Detection of food-born allergens with aptamer-based biosensors. TrAC Trends Anal. Chem. (Reference Ed.) 103, 126–136. https://doi.org/10.1016/j.trac.2018.04.001.

Killiny, N., Nehela, Y., 2017. Metabolomic response to huanglongbing: role of carboxylic compounds in citrus sinensis response to candidatus liberibacter asiaticus and its vector, Diaphorina citri. Mol. Plant Microbe Interact. 30, 666–678. https://doi.org/10.1094/MPMI-05-17-0106-R.

Killiny, N., Nehela, Y., Hijaz, F., Vincent, C.I., 2018. A plant pathogenic bacterium exploits the tricarboxylic acid cycle metabolic pathway of its insect vector. Virulence 9, 99–109. https://doi.org/10.1080/21505594.2017.1339008.

Kim, J., Wang, N., 2009. Characterization of copy numbers of 16S rDNA and 16S rRNA of Candidatus Liberibacter asiaticus and the implication in detection in planta. using quantitative PCR 4, 2–5. https://doi.org/10.1186/1756-0500-2-37.

Kim, Y.S., Raston, N.H.A., Gu, M.B., 2016. Aptamer-based nanobiosensors. Biosens. Bioelectron. 76, 2–19.

Kliot, A., Kontsedalov, S., Lebedev, G., Brumin, M., Cathrin, P.B., Marubayashi, J.M., Skaljac, M., Belausov, E., Czosnek, H., Ghanim, M., 2014. Fluorescence in situ hybridizations (FISH) for the localization of viruses and endosymbiotic bacteria in plant and insect tissues. JoVE 1–8. https://doi.org/10.3791/51030.

Kogenaru, S., Yan, Q., Riera, N., Roper, M.C., Deng, X., Ebert, T.A., Rogers, M., Irey, M. E., Pietersen, G., Rush, C.M., Wang, N., 2014. Repertoire of novel sequence signatures for the detection of Candidatus Liberibacter asiaticus by quantitative real-time PCR. BMC Microbiol. 14, 1–11. https://doi.org/10.1186/1471-2180-14-39.

Kou, X., Tong, L., Shen, Y., Zhu, W., Yin, L., Huang, S., Zhu, F., Chen, G., Ouyang, G., 2020. Smartphone-assisted robust enzymes@MOFs-based paper biosensor for point-of-care detection. Biosens. Bioelectron. 156, 112095. https://doi.org/10.1016/j.bios.2020.112095.

Kumar, N., Wang, W., Ortiz-marquez, J.C., Catalano, M., Gray, M., Biglari, N., Hikari, K., Ling, X., Gao, J., Van, T., Burch, K.S., 2020. Biosensors and Bioelectronics Dielectrophoresis assisted rapid , selective and single cell detection of antibiotic resistant bacteria with G-FETs. Biosens. Bioelectron. 156, 112123. https://doi.org/10.1016/j.bios.2020.112123.

Kurouski, D., Washington, J., Ozbil, M., Prabhakar, R., Shekhtman, A., Lednev, I.K., 2012. Disulfide bridges remain intact while native insulin converts into amyloid fibrils. PLoS One 7, 1–9. https://doi.org/10.1371/journal.pone.0036989.

Lee, G.-Y., Bong, J.-H., Jung, J., Kang, M.-J., Jose, J., Pyun, J.-C., 2020. Application of a thermophoretic immunoassay in the diagnosis of lupus using outer membrane particles from E. coli. Biosens. Bioelectron. 156, 112110. https://doi.org/10.1016/j.bios.2020.112110.

Lee, S.-H., Ahn, J.-Y., Lee, K.-A., Um, H.-J., Sekhon, S.S., Park, T.S., Min, J., Kim, Y.-H., 2015. Analytical bioconjugates, aptamers, enable specific quantitative detection of Listeria monocytogenes. Biosens. Bioelectron. 68, 272–280.

Leonard, M.T., Fagen, J.R., Davis-richardson, A.G., Davis, M.J., 2012. Complete Genome Sequence of Liberibacter Crescens BT-1 271–283. https://doi.org/10.4056/sigs.3326772.

Li, S., Ma, F., Bachman, H., Cameron, C.E., Zeng, X., Huang, T.J., 2016. Acoustofluidic bacteria separation. J. Micromech. Microeng. 27, 15031.

Li, W., Hartung, J.S., Levy, L., 2006. Quantitative real-time PCR for detection and identification of Candidatus Liberibacter species associated with citrus. huanglongbing 66, 104–115. https://doi.org/10.1016/j.mimet.2005.10.018.

Li, W., Levy, L., Hartung, J.S., 2009. Quantitative distribution of ' candidatus liberibacter asiaticus' in citrus plants with citrus huanglongbing. Phytopathology 99, 139–144. https://doi.org/10.1094/phyto-99-2-0139.

Li, W., Li, D., Twieg, E., Hartung, J.S., Levy, L., 2008. Optimized quantification of unculturable candidatus liberibacter spp. in host plants using real-time PCR. Plant Dis. 92, 854–861. https://doi.org/10.1094/pdis-92-6-0854.

Lillis, B., Hurley, E., Berney, H., Duane, R., Manning, M., Mathewson, A., Sheehan, M.M., 2006. Investigation into the effect that probe immobilisation method type has on the analytical signal of an EIS DNA biosensor. Biosens. Bioelectron. 22, 1289–1295. https://doi.org/10.1016/j.bios.2006.05.021.

Lin, H., Chen, C., Doddapaneni, H., Duan, Y., Civerolo, E.L., Bai, X., Zhao, X., 2010. A new diagnostic system for ultra-sensitive and speci fi c detection and quanti fi cation of Candidatus Liberibacter asiaticus , the bacterium associated with citrus Huanglongbing. J. Microbiol. Methods 81, 17–25. https://doi.org/10.1016/j.mimet.2010.01.014.

Lin, H., Han, C.S., Liu, B., Lou, B., Bai, X., Deng, C., Civerolo, E.L., Gupta, G., 2013. Complete genome sequence of a Chinese strain of "Candidatus Liberibacter asiaticus. Genome Announc. 1, 2–3. https://doi.org/10.1128/genomeA.00184-13.

Lin, H., Lou, B., Glynn, J.M., Doddapaneni, H., Civerolo, E.L., Chen, C., Duan, Y., Zhou, L., Vahling, C.M., 2011. The Complete Genome Sequence of ' Candidatus Liberibacter Solanacearum ', the Bacterium Associated with Potato Zebra Chip Disease, vol. 6. https://doi.org/10.1371/journal.pone.0019135.

Lin, L.H., Ntambo, M.S., Rott, P.C., Wang, Q.N., Lin, Y.H., Fu, H.Y., Gao, S.J., 2018. Molecular detection and prevalence of Xanthomonas albilineans, the causal agent of sugarcane leaf scald, in China. Crop Protect. 109, 17–23. https://doi.org/10.1016/j.cropro.2018.02.027.

Liu, X., Marrakchi, M., Xu, D., Dong, H., Andreescu, S., 2016. Biosensors based on modularly designed synthetic peptides for recognition, detection and live/dead differentiation of pathogenic bacteria. Biosens. Bioelectron. 80, 9–16. https://doi.org/10.1016/j.bios.2016.01.041.

López, M.M., Llop, P., Olmos, A., Marco-Noales, E., Cambra, M., Bertolini, E., 2009. Are molecular tools solving the challenges posed by detection of plant pathogenic bacteria and viruses? Curr. Issues Mol. Biol. 11, 13–46.

Lv, M., Liu, Y., Geng, J., Kou, X., Xin, Z., Yang, D., 2018. Engineering nanomaterials-based biosensors for food safety detection. Biosens. Bioelectron. 106, 122–128. https://doi.org/10.1016/j.bios.2018.01.049.

MacLean, A.M., Sugio, A., Makarova, O.V., Findlay, K.C., Grieve, V.M., Toth, R., Nicolaisen, M., Hogenhout, S.A., 2011. Phytoplasma effector SAP54 induces indeterminate leaf-like flower development in arabidopsis plants. Plant Physiol. 157, 831–841. https://doi.org/10.1104/pp.111.181586.

Madufor, N.J.K., Perold, W.J., Opara, U.L., 2018. Detection of plant diseases using biosensors: a review. Acta Hortic. 1201, 83–90. https://doi.org/10.17660/ActaHortic.2018.1201.12.

Maffei, M.E., 2010. Sites of synthesis, biochemistry and functional role of plant volatiles. South Afr. J. Bot. 76, 612–631. https://doi.org/10.1016/j.sajb.2010.03.003.

Manhas, F., Pereira, V., 2010. Investigation of the Stages of Citrus Greening Disease Using Micro Synchrotron Radiation X-Ray Fluorescence in Association with Chemometric Tools † 351–355. https://doi.org/10.1039/b920980h.

Manhas, F., Pereira, V., Marcondes, D., Pereira, B., Pereira-filho, E.R., Leonardo, A., Sá, M. De, Russo, T., Camponez, M., Kelly, P., Freitas-astúa, J., 2011. Laser-induced Fluorescence Imaging Method to Monitor Citrus Greening Disease, vol. 79, pp. 90–93. https://doi.org/10.1016/j.compag.2011.08.002.

Martinelli, F., Reagan, R.L., Uratsu, S.L., Phu, M.L., Albrecht, U., Zhao, W., Davis, C.E., Bowman, K.D., Dandekar, A.M., 2013. Gene Regulatory Networks Elucidating Huanglongbing Disease Mechanisms, vol. 8. https://doi.org/10.1371/journal.pone.0074256.

Masdor, N.A., Altintas, Z., Shukor, M.Y., Tothill, I.E., 2019. Subtractive inhibition assay for the detection of Campylobacter jejuni in chicken samples using surface plasmon resonance. Sci. Rep. 9, 1–10.

Mishra, A., Karimi, D., Ehsani, R., Albrigo, L.G., 2011. Evaluation of an active optical sensor for detection of Huanglongbing (HLB) disease. Biosyst. Eng. 110, 302–309. https://doi.org/10.1016/j.biosystemseng.2011.09.003.

Mohanty, S.P., Hughes, D.P., Salathé, M., 2016. Using deep learning for image-based plant disease detection. Front. Plant Sci. 7, 1–10. https://doi.org/10.3389/fpls.2016.01419.

Morgan, J.K., Zhou, L., Li, W., Shatters, R.G., Keremane, M., Duan, Y., 2012. Improved real-time PCR detection of ' Candidatus Liberibacter asiaticus ' from citrus and psyllid hosts by targeting the intragenic tandem-repeats of its prophage genes. Mol. Cell. Probes 26, 90–98. https://doi.org/10.1016/j.mcp.2011.12.001.

Mudgal, N., Yupapin, P., Ali, J., Singh, G., 2020. BaTiO 3-graphene-affinity layer–based surface plasmon resonance (SPR) biosensor for Pseudomonas bacterial detection. Plasmonics 1–9.

Muniandy, S., Teh, S.J., Thong, K.L., Thiha, A., Dinshaw, I.J., Lai, C.W., Ibrahim, F., Leo, B.F., 2019. Carbon nanomaterial-based electrochemical biosensors for foodborne bacterial detection. Crit. Rev. Anal. Chem. 49, 510–533. https://doi.org/10.1080/10408347.2018.1561243.

Nagamine, K., Hase, T., Notomi, T., 2002. Accelerated reaction by loop-mediated isothermal ampli ® cation using loop primers, pp. 223–229. https://doi.org/10.1006/mcpr.2002.0415.

Nayak, M., Kotian, A., Marathe, S., Chakravortty, D., 2009. Detection of microorganisms using biosensors-A smarter way towards detection techniques. Biosens. Bioelectron. 25, 661–667. https://doi.org/10.1016/j.bios.2009.08.037.

Nehela, Y., Hijaz, F., Elzaawely, A.A., El-Zahaby, H.M., Killiny, N., 2018. Citrus phytohormonal response to *Candidatus* Liberibacter asiaticus and its vector *Diaphorina citri*. Physiol. Mol. Plant Pathol. 102, 24–35. https://doi.org/10.1016/j.pmpp.2017.11.004.

Nehela, Y., Hijaz, F., Elzaawely, A.A., El-Zahaby, H.M., Killiny, N., 2016. Phytohormone profiling of the sweet orange (Citrus sinensis (L.) Osbeck) leaves and roots using GC-MS-based method. J. Plant Physiol. 199, 12–17. https://doi.org/10.1016/j.jplph.2016.04.005.

Nehela, Y., Killiny, N., 2020a. Melatonin is involved in citrus response to the pathogen huanglongbing via modulation of phytohormonal biosynthesis. Plant Physiol. 184, 2216–2239. https://doi.org/10.1104/pp.20.00393.

Nehela, Y., Killiny, N., 2020b. Revisiting the complex pathosystem of huanglongbing: deciphering the role of citrus metabolites in symptom development. Metabolites 10, 1–25. https://doi.org/10.3390/metabo10100409.

Nehela, Y., Killiny, N., 2019. 'Candidatus liberibacter asiaticus' and its vector, Diaphorina citri, augment the tricarboxylic acid cycle of their host via the g-aminobutyric acid shunt and polyamines pathway. Mol. Plant Microbe Interact. 32, 413–427. https://doi.org/10.1094/MPMI-09-18-0238-R.

Nehela, Y., Killiny, N., 2018. Multiple phytohormonal signaling mediates citrus response to the bacterial pathogen Candidatus Liberibacter asiaticus. In: International Congress of Plant Pathology (ICPP) 2018: Plant Health in A Global Economy. APSNET.

Neumann, M., Klatt, B., Hallau, L., Kersting, K., Bauckhage, C., 2016. Cell Phone Image-Based Plant Disease Classification. Biometrics: Concepts, Methodologies, Tools, and Applications. https://doi.org/10.4018/978-1-5225-0983-7.ch032.

Nocker, A., Camper, A.K., 2009. Novel approaches toward preferential detection of viable cells using nucleic acid amplification techniques. FEMS Microbiol. Lett. 291, 137–142.

Noi, K., Iijima, M., Kuroda, S., Ogi, H., 2019. Ultrahigh-sensitive wireless QCM with bio-nanocapsules. Sensor. Actuator. B Chem. 293, 59–62.

Nouraei, M., Martin, V., 2006. Part II: the karguzar and security, the trade routes of Iran and foreign subjects 1900-1921. J. R. Asiatic Soc. 16 (1), 29–41. https://doi.org/10.1017/S1356186305005638.

Nunes Pauli, G.E., De La Escosura-Muñiz, A., Parolo, C., Helmuth Bechtold, I., Merkoçi, A., 2015. Lab-in-a-syringe using gold nanoparticles for rapid immunosensing of protein biomarkers. Lab Chip 15, 399–405. https://doi.org/10.1039/c4lc01123f.

Pagliaccia, D., Shi, J., Pang, Z., Hawara, E., Clark, K., Thapa, S.P., De Francesco, A., Liu, J., Tran, T.T., Bodaghi, S., Folimonova, S.Y., Ancona, V., Mulchandani, A., Coaker, G., Wang, N., Vidalakis, G., Ma, W., 2017. A pathogen secreted protein as a detection marker for citrus huanglongbing. Front. Microbiol. 8 https://doi.org/10.3389/fmicb.2017.02041.

Palchetti, I., Mascini, M., 2008. Electroanalytical Biosensors and Their Potential for Food Pathogen and Toxin Detection, pp. 455–471. https://doi.org/10.1007/s00216-008-1876-4.

Park, S.N., Lim, Y.K., Freire, M.O., Cho, E., Jin, D., Kook, J.K., 2012. Antimicrobial effect of linalool and α-terpineol against periodontopathic and cariogenic bacteria. Anaerobe 18, 369–372. https://doi.org/10.1016/j.anaerobe.2012.04.001.

Parolo, A., Merkoc, C., Escosura-mun, A. De, 2013. Biosensors and Bioelectronics Enhanced lateral flow immunoassay using gold nanoparticles loaded with enzymes, 40, pp. 412–416. https://doi.org/10.1016/j.bios.2012.06.049.

Paternolli, C., Antonini, M., Ghisellini, P., Nicolini, C., 2004. Recombinant cytochrome P450 immobilization for biosensor applications. Langmuir 20, 11706–11712. https://doi.org/10.1021/la048081q.

Peluffo, L., Lia, V., Troglia, C., Maringolo, C., Norma, P., Escande, A., Esteban Hopp, H., Lytovchenko, A., Fernie, A.R., Heinz, R., Carrari, F., 2010. Metabolic profiles of sunflower genotypes with contrasting response to Sclerotinia sclerotiorum infection. Phytochemistry 71, 70–80. https://doi.org/10.1016/j.phytochem.2009.09.018.

Petrellis, N., 2017. A smart phone image processing application for plant disease diagnosis. In: 2017 6th Int. Conf. Mod. Circuits Syst. Technol. MOCAST, vol. 2017, pp. 1–4. https://doi.org/10.1109/MOCAST.2017.7937683.

Pourreza, A., Suk, W., Ehsani, R., Schueller, J.K., Raveh, E., 2015. An optimum method for real-time in-field detection of Huanglongbing disease using a vision sensor. Comput. Electron. Agric. 110, 221–232. https://doi.org/10.1016/j.compag.2014.11.021.

Prasad, S., Xu, J., Zhang, Y., Wang, N., 2016. SEC-translocon dependent extracytoplasmic proteins of Candidatus liberibacter asiaticus. Front. Microbiol. 7, 1–9. https://doi.org/10.3389/fmicb.2016.01989.

Rad, F., Mohsenifar, A., Tabatabaei, M., Safarnejad, M.R., Shahryari, F., Safarpour, H., Foroutan, A., Mardi, M., Davoudi, D., Fotokian, M., 2012. Detection of Candidatus Phytoplasma aurantifolia with a quantum dots fret-based biosensor. J. Plant Pathol. 94, 525–534.

Raddadi, N., Attilio Bianco, P., Tedeschi, R., Gonella, E., Mandrioli, M., Pizzinat, A., Daffonchio, D., Crotti, E., Camerota, C., Alma, A., 2011. 'Candidatus Liberibacter europaeus' sp. nov. that is associated with and transmitted by the psyllid Cacopsylla pyri apparently behaves as an endophyte rather than a pathogen. Environ. Microbiol. 13, 414–426. https://doi.org/10.1111/j.1462-2920.2010.02347.x.

Ramamurthy, T., Ghosh, A., Pazhani, G.P., Shinoda, S., 2014. Current perspectives on viable but non-culturable (VBNC) pathogenic bacteria. Front. public Heal. 2, 103.

Rani, A., Donovan, N., Mantri, N., 2019. Review: the future of plant pathogen diagnostics in a nursery production system. Biosens. Bioelectron. 145, 111631. https://doi.org/10.1016/j.bios.2019.111631.

Rayfuse, R., Weisfelt, N., 2012. The Challenge of Food Security 327, 812–819. https://doi.org/10.4337/9780857939388.

Richter, Ł., Albrycht, P., Księżopolska-Gocalska, M., Poboży, E., Bachliński, R., Sashuk, V., Paczesny, J., Hołyst, R., 2020. Fast and efficient deposition of broad range of analytes on substrates for surface enhanced Raman spectroscopy. Biosens. Bioelectron. 156, 112124. https://doi.org/10.1016/j.bios.2020.112124.

Rigano, L.A., Malamud, F., Orce, I.G., Filippone, M.P., Marano, M.R., Morais, A., Castagnaro, A.P., Vojnov, A.A., 2014. Rapid and Sensitive Detection of Candidatus Liberibacter Asiaticus by Loop Mediated Isothermal Amplification Combined with a Lateral Flow Dipstick, pp. 1–9.

Roberts, R., Steenkamp, E.T., Pietersen, G., 2015. Three novel lineages of 'Candidatus liberibacter africanus' associated with native rutaceous hosts of trioza erytreae in South Africa. Int. J. Syst. Evol. Microbiol. 65, 723–731. https://doi.org/10.1099/ijs.0.069864-0.

Ronkainen, N.J., Brian, H., Heineman, W.R., 2010. Electrochemical Biosensors 1747–1763. https://doi.org/10.1039/b714449k.

Rubab, M., Shahbaz, H.M., Olaimat, A.N., Oh, D.H., 2018. Biosensors for rapid and sensitive detection of Staphylococcus aureus in food. Biosens. Bioelectron. 105, 49–57. https://doi.org/10.1016/j.bios.2018.01.023.

Sadanandom, A., Napier, R.M., 2010. Biosensors in plants. Curr. Opin. Plant Biol. 13, 736–743. https://doi.org/10.1016/j.pbi.2010.08.010.

Sadani, K., Nag, P., Mukherji, S., 2019. LSPR based optical fiber sensor with chitosan capped gold nanoparticles on BSA for trace detection of Hg (II) in water, soil and food samples. Biosens. Bioelectron. 134, 90–96. https://doi.org/10.1016/j.bios.2019.03.046.

Sanchez, L., Pant, S., Xing, Z., Mandadi, K., Kurouski, D., 2019. Rapid and noninvasive diagnostics of Huanglongbing and nutrient deficits on citrus trees with a handheld Raman spectrometer. Anal. Bioanal. Chem. 411, 3125–3133. https://doi.org/10.1007/s00216-019-01776-4.

Sankaran, S., Ehsani, R., 2011. Visible-near infrared spectroscopy based citrus greening detection : evaluation of spectral feature extraction techniques. Crop Protect. 30, 1508–1513. https://doi.org/10.1016/j.cropro.2011.07.005.

Sankaran, S., Mishra, A., Ehsani, R., Davis, C., 2010. A review of advanced techniques for detecting plant diseases. Comput. Electron. Agric. 72, 1–13. https://doi.org/10.1016/j.compag.2010.02.007.

Savary, S., Ficke, A., Aubertot, J.-N., Hollier, C., 2012. Crop losses due to diseases and their implications for global food production losses and food security. Food Secur 4, 519–537. https://doi.org/10.1007/s12571-012-0200-5.

Sayad, A.A., Ibrahim, F., Uddin, S.M., Pei, K.X., Mohktar, M.S., Madou, M., Thong, K.L., 2016. A microfluidic lab-on-a-disc integrated loop mediated isothermal amplification for foodborne pathogen detection. Sensor. Actuator. B Chem. 227, 600–609. https://doi.org/10.1016/j.snb.2015.10.116.

Scala, A., Allmann, S., Mirabella, R., Haring, M.A., Schuurink, R.C., 2013. Green Leaf Volatiles : A Plant ' S Multifunctional Weapon against Herbivores and Pathogens, pp. 17781–17811. https://doi.org/10.3390/ijms140917781.

Scala, V., Pucci, N., Loreti, S., 2018. The diagnosis of plant pathogenic bacteria: a state of art. Front. Biosci. - Elit. 10, 449–460.

Schofield, D.A., Bull, C.T., Rubio, I., Patrick Wechter, W., Westwater, C., Molineux, I.J., 2013. "Light-tagged" bacteriophage as a diagnostic tool for the detection of phytopathogens. Bioengineered 4, 50–54. https://doi.org/10.4161/bioe.22159.

Sharifi, S., Vahed, S.Z., Ahmadian, E., Dizaj, S.M., Eftekhari, A., Khalilov, R., Ahmadi, M., Hamidi-Asl, E., Labib, M., 2020. Detection of pathogenic bacteria via nanomaterials-modified aptasensors. Biosens. Bioelectron. 150, 111933. https://doi.org/10.1016/j.bios.2019.111933.

Sharma, P., Sharma, S., 2016. Current Trends in Plant Disease Diagnostics and Management Practices, pp. 237–264. https://doi.org/10.1007/978-3-319-27312-9.

Shi, Q., Febres, Vicente J., Khalaf, A., 2014. Lflg22, a pathogen-associated molecular pattern (PAMP) of candidatus liberibacter asiaticus, initiated differential PAMP-triggered immunity (PTI) in grapefruit and sun chu sha. Rev. Gen. Psychol. 9 (2), 0–1.

Shokrollah, H., Lee, T., Sijam, K., Nor, S., Abdullah, A., 2011. Potential use of selected citrus rootstocks and interstocks against HLB disease in Malaysia. Crop Protect. 30, 521–525. https://doi.org/10.1016/j.cropro.2010.09.005.

Skottrup, P.D., Nicolaisen, M., Justesen, A.F., 2008. Towards on-site pathogen detection using antibody-based sensors. Biosens. Bioelectron. 24, 339–348. https://doi.org/10.1016/j.bios.2008.06.045.

Srivastava, A., Sharma, S.K., 2010. Development of a robotic navigator to assist the farmer in field. Proc. Int. MultiConference Eng. Comput. Sci. 2010, 1009–1012. IMECS 2010 II.

Stiefel, P., Schmidt-Emrich, S., Maniura-Weber, K., Ren, Q., 2015. Critical aspects of using bacterial cell viability assays with the fluorophores SYTO9 and propidium iodide. BMC Microbiol. 15, 1–9.

Strange, R.N., Scott, P.R., 2005. Plant disease: a threat to global food security. Annu. Rev. Phytopathol. 43, 83–116. https://doi.org/10.1146/annurev.phyto.43.113004.133839.

Sturbaum, G.D., Klonicki, P.T., Marshall, M.M., Jost, B.H., Clay, B.L., Sterling, C.R., 2002. Immunomagnetic separation (IMS)-fluorescent antibody detection and IMS-PCR detection of seeded Cryptosporidium parvum oocysts in natural waters and their limitations. Appl. Environ. Microbiol. 68, 2991–2996.

Su, D.-S., Chen, P.-Y., Chiu, H.-C., Han, C.-C., Yen, T.-J., Chen, H.-M., 2019. Disease antigens detection by silicon nanowires with the efficiency optimization of their antibodies on a chip. Biosens. Bioelectron. 141 https://doi.org/10.1016/j.bios.2019.03.042.

Sugio, A., Kingdom, H.N., MacLean, A.M., Grieve, V.M., Hogenhout, S.A., 2011. Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and defense hormone biosynthesis. Proc. Natl. Acad. Sci. U.S.A. 108, E1254–63. https://doi.org/10.1073/pnas.1105664108.

Teixeira, D.C., Saillard, C., Couture, C., Martins, E.C., Wulff, N.A., Eveillard-jagoueix, S., Yamamoto, P.T., Ayres, A.J., Bove, J.M., 2008. Distribution and quantification of Candidatus Liberibacter americanus , ~ o Paulo State , Brasil , agent of huanglongbing disease of citrus in Sa in leaves of an affected sweet orange tree as determined by PCR 22, pp. 139–150. https://doi.org/10.1016/j.mcp.2007.12.006.

Thompson, S.M., Johnson, C.P., Lu, A.Y., Frampton, R.A., Sullivan, K.L., Fiers, M.W.E.J., Crowhurst, R.N., Pitman, A.R., Scott, I.A.W., Wen, A., Gudmestad, N.C., Smith, G.R., 2015. Genomes of "candidatus liberibacter solanacearum" haplotype a from New Zealand and the United States suggest significant genome plasticity in the species. Phytopathology 105, 863–871. https://doi.org/10.1094/PHYTO-12-14-0363-FI.

Tlili, C., Sokullu, E., Safavieh, M., Tolba, M., Ahmed, M.U., Zourob, M., 2013. Bacteria Screening, Viability, and Con Fi Rmation Assays Using Bacteriophage-Impedimetric/Loop-Mediated Isothermal Ampli Fi Cation Dual-Response Biosensors. https://doi.org/10.1021/ac302699x.

Umasankar, Y., Ramasamy, R.P., 2013. Highly sensitive electrochemical detection of methyl salicylate using electroactive gold nanoparticles. Analyst 138, 6623. https://doi.org/10.1039/c3an01295f.

Valderrama, W.B., Dudley, E.G., Doores, S., Cutter, C.N., 2016. Commercially available rapid methods for detection of selected food-borne pathogens. Crit. Rev. Food Sci. Nutr. 56, 1519–1531.

Vanegas, D.C., Gomes, C.L., Cavallaro, N.D., Giraldo-Escobar, D., McLamore, E.S., 2017. Emerging biorecognition and transduction schemes for rapid detection of pathogenic bacteria in food. Compr. Rev. Food Sci. Food Saf. 16, 1188–1205. https://doi.org/10.1111/1541-4337.12294.

Vaseghi, A., Bakhshinejad, B., Safaie, N., Ashrafi Parchin, R., Sadeghizadeh, M., 2013a. PCR Amplification of the hrcV Gene through Specific Primers for Detecting Pseudomonas syringae Pathovars. https://doi.org/10.1007/s11274-013-1438-6.

Vaseghi, A., Safaie, N., Bakhshinejad, B., Mohsenifar, A., Sadeghizadeh, M., 2013b. Sensors and actuators B : chemical detection of Pseudomonas syringae pathovars by thiol-linked DNA – gold nanoparticle probes. Sensor. Actuator. B Chem. 181, 644–651. https://doi.org/10.1016/j.snb.2013.02.018.

Vashist, S.K., Luppa, P.B., Yeo, L.Y., Ozcan, A., Luong, J.H.T., 2015. Emerging technologies for next-generation point-of-care testing. Trends Biotechnol. 33, 692–705. https://doi.org/10.1016/j.tibtech.2015.09.001.

Virkler, K., Lednev, I.K., 2009. Blood species identification for forensic purposes using Raman spectroscopy combined with advanced statistical analysis. Anal. Chem. 81, 7773–7777. https://doi.org/10.1021/ac901350a.

Wang, C.-H., Lien, K.-Y., Wu, J.-J., Lee, G.-B., 2011. Lab on a Chip PAPER A Magnetic Bead-Based Assay for the Rapid Detection of Methicillin-Resistant Staphylococcus aureus by Using a Microfluidic System with Integrated Loop- Mediated Isothermal Amplification †, pp. 1521–1531. https://doi.org/10.1039/c0lc00430h.

Wang, N., Pierson, A., Setubal, E., Carlos, João, Xu, J., Levy, J.G., Zhang, Y., Li, J., Rangel, L.T., Martins, J., 2017. The candidatus liberibacter–host interface: insights into pathogenesis mechanisms and disease control. Annu. Rev. Phytopathol. 55, 451–482. https://doi.org/10.1146/annurev-phyto-080516-035513.

Wang, X., Zhu, P., Pi, F., Jiang, H., Shao, J., Zhang, Y., Sun, X., 2016. A Sensitive and simple macrophage-based electrochemical biosensor for evaluating lipopolysaccharide cytotoxicity of pathogenic bacteria. Biosens. Bioelectron. 81, 349–357. https://doi.org/10.1016/j.bios.2016.03.007.

Wang, Z., Yue, T., Yuan, Y., Cai, R., Niu, C., Guo, C., 2013. Development and evaluation of an immunomagnetic separation–ELISA for the detection of Alicyclobacillus spp. in apple juice. Int. J. Food Microbiol. 166, 28–33.

West, J.S., Heard, S., 2014. Detection and diagnostics of plant pathogens. Detect. Diagnostics Plant Pathog. https://doi.org/10.1007/978-94-017-9020-8, 1–200.

Wu, F., Deng, X., Liang, G., Wallis, C., Trumble, J.T., Prager, S., Chen, J., 2015a. De novo genome sequence of "Candidatus Liberibacter solanacearum" from a single potato psyllid in California. Genome Announc. 3, 14–15. https://doi.org/10.1128/genomeA.01500-15.

Wu, F., Zheng, Z., Deng, X., Cen, Y., Liang, G., Chen, J., 2015b. Draft genome sequence of "candidatus liberibacter asiaticus" from Diaphorina citri in Guangdong, China. Genome Announc. 3, 14–15. https://doi.org/10.1128/genomeA.01316-15.

Wu, F., Zheng, Z., Deng, X., Cen, Y., Liang, G., Chen, J., 2015c. Draft genome sequence of "candidatus liberibacter asiaticus" from Diaphorina citri in Guangdong, China. Genome Announc. 3, 119–120. https://doi.org/10.1128/genomeA.01316-15.

Wulff, N.A., Zhang, S., Setubal, J.C., Almeida, N.F., Martins, E.C., Harakava, R., Kumar, D., Rangel, L.T., Foissac, X., Bové, J.M., Gabriel, D.W., Fundecitrus, D.C., Fitopatológica, L.D.B., Biológico, I., Paulo, S., 2014. The complete genome sequence of ' candidatus liberibacter americanus. Associated with Citrus Huanglongbing 27, 163–176.

Yan, Q., Sreedharan, A., Wei, S., Wang, J., Pelz-Stelinski, K., Folimonova, S., Wang, N., 2013. Global gene expression changes in CandidatusLiberibacter asiaticus during the transmission in distinct hosts between plant and insect. Mol. Plant Pathol. 14, 391–404. https://doi.org/10.1111/mpp.12015.

Yang, F., Chang, T.L., Liu, T., Wu, D., Du, H., Liang, J., Tian, F., 2019. Label-free detection of Staphylococcus aureus bacteria using long-period fiber gratings with functional polyelectrolyte coatings. Biosens. Bioelectron. 133, 147–153. https://doi.org/10.1016/j.bios.2019.03.024.

Yanling, C., Li, T., Zheng, Z., Xu, M., Deng, X., 2019. Draft Whole-Genome Sequence of a "Candidatus Liberibacter Asiaticus" Strain from Yunnan. China Yanling, pp. 17–19.

Yao, K.S., Li, S.J., Tzeng, K.C., Cheng, T.C., Chang, C.Y., Chiu, C.Y., Liao, C.Y., Hsu, J.J., Lin, Z.P., 2009. Fluorescence Silica Nanoprobe as a Biomarker for Rapid Detection of Plant Pathogens, 82, pp. 513–516. https://doi.org/10.4028/www.scientific.net/AMR.79-82.513.

Zeng, C., Huang, X., Xu, J., Li, G., Ma, J., Ji, H., Zhu, S., Chen, H., 2013. Rapid and sensitive detection of maize chlorotic mottle virus using surface plasmon resonance-based biosensor. Anal. Biochem. 440, 18–22. https://doi.org/10.1016/j.ab.2013.04.026.

Zeng, Z.C., Hu, S., Huang, S.C., Zhang, Y.J., Zhao, W.X., Li, J.F., Jiang, C., Ren, B., 2016. Novel electrochemical Raman spectroscopy enabled by water immersion objective. Anal. Chem. 88, 9381–9385. https://doi.org/10.1021/acs.analchem.6b02739.

Zhang, H., Miller, B.L., 2019. Immunosensor-based label-free and multiplex detection of influenza viruses: state of the art. Biosens. Bioelectron. 141, 111476. https://doi.org/10.1016/j.bios.2019.111476.

Zhang, X., Xie, G., Gou, D., Luo, P., Yao, Y., Chen, H., 2019. A novel enzyme-free electrochemical biosensor for rapid detection of Pseudomonas aeruginosa based on high catalytic Cu-ZrMOF and conductive Super P. Biosens. Bioelectron. 142, 111486. https://doi.org/10.1016/j.bios.2019.111486.

Zhao, W., Lu, J., Ma, W., Xu, C., Kuang, H., Zhu, S., 2011. Biosensors and Bioelectronics Rapid on-site detection of Acidovorax avenae subsp . citrulli by gold-labeled DNA strip sensor. Biosens. Bioelectron. 26, 4241–4244. https://doi.org/10.1016/j.bios.2011.04.004.

Zhao, Y., Liu, L., Kong, D., Kuang, H., Wang, L., Xu, C., 2014. Dual amplified electrochemical immunosensor for highly sensitive detection of Pantoea stewartii sbusp. stewartii. ACS Appl. Mater. Interfaces 6, 21178–21183. https://doi.org/10.1021/am506104r.

Zheng, Z., Deng, X., Chen, J., 2014a. Draft genome sequence of "candidatus liberibacter asiaticus" from California. Genome 2, 4–5. https://doi.org/10.1128/genomeA.00999-14 (Copyright).

Zheng, L., Cai, G., Wang, S., Liao, M., Li, Y., Lin, J., 2019. A microfluidic colorimetric biosensor for rapid detection of Escherichia coli O157:H7 using gold nanoparticle aggregation and smart phone imaging. Biosens. Bioelectron. 124–125, 143–149. https://doi.org/10.1016/j.bios.2018.10.006.

Zheng, Z., Clark, N., Keremane, M., Lee, R., Wallis, C., Deng, X., Chen, J., 2014b. Whole-Genome Sequence of "Candidatus Liberibacter Solanacearum" Strain R1 from California 2, pp. 2–3. https://doi.org/10.1128/genomeA.01353-14 (Copyright).

Zheng, Z., Sun, X., Deng, X., Chen, J., 2016. Whole-genome sequence of "Candidatus Liberibacter asiaticus" from a huanglongbing-affected citrus tree in central Florida. Genome Announc. 3, 7–8. https://doi.org/10.1128/genomeA.00169-15.

Zhou, J., Qi, Q., Wang, C., Qian, Y., Liu, G., Wang, Y., Fu, L., 2019. Surface plasmon resonance (SPR) biosensors for food allergen detection in food matrices. Biosens. Bioelectron. 142, 111449. https://doi.org/10.1016/j.bios.2019.111449.