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LETTER TO THE EDITOR

PCR DETECTION AND IDENTIFICATION OF PLANT-PATHOGENIC BACTERIA: UPDATED REVIEW OF PROTOCOLS (1989-2007)

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SUMMARY

PCR-based methods offer advantages over more traditional diagnostic tests, in that organisms do not need to be cultured prior to their detection and protocols are highly sensitive and rapid. Consequently, there is a shift in research towards DNA-based techniques. Although reports already exist on a variety of PCR-based fingerprinting assays used to analyse the genetic diversity of bacterial populations and define their relationships, this review focuses on the general use of PCR in phytobacteriology for detection and diagnosis purposes. An updated and detailed list of published PCR protocols for detection and identification of plant-pathogenic bacteria is presented and discussed, aimed at facilitating access to information that could be particularly useful for diagnostic laboratories. This compilation includes and discusses 246 articles published between 1989 and 2007 addressing 23 genera, more than 50 species, 10 subspecies and more than 40 pathovars.

Key words: co-operational PCR; multiplex PCR; nested-PCR; real-time PCR.

INTRODUCTION

Control of diseases caused by plant-pathogenic bacteria usually requires accurate detection, followed by proper identification of the causal organism. Although presumptive diagnosis of bacterial diseases can be relatively simple when typical symptomatology is evident, symptoms in plants are not always specific and can be confused with those caused by other biotic or abiotic agents. On the other hand, detection of bacteria in symptomless plant material for preventive control is necessary but can be extremely difficult, since low populations with uneven distribution of the pathogen can occur, so highly sensitive protocols are required. Nucleic-acid based tests offer greater sensitivity, specificity, reliability and may be quicker than many conventional methods used to detect plant-pathogenic bacteria in different plant hosts and environments. With the development of polymerase chain reaction (PCR), and especially real-time PCR, such high sensitivity is achieved, improving the accuracy of pathogen detection and identification (Mullis, 1987; Holland *et al.*, 1991; Vincelli and Tisserat, 2008).

Globalisation implies that state borders have become more open due to increase in free-trade agreements, and this can facilitate the introduction and dissemination of foreign pathogens. This, in turn, leads to emerging diseases, which are a growing reality for phytopathologists worldwide. A guiding principle for disease prevention is that when key inoculum sources have been identified, effective measures must be taken to prevent further spread and subsequent disease outbreaks. Consequently, detection of the causal organisms becomes essential, as most bacterial diseases are transmitted through contaminated seeds or propagative plant material. Plant quarantine polices and regulations have been implemented in many countries to avoid pathogens from spreading and/or to prevent exotic pathogens from being introduced with plant material. To achieve this goal, complex control systems have been designed, which often include guidelines for rapid, sensitive and specific pathogen detection and diagnosis and among them, PCR is the technique of choice for rapid screening.

Compared to conventional diagnostic methods, PCR offers several advantages, because organisms do not need to be cultured prior to detection; moreover it is highly sensitive, relatively simple and fast to perform. There has been a shift towards DNA-based protocols developed for diagnostic purposes as well as for etiological or epidemiological studies, as reported by reviews published over the past fifteen years (Henson and French, 1993; Louws *et al.*, 1999; López *et al.*, 2003; Schaad *et al.*, 2003; Alvarez, 2004; López *et al.*, 2006; Vincelli and Tisseral, 2008; López *et al.*, 2009). Application of PCR techniques in diagnostic laboratories for routine purposes is also increasing and will continue in the near future, especially for the rapid screening of

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samples. PCR is now considered a routine technique and recommended in most protocols recently developed by the European Union and the European and Mediterranean Plant Protection Organization (EPPO) (Anonymous 2004a, 2004b; 2005a, 2005b; 2006a, 2006b, 2006c, 2006d; 2007; López *et al.*, 2006).

Taxonomy of plant-pathogenic bacteria has been extensively revised in recent years. Therefore, in the present compilation, the names utilised are those recorded in the "List of Names of Plant Pathogenic Bacteria, 1864-2004" of the International Society for Plant Pathology (ISPP) (http://isppweb.org/names_bacterial.asp) and have been used to classify the listed publications. However, when the original bacterial genus or species differs from the one in the ISPP list (due to different reasons and the fact that some of the cited articles were published before the latest taxonomic revisions appeared) both the originally cited name and its current nomenclature, according to the ISPP, are indicated.

A wide range of plant-pathogenic bacteria can be currently detected by PCR in numerous hosts or environmental samples (Schaad *et al.*, 2001). This compilation provides an updated listing of PCR published protocols for detection and identification of phytopathogenic bacteria, which could be especially useful for diagnosis laboratories. It contains a non-exhaustive list of 246 references related to PCR protocols published from 1989 up to 2007, which refers to 23 bacterial genera including more than 50 species, 10 subspecies and more than 40 pathovars.

This work summarizes essential data from each of the published protocols and, in order to facilitate searches, information is presented according to each bacterial genus in a Table, which comprises the following information: ISPP accepted nomenclature for the target bacteria and name of the bacteria in the original article, primers name and target DNA, variants utilised in the PCR protocol, type of sample and treatment prior to amplification, reference and observations about the method. Protocols for specific detection of bacterial species, alphabetically ordered, appear first, followed by those designed for the simultaneous detection of two or more species, or for other genera that could also be present in a given host. References for each species, subspecies and pathovar are listed in chronological order. The discussion concentrates on the target sequences utilised for primer design as well as on the different DNA extraction protocols or PCR variants utilised.

DISCUSSION

This review presents references and details of most of the available PCR protocols published between 1989 and 2007 for specific detection and identification of plant-pathogenic bacteria. A variety of PCR-based fingerprinting techniques have been described for classification and typing of plant-pathogenic bacteria (Louws *et al.*, 1999), such as randomly amplified polymorphic DNA (RAPD) (Wang *et al.*, 1993), repetitive sequence-based (rep-PCR) (Versalovic *et al.*, 1998; Louws *et al.*, 1994, 1995, 1998), amplified fragment length polymorphism (AFLP) (Janssen *et al.*, 1996), restriction fragment length polymorphism (RFLP) (Darrase *et al.*, 1994; Manceau and Horvais, 1997; Mkandawire *et al.*, 2004) and others. However, the present compilation focuses solely on the PCR protocols available for routine detection, diagnosis or identification of plant-pathogenic bacteria.

One can appreciate from the Table, that the number of references to the different genera is highly variable and not only related to the number of described species or pathovars in every genus, but also to the economic importance of the diseases they cause, their distribution, whether local or widespread, and their status as quarantine organisms. We found more than 50 protocols for species of the genus *Xanthomonas*, more than 40 for *Pseudomonas* spp., 20 for *Ralstonia* spp., 19 for *Clavibacter* and *Agrobacterium* spp., 16 for *Erwinia* and *Xylella* spp., 12 for *Pectobacterium* spp., 11 for "*Candidatus* Liberibacter" spp., nine for *Burkholderia* spp., seven for *Streptomyces* and *Pantoea* spp., six for *Dickeya* and *Xylophylus* spp., four for *Leifsonia* spp., three for *Acidovorax* spp., and only one or two protocols for species of other genera.

Depending on the choice of PCR primers, both narrow and broad specificity can be obtained, allowing detection of a single pathogen or of several members of a group of related pathogens. Primer design requires knowledge of the target DNA sequences and the past two decades have witnessed reports of primers used to identify many plant-pathogenic bacteria (Schaad *et al.*, 2001), multiple strategies being developed to design primers for specific detection and disease diagnosis. Among them, the DNA sequences from known pathogenicity/virulence genes have been used as targets to design specific primers, as those described by Bereswill *et al.* (1994), Darrasse *et al.* (1994), Dreier *et al.* (1995), Leite *et al.* (1995), Nassar *et al.* (1996), Stange *et al.* (1996), Sato *et al.* (1997), Burkhalid *et al.* (1998), Sorensen *et al.* (1998), Kerkoud *et al.* (2002), Loreti and Gallelli (2002), Zaccardelli *et al.* (2005, 2007) or Cullen and Lees (2007).

In other primers reported here, sequences from pathogenicity-related genes in principle specific to a pathogen, or to a group of pathogens, have been employed, such as the *pel* gene of soft-rot diseases caused by pectolytic species or subspecies of the genus *Pectobacterium* (Darrase *et al.*, 1994; Louws *et al.*, 1999), or belonging to a cluster of genes involved in the virulence systems of different bacterial families (*hrp*, *pth* and *vir* genes). The utility of PCR primers that employ specific

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sequences from known pathogenicity genes has been demonstrated for a wide range of bacterial species, although there are also examples of the need to design new primers after the discovery of strains that lack some pathogenicity genes, previously considered universal. For example, the phaseolotoxin gene was considered an excellent target for *Pseudomonas savastanoi* pv. *phaseolicola* detection and several sets of primers were designed on its sequence (Prosen *et al.*, 1993; Schaad *et al.*, 1995, 2007; Audy *et al.*, 1996; Sawada *et al.*, 1997). However, the discovery of nontoxigenic strains of this pathovar showed that these primers were not as specific as expected (Rico *et al.*, 2003). Also, the *nec1* gene was previously proposed for *Streptomyces* pathogenicity testing (Burkhalid *et al.*, 1998), but recent works suggest that the gene seems to play a subsidiary role in pathogenicity and is missing from some pathogenic strains (Wanner, 2004, 2006).

Primers have also been designed on plasmid sequences like those from the Ti plasmid of *Agrobacterium* species (Nesme *et al.*, 1989; Bereswill *et al.*, 1992; Dong *et al.*, 1992; Hartung *et al.*, 1993, 1996; Firrao and Locci, 1994; Sawada *et al.*, 1995; Verdier *et al.*, 1998), although in general plasmid stability must be previously evaluated in order to avoid false negative results. It is assumed that if the plasmid genes encode essential fitness or pathogenicity traits they are stable (Eastwell *et al.*, 1995). Nevertheless, primers targeted to a plasmid reported as universal, sometimes were not found useful for detecting all virulent strains of a group, for example those based on the pEA29 plasmid of *Erwinia amylovora* (Llop *et al.*, 2006).

The ribosomal DNA operon has also frequently been used to design primers that allow highly sensitive detection, but due to its universal nature, the level of discrimination lies at the species or genus levels. The internally transcribed spacer region (ITS) between the 16S and 23S rRNA genes appears to be more variable than 16S and 23S rRNA genes and was used to design primers by Li and De Boer (1995), Kim and Song (1996), Maes *et al.* (1996b), Takeuchi *et al.* (1997), Pan *et al.* (1998), Whitby *et al.* (1998), McDowell *et al.* (2001), Walcott *et al.* (2002), Song *et al.* (2004), Grall *et al.* (2005), Sayler *et al.* (2006) or Grisham *et al.* (2007), among others. However, some primers from rRNA genes, such as those designed for *E. amylovora* (Maes *et al.*, 1996a) showed problems due to lack of specificity because they also amplified another *Erwinia* species (Roselló *et al.*, 2007). The rDNA sequences from unknown bacteria associated with plant disease can be amplified by PCR, subjected to sequence analysis and compared with strains in the RDP database (Ribosomal Database Project) (http://rdp.cme.msu.edu) (Maidak *et al.*, 1999), providing a phylogenetic framework to identify the causal agent.

In other cases, DNA fragments specific to a particular species have been cloned by subtractive hybridisation and used to design primers to detect some organisms (Seal *et al.*, 1992a; Manceau *et al.*, 2005). Furthermore, as the field of genomics progresses, more genome sequences become available and specific primers can be designed to target unique regions of the genome of a given pathogen. Nevertheless, only in very few cases it is reported that these newly available sequences have been employed to design specific primers (López *et al.*, 2008). It is also necessary to check the reliability of the information available in the sequence databases on which the design of specific primers is based, because Arahal *et al.* (2004) found mistakes in primers designed for *Ralstonia solanacearum* and *Clavibacter michiganensis* subsp. *sepedonicus*, when comparing their sequences to those of the databases.

A low copy number of initial target DNA sequences makes the first amplification cycles critical and PCR inhibitors can result in false negatives, which could have a major impact, especially in quarantine settings. In this context, sample preparation is critical, and target DNA should be made as available as possible for amplification. Plant-derived compounds and the presence of different substances, like copper products (Minsavage *et al.*, 1994; Hartung *et al.*, 1996), have been reported as inhibitors of PCR. To avoid this, some PCR protocols reported here submit the samples to some physical or chemical treatments before amplification. Preparation methods listed include dilution, separation and concentration of cells by centrifugation or washing/centrifugation of plant tissue (Maes *et al.*, 1996b; Smid *et al.*, 1995; Pan *et al.*, 1997), or immunomagnetic separation to enhance sensitivity and specificity (van der Wolf *et al.*, 1996; Walcott and Gitaitis, 2000; Walcott *et al.*, 2002; Khoodoo *et al.*, 2005), etc.

Removal of PCR inhibitors from samples using simple procedures is also reported, including treatment with cation-exchange resins (Jacobsen and Rasmussen, 1992) or polyvinyl-pyrrolidone (PVP), which binds to phenolic compounds (Leite *et al.*, 1995; Maes *et al.*, 1996a; Fegan *et al.*, 1998; Pan *et al.*, 1998; Robène-Soustrade *et al.*, 2006). Besides, an increasing number of commercial kits are available for DNA purification from plant material (López *et al.*, 2006) and simple DNA extraction protocols are advised for many targets (Llop *et al.*, 1999). The design of internal PCR controls, based on sequences from the bacteria or from the plant, has also improved sensitivity and avoided false negatives (Pastrik, 2000; Weller *et al.*, 2000; Cubero *et al.*, 2001, 2002; Glick *et al.*, 2002; Pastrik et *al.*, 2002). On the other hand, simply treating the sample at high temperatures for a few minutes has often been used as an adequate pre-amplification treatment for detection of the target sequence from pure bacterial cultures (Seal *et al.*, 1992a, 1992b; Schulz *et al.*, 1993; Sato *et al.*, 1997; Boudazin *et al.*, 1999; Weller *et al.*, 2000; Weller and Stead, 2002; Tan *et al.*, 2003; Kawaguchi *et al.*, 2005;

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Lee *et al.*, 2006; Milijasevic *et al.*, 2006).

Enrichment of the pathogen in a liquid or solid medium can increase its population prior to PCR processing (López *et al.*, 2003). When the sample is first plated on solid medium and micro-colonies are recovered and amplified the method has been named BIO-PCR (Schaad *et al.*, 1995). In general, these enrichment methods facilitate target detection by increasing their numbers and decreasing inhibitors and have proven successful in detecting and identifying bacteria in seeds, soil samples and symptomless plant tissues (Schaad *et al.*, 1995, 1999, 2007; Ito *et al.*, 1998; Manulis *et al.*, 1998; Wang *et al.*, 1999; Penyalver *et al.*, 2000; Weller *et al.*, 2000; Sakthivel *et al.*, 2001; Weller and Stead, 2002; Bertolini *et al.*, 2003b). They are applicable to culturable and fast-growing bacteria and can also detect viable but not culturable cells (VBNC), which are well documented in environmental samples (Roszak and Colwell, 1987) and could constitute a risk as an inoculum source of plant pathogens (Alexander *et al.*, 1999; Ghezzi and Steck, 1999; Grey and Steck, 2001; Ordax *et al.*, 2006, 2009). In this respect, nine-month-old VBNC *E. amylovora* cells detected by PCR became culturable and recovered pathogenicity after brief enrichment in liquid medium (Ordax *et al.*, 2006).

Several variants have been developed to improve sensitivity of conventional PCR. Among the first described, nested-PCR, with both internal and external primers to the target sequence, was reported to increase sensitivity and reduce the effect of inhibitors (Honeycut *et al.*, 1995; McManus and Jones, 1995; Schaad *et al.*, 1995; Hartung *et al.*, 1996; Roberts *et al.*, 1996; Lee *et al.*, 1997b; Mahuku and Goodwin, 1997; Manulis *et al.*, 1998; Poussier and Luisetti, 2000; Pradhanang *et al.*, 2000; Botha *et al.*, 2001; Poussier *et al.*, 2002; Kang *et al.*, 2003; Anonymous, 2004a; Song *et al.*, 2004; Moltmann and Zimmermann, 2005; Falloon *et al.*, 2006; Robène-Soustrade *et al.*, 2006; Cullen and Lees, 2007). However, in nested-PCR the risk of cross-contamination in routine analysis of large numbers of samples is increased by the introduction of a second round of amplification and the simultaneous manipulation of the previously amplified products. To avoid these problems, nested-PCR in a single closed tube has been developed (Llop *et al.*, 2000; Bertolini *et al.*, 2003b).

A new method named co-operational polymerase chain reaction (Co-PCR) (Spanish patent 31 October 2000; P20002613) has been described for highly sensitive detection of plant viruses and bacteria (Olmos *et al.*, 2002; Caruso *et al.*, 2003; Marco-Noales *et al.*, 2008). Co-PCR is based on the simultaneous action of three or more primers that produce three or more amplicons by the combination of the primers and the co-operational action of amplicons for the production of the largest fragment amplified by the external primers. As it is performed in a single reaction, it minimizes contamination risks and has a level of sensitivity similar to nested-PCR and real-time PCR.

Multiplex PCR protocols using specific primers have also been set up for simultaneous detection of two genes of the same bacterial pathogen, thus limiting false positives, (Haas *et al.*, 1995; Arnold *et al.*, 1996; Kawaguchi *et al.*, 2005; Rico *et al.*, 2006), or allowing amplification of several pathogenic bacteria in seed or plant material (Haas *et al.*, 1995; Smid *et al.*, 1995; Arnold *et al.*, 1996; Audy *et al.*, 1996; Mills *et al.*, 1997; Fegan *et al.*, 1998; Toth *et al.*, 1998; Catara *et al.*, 2000; Glick *et al.*, 2002; Berg *et al.*, 2005; Kawaguchi *et al.*, 2005; Kabadjova-Hristova *et al.*, 2006; Peters *et al.*, 2007), or even detection of one bacterium and four viruses in olive plants (Bertolini *et al.*, 2003a).

Further advances have also been made through the use of real-time PCR, which offers advantages over conventional PCR because data are available in real-time, do not require time consuming post-PCR processing and can be quantitative. Moreover, it is a high-throughput technique for many plant pathogens from different sample types (Schaad *et al.*, 2003; Alvarez, 2004; Gitaitis and Walcott, 2007). The ability to quantify pathogen populations using quantitative real-time PCR holds great potential for epidemiological studies, for determining seed-borne or plant-borne inoculum and for establishing and monitoring transmission thresholds as bases for improved disease management (Gitaitis and Walcott, 2007).

Real-time PCR and melting curve analysis (MCA) are state-of-the-art techniques for quantifying nucleic acids, mutation detection, genotyping analysis as well as for detection and diagnosis purposes. Many different systems have been developed, including probe-based methods, such as TaqMan Probes, molecular beacons (Fanelli *et al.*, 2007), Scorpion primers (De Bellis *et al.*, 2007), etc. In general, the protocols developed are based on hybridisation of the probe to the target amplicon, thus achieving maximum sensitivity and confirming the identity of the amplified product (Schaad *et al.*, 1999; Weller *et al.*, 2000; Oliveira *et al.*, 2002; Schaad *et al.*, 2002; Weller and Stead, 2002; Bach *et al.*, 2003; Ozakman and Schaad, 2003; Salm and Geider, 2004; Baumgartner and Warren, 2005; Cubero and Graham, 2005; Fatmi *et al.*, 2005; Anonymous, 2006b; Francis *et al.*, 2006; Koyama *et al.*, 2006; Li *et al.*, 2006b; Cullen and Lees, 2007; De Bellis *et al.*, 2007; Dreo *et al.*, 2007; Fanelli *et al.*, 2007; Li *et al.*, 2007; Schaad *et al.*, 2007; Weller *et al.*, 2007; Zhao *et al.*, 2007). In addition, Koyama *et al.* (2006) developed competitive quenching probes. This new method uses a special fluorescent dye whose fluorescence is quenched by the guanine bases in DNA. The conventional real-time PCR requires real-time measurement of fluorescence intensity during DNA amplification, whereas this novel method only requires measurement of fluorescence intensity before

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and after amplification.

Real-time PCR, which can provide accurate and rapid detection of bacterial pathogens, is becoming the gold standard for diagnosis of plant-pathogenic bacteria, as well as of other organisms. Although only 27 available real-time protocols are referred to here, one should bear in mind that their number has increased from only one in 1999 (Schaad *et al.*, 1999) to seven in 2006 (Anonymous, 2006b; Atallah and Stevenson, 2006; Berg *et al.*, 2006; Francis *et al.*, 2006; Koyama *et al.*, 2006; Li *et al.*, 2006b; Sayler *et al.*, 2006) and nine in 2007 (Cullen and Lees, 2007; De Bellis *et al.*, 2007; Dreo *et al.*, 2007; Fanelli *et al.*, 2007; Grisham *et al.*, 2007; Li *et al.*, 2007; Schaad *et al.*, 2007; Weller *et al.*, 2007; Zhao *et al.*, 2007).

In this compilation, most of the real-time PCR protocols have utilised TaqMan® probes (Applied Biosystems, USA), which provide greater sensitivity and specificity. An alternative to probe-based methods is the use of DNA intercalating dyes that bind to double-stranded DNA. Dyes have much higher fluorescence when bound to double-stranded DNA compared to the unbound state. SYBR Green I became the most widely used DNA dye for real-time PCR applications because of cost efficiency, generic detection of amplified DNA, and its ability to differentiate PCR products by melting curve analysis. Several protocols in the present compilation utilised SYBR Green (Mavdorieva *et al.*, 2004; Salm and Geider, 2004; Atallah and Stevenson, 2006; Sayler *et al.*, 2006; Grisham *et al.*, 2007).

In our experience, it is easy to adapt existing conventional PCR protocols to a real-time PCR assays by using SYBR® Green Master Mix (Qiagen, USA) and utilising them for identification of bacterial cultures. However, there are disadvantages with the use of SYBR Green I, such as inhibition of PCR amplification in a concentration-dependent manner, effects on DNA melting temperature and preferential binding to certain DNA sequences. The drawback of using SYBR Green I for melting curve analysis is that the melting temperature is highly dependent on the concentration of the dye (Ririe *et al.*, 1997) and the DNA (Xu *et al.*, 2000).

Loop-mediated isothermal amplification (LAMP) is another DNA amplification method, based on auto-cycling strand displacement DNA synthesis by a DNA polymerase, which has high strand displacement activity, and a set of specially designed inner and outer primers. Typically, amplification is completed within 30 min using a simple water bath, which is kept constantly at 65°C (Notomi *et al.*, 2000). LAMP-based detection could be as sensitive as a conventional PCR assay for practical diagnosis. The product is rapidly detected on nylon membranes by staining, replacing conventional electrophoresis and visualization of DNA bands under UV illumination. Thus, this method does not depend upon a thermal cycler and electrophoresis apparatus (Okuda *et al.*, 2005; Li *et al.*, 2007).

Accurate detection or diagnosis of plant pathogenic-bacteria often requires multiple complementary tests to achieve definitive identification (Alvarez, 2004; López *et al.*, 2006). Besides, PCR-based approaches require thorough studies of target pathogens to both characterize their diversity and identify common stable markers for designing specific primers. It is necessary to indicate that, although most protocols are claimed to be specific, they must be validated against a large collection of strains of the target bacterium and other pathogens of the same host, as well as against organisms of its environment, before they can be used as standards. The reliablity of the protocols will eventually be demonstrated after years of use, building confidence in their accuracy and robustness in international ring tests among laboratories (Alvarez, 2004). Inter-laboratory evaluations of new detection or diagnostic protocols provide essential information on repeatability and reproducibility, ease of implementation, use and interpretation of tests, giving an indication of their robustness in routine analysis of large numbers of samples. A standard protocol must subsequently be established and optimized based on results (López *et al.*, 2003, 2008; Alvarez, 2004). In this sense the diagnostic protocols for detection of some European Union quarantine bacteria, as *Clavibacter michiganensis* subsp. *sepedonicus*, *Xanthomonas fragariae* and *E. amylovora*, have been validated by ring tests in the DIAGPRO project financed by the "Standard, Measurements and Testing" programme of the European Union, before being adopted by the EPPO.

As more PCR-based methods for detection of phytopathogenic bacteria become available, their use will progressively increase not only for identification purposes, but also for studying pathogen populations in their biology, ecology, and host-pathogen interactions, thus expanding our knowledge of the hidden part of the life cycle of plant pathogenic bacteria.

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Table 1. Name of primers and target DNA, sample treatment in the original article, variant of PCR protocol, reference and observations for protocols for the different genera of plant pathogenic bacteria according to ISPP nomenclature. When the nomenclature reported in articles differs, the originally cited names of bacteria are indicated on the right side of the table.

Genus *Acidovorax*					
Species/subspecies	Primer name Target DNA	Variant of PCR Protocol	Sample (treatment)	Reference	Synonyms/observations
A. avenae subsp. *avenae*	**Aaaf3/Aaar2 (external)** ITS region + **Aaaf5/Aaar2 (internal)** ITS region	Nested BIO	Seed (washes enrichment)	Song *et al.,* 2004	DNA extraction recommended if high level of other microflora is found after enrichment.
A. avenae subsp. *citrulli*	**WFB1/WFB2** 16S rRNA gene	Conventional	Bacteria (lysed) or crude extract and immunocapture	Walcott and Gitaitis, 2000	
A. avenae	**R16-1/R23-2R** ITS region	Conventional	Bacteria (DNA extraction)	Kim and Song, 1996	*Pseudomonas avenae* *Burkholderia glumae* (*Pseudomonas glumae*), *Pantoea agglomerans* (*Erwinia herbicola*), *Pseudomonas fuscovaginae, Pseudomonas syringae* pv. *syringae* and *Xanthomonas oryzae* (pathovars *oryzae* and *oryzicola*) also amplified and differentiated by primary and secondary fragments.
Genus *Agrobacterium*					
Species/biovars	Primer name Target DNA	Variant of PCR Protocol	Sample (treatment)	Reference	Synonyms/observations
Agrobacterium spp.	**tms2F1/tms2R2** **tms2B** *tms2* gene	Semi-nested	Bacteria from soil (DNA extraction)	Pulawska and Sobiczewski, 2005; Sobiczewski *et al.,* 2005	Tumour-inducing strains.
A. tumefaciens	**FGPtmr 530/FGPtmr 701** T-DNA **FGP vir B_{11+12}/FGP vir B 15** Intercistronic *vir* B/G region	Conventional	Bacteria (DNA extraction)	Nesme *et al.,* 1989	
A. tumefaciens	**tms2A/tms2B** pTi *tms2* gene **RBF/RBR** Nopaline type T-DNA **ocsF/ocsR** Octopine type T-DNA **virD2A, virD2C´, virD2E´** *vir* D2 gene	Conventional	Bacteria (boiled)	Tan *et al.,* 2003	

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A. tumefaciens	**FGP vir B_{11+12}/FGP vir B 15** Intercistronic *vir* B/G region	Conventional	Plant roots (DNA extraction)	Puopolo *et al.*, 2007	
A. radiobacter	**Primers** **rol-F/rol-R** **Probe** **rol-Pr** Ri-plasmid	Real-time (TaqMan)	Bacteria (boiled)	Weller and Stead, 2002	*Agrobacterium* biovar 1
A. vitis	**Tm 4 ipt, IS866, S4 6b/vis** T-DNA	Conventional	Bacteria (boiled)	Schulz *et al.*, 1993	
A. vitis	**virA** *virA* region **6a** *6a* gene **pehA** Pectin enzyme hydrolase gene	Conventional	Bacteria (lysed) or plant tissue (DNA extraction)	Eastwell *et al.*, 1995	
A. vitis	**VCF/VCR** *vir*C gene **PGF/PGR** Polygalacturonase gene **VirE2PF/VirE2PR** *vir*E2 gene **VisF/VisR** pTiS4 vitopine synthase gene	Conventional	Bacteria (lysed)	Szegedi and Bottka, 2002	
A. tumefaciens *A. rhizogenes*	**UF** Universal agrobacteria 23S rRNA gene **BIR** *A. tumefaciens* specific 23S rRNA gene **B2R** *A. rhizogenes* specific 23S rRNA gene **AvR** *A. vitis* specific 23S rRNA gene **ArR** *A. rubi* specific 23S rRNA gene	Multiplex	Bacteria from soil or plant tissue (DNA extraction)	Pulawska *et al.*, 2006	*Agrobacterium* biovar 1 *Agrobacterium* biovar 2

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A. tumefaciens *A. vitis*	**Wide 1/Wide 2 (WHR)** T-DNA **Narrow 1/Narrow 2 (NHR)** T-DNA	Conventional	Bacteria (DNA extraction)	Dong *et al.*, 1992	*A. tumefaciens* biovar 1 *A. tumefaciens* biovar 3
A. tumefaciens *A. vitis*	**FGPtmr 530/FGPtmr 701** T-DNA **FGP vir B_{11+12}/FGP vir B 15** Intercistronic *vir* B/G region **VCF/VCR** *vir* C gene	Conventional	Bacteria (boiled) or plant tissue (DNA extraction)	Cubero *et al.*, 1999	
A. tumefaciens *A. vitis*	**VCF/VCR** *vir*C **VisF/VisR** pTiS4 vitopine synthase gene **TF/TR** *6b* gene of *A. vitis* octopine pTis **NF/NR** *6b* gene of *A. vitis* nopaline pTis **ttuCfw/ttuCrev** *A. vitis* tartrate deshydrogenase gene	Conventional	Bacteria (lysed)	Szegedi *et al.*, 2005	
A. vitis *A. radiobacter*	**Ab3-F3/Ab3-R4** *Agrobacterium* and *Rhizobium* 16S rRNA gene **VCF3/VCR3** *vir*C gene	Multiplex	Bacteria (boiled)	Kawaguchi *et al.*, 2005	*A. tumefaciens* biovar 3 (tumorigenic *A. vitis*) *A. radiobacter* biovar 3 (nonpathogenic *A. vitis*)
A. tumefaciens *A. rhizogenes* *A. vitis*	**A, C´, E´** *vir* D2 gene **CYT/CYT´** *ipt* oncogene	Conventional Multiplex	Bacteria (DNA extraction or boiled)	Haas *et al.*, 1995	
A. tumefaciens *A. rhizogenes* *A. vitis*	**VCF/VCR** *vir*C gene	Conventional	Bacteria (cells lysates or DNA extraction)	Sawada *et al.*, 1995	*Agrobacterium* biovar 1 (Ti or Ri plasmid) *Agrobacterium* biovar 2 (Ti or Ri plasmid) *Agrobacterium* biovar 3 (Ti plasmid)
A. tumefaciens *A. rhizogenes*	**VirE2PF/VirE2PR** *vir* E2 gene	Conventional	Bacteria (DNA extraction)	Genov *et al.*, 2006	

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A. vitis	**VisF/VisR** Vitopine synthase gene				
A. tumefaciens *A. rhizogenes* *A. vitis* *A. rubi*	**FGPS6, FGPS1509´, FGPL 132´** Chromosomal genes **FGPtmr 530, FGPtmr 701, FGPnos975, FGPnos1236´, FGPvir**A2275, FGP*vir*$B_2$164´ Ti plasmid genes	PCR-RFLP	Bacteria (DNA extraction)	Ponsonnet and Nesme, 1994	*Agrobacterium* biovar 1 *Agrobacterium* biovar 2 *Agrobacterium* biovar 3
A. tumefaciens *A. tumefaciens* *A. rhizogenes* *A. vitis* *A. rhizogenes* (nonpathogenic)	**VCF3/VCR3** *vir*C gene **VCF5/VCR5** *vir*C gene	Conventional	Bacteria (cells directly added to PCR mix)	Suzaki *et al.,* 2004	*A. tumefaciens* biovar 1 (Ti plasmid) *A. rhizogenes* biovar 1 (Ri plasmid) *A. tumefaciens* biovar 2 (Ti plasmid) *A. tumefaciens* biovar 3 (Ti plasmid) *A. radiobacter* biovar 2
Genus *Brenneria*					
Species	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
B. salicis	**Es1A/Es4B** 16S rRNA gene	Conventional	Bacteria (boiled) and plant vascular fluid (DNA extraction)	Hauben *et al.,* 1998	
Genus *Burkholderia*					
Species	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
B. andropogonis	**Pf/Pr** 16S rRNA gene	Conventional	Bacteria (DNA extraction)	Bagsic *et al.,* 1995	*Pseudomonas andropogonis*
B. caryophylli	**P1240-5´/P480-5** 16S rRNA gene	Conventional and BOX-PCR	Bacteria (boiled)	Anon., 2006a	Amplify also other species (but shows a distinct profile for *B. caryophilli*). Adviced in the EPPO protocol.
B. cepacia	**PSL1/PSR1** 16S rRNA gene **PSL/PSR** 16S rRNA gene **G1/G2** ITS region	Conventional and RFLP	Bacteria (DNA extraction)	Whitby *et al.,* 1998; McDowell *et al.,* 2001	
B. gladioli	**CMG16-1/G-16-2** 16S rRNA gene **CMG-23-1/G-23-2** 23S rRNA gene	Conventional	Bacteria (DNA extraction)	Bauernfeind *et al.,* 1998	

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B. gladioli	**Eub-16-1** Eubacteria 16S rDNAs **Gl-16-2** *B. gladioli* 16S rRNA gene	Conventional	Bacteria (DNA extraction)	Bauernfeind *et al.*, 1999	
B. glumae	**R16-1/R23-2R** ITS region	Conventional	Bacteria (DNA extraction)	Kim and Song, 1996	*Pseudomonas glumae* *Pantoea agglomerans* (*Erwinia herbicola*), *Pseudomonas fuscovaginae, Pseudomonas syringae* pv. *syringae* and *Xanthomonas oryzae* (pathovars *oryzae* and *oryzicola*) also amplified and differentiated by primary and secondary fragments.
B. glumae	**GL-13f/GL-14r** ITS region	Conventional	Bacteria or plant tissue (boiled)	Takeuchi *et al.*, 1997	
B. glumae	**Forward/Reverse** ITS region	Real-time (SBYR® Green Master Mix)	Seed washes and plants (without extraction step)	Sayler *et al.*, 2006	
B. plantarii	**PL-12f/PL-11r** ITS region	Conventional	Bacteria or plant tissue (boiled)	Takeuchi *et al.*, 1997	
Genus *Clavibacter*					
Species/subspecies	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
Clavibacter and *Rathayibacter* (genus specific)	**R16FO/CBR16R1** **+** **CBR16F2/CBR16R2** 16S rDNA	Nested	Bacteria (DNA extraction)	Lee *et al.*, 1997a	Restriction enzyme analysis required for differentiation species and subspecies inside both genera.
C. michiganensis subsp. *insidiosus*	**CIRS-1/CIRS2** Insertion element	Conventional	Plant tissue and seeds (DNA extraction)	Samac *et al.*, 1998	
C. michiganensis subsp. *michiganensis*	**CMM-5/CMM-6** *Pat-1* gene plasmid DNA	Conventional	Plant tissue and seeds (DNA extraction) bacteria (boiled)	Dreier *et al.*, 1995	
C. michiganensis subsp. *michiganensis*	**CM_3/CM_4** DNA fragment from a cloned pathogenic isolate	Conventional	Bacteria, seeds (alkaline lysis and boiled)	Santos *et al.*, 1997	
C. michiganensis subsp. *michiganensis*	**CMM5/CMM6** *Pat-1* gene plasmid DNA **PSA-4/PSA-R** 16S-23S rDNA spacer region	Conventional	Bacteria (boiled)	Anon., 2005a; Milijasevic *et al.*, 2006	Recommended in the EPPO protocol.
C. michiganensis subsp. *michiganensis*	**CMM-5/CMM-6** *Pat-1* gene plasmid DNA	Conventional	Bacteria (DNA extraction)	Hadas *et al.*, 2005	

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	CM_3/CM_4 DNA fragment from a cloned pathogenic isolate	BIO			
C. michiganensis subsp. *sepedonicus*	**A47A/A47B** CS1 plasmid sequence fragment	Conventional	Bacteria (untreated)	Firrao and Locci, 1994	
C. michiganensis subsp. *sepedonicus*	**CMS-6/CMS-7** CS1 plasmid sequence fragment	Conventional Competitive (*Arabidopsis* genomic DNA as internal standard)	Plant tissue (DNA extraction)	Schneider *et al.,* 1993 Hu *et al.,* 1995	Both authors used the same primers but the second protocol can be quantitative.
C. michiganensis subsp. *sepedonicus*	**Spif/Sp5r** 16S-23S rDNA spacer region	Conventional	Bacteria, potato tubers (DNA extraction)	Li and De Boer, 1995	
C. michiganensis subsp. *sepedonicus*	**CSRS-C** Inverted repeat plasmid CS1	Conventional	Bacteria, plant tissue (alkaline treatment)	Slack *et al.,* 1996	
C. michiganensis subsp. *sepedonicus*	**Nested** **CMSIF1/CMSIR1** **+** **CMSIF2/CMSIR2** Insertion element	Nested	Bacteria, potato tubers (DNA extraction)	Lee *et al.,* 1997b	
C. michiganensis subsp. *sepedonicus*	**CMS50F/CMS50R** **CMS72F/CMS72R** **CMS85F/CMS85R** Three primer sets for single or multiplex PCR Chromosomal DNA (unknown)	Conventional Multiplex	Bacteria, potato tubers (DNA extraction)	Mills *et al.,* 1997	
C. michiganensis subsp. *sepedonicus*	**Primers** **Cms 50-2F/Cms 133R** Chromosomal DNA (unknown) **Probe** **Cms 50-53T**	Real-time (TaqMan) BIO+TaqMan	Bacteria (untreated)	Schaad *et al.,* 1999	BIO implies enrichment in solid medium.
C. michiganensis subsp. *sepedonicus*	**PSA-1/PSA-R** 16S-23S rDNA spacer region **NS-7-F/NS-8-R** DNA from potato, eggplant and tomato	Conventional Multiplex (Coamplification of host DNA as internal control)	Bacteria (untreated), potato tissue (DNA extraction)	Pastrik, 2000	

C. michiganensis subsp. *sepedonicus*	See: Schneider *et al.,* 1993; Firrao and Locci, 1994, Li and De Boer, 1995, Slack *et al.,* 1996, Mills *et al.,* 1997, Schaad *et al.,* 1999; Pastrik, 2000	Conventional Real-time (TaqMan)		Anon., 2006b	Recommended in the EPPO protocol
C. michiganensis subspecies: *insidiosus, michiganensis sepedonicus, nebraskensis, tessellarius*	**CMR16F1/CMR16R1** + **CMR16F2/CMR16R2** 16S rRNA gene **CMR16F1/CMR16R1**	Nested Conventional	Bacteria, potato tubers (DNA extraction)	Lee *et al.,* 1997b	Restriction analysis required for differentiation of *C. michiganensis* subsp. *sepedonicus.*
C. michiganensis subspecies: *insidiosus, michiganensis sepedonicus, nebraskensis, tessellarius*	**Universal all subspecies PAS-R/ Subspecies-specific PSA-1** (*C. m.* subsp. *sepedonicus)* **PSA-4** (*C. m.* subsp. *michiganensis)* **PSA-5** (*C. m.* subsp. *insidiosus)* **PSA-2** (*C. m.* subsp. *tesalarius)* **PSA-7** (*C. m.* subsp. *nebraskensis)* 16S-23S rDNA spacer region	Conventional	Bacteria (DNA extraction)	Pastrik and Rainey, 1999	*C. michiganensis* subsp. *insidiosus* and *nebraskensis* yield same band. RAPD-PCR for distinguishing subspecies.
C. michiganensis subspecies: *insidiosus, michiganensis sepedonicus, nebraskensis, tessellarius*	**Primers FP Cm/RP Cm** Common ITS in all subspecies **Subspecies specific probes Cms probe Cmm probe Cmn probe Cmi probe Cmt probe**	Real-time (TaqMan)	Bacteria (DNA extraction)	Bach *et al.,* 2003	
Genus *Curtobacterium*					
Species/pathovars	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
C. flaccumfaciens pv. *flaccumfaciens*	**CF4/CF5** Chromosomal DNA (unknown)	Conventional	Bacteria (DNA extraction)	Guimaraes *et al.,* 2001	

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C. flaccumfaciens pv. *flaccumfaciens*	**CffFOR2/CffREV4** Chromosomal DNA (unknown)	Conventional	Bacteria (DNA extraction) or seeds	Tegli *et al.,* 2002	
Genus *Dickeya*					
Species	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
Dickeya sp.	**ADE1/ADE2** *pel*ADE gen	Conventional	Bacteria (DNA extraction)	Nassar *et al.,* 1996	*Erwinia chrysanthemi* Restriction analysis results correlate with pathovar and biovar.
Dickeya sp.	**5A/5B** pT8-1, *idg* and *pecS* genes	Conventional	Not indicated	Chao *et al.,* 2006	*E. chrysanthemi*
Dickeya sp.	**PelZ-1-F/pelZ-1-S** *Zantedeschia aethiopica pelZ* gene (including an *AhdI* restriction site)	Conventional and RFLP	Bacteria (boiled)	Lee *et al.,* 2006	*E. chrysanthemi* Restriction analysis allows discrimination of *Z. aeothiopica* isolates from other hosts.
Dickeya sp. *Pectobacterium atrosepticum*	**ERWFOR/ATROREV** Metalloprotease genes (specific for *P. atrosepticum*) **ERWFOR+CHRREV +ATROREV** (Simultaneous detection of *Dickeya* spp. and *P. atrosepticum*)	Conventional Multiplex	Bacteria (boiled), potato tubers (centrifugation and lysis buffer)	Smid *et al.,* 1995	*E. chrysanthemi* *E. carotovora* subsp. *atroseptica* Specificity of multiplex PCR is lower than single assay, whereas an undesirable band can be also obtained with *P. carotovorum* subsp. *carotovorum.*
Dickeya dianthicola	**Dcd For/Dcd Rev** *pel*ADE gene + **Pca For/Pca Rev** Chromosomal DNA (unknown)	Multiplex	Enriched tubers extracts microsphere immunoassay (MIA)	Peters *et al.,* 2007	*P. atrosepticum* also amplified.
Dickeya sp. *Pectobacterium carotovorum* subsp. *carotovorum* *P. atrosepticum*	**SR3F/SR1cR** 16S rRNA gene	Conventional and RFLP	Purified isolate suspension or enriched microplant tissue (untreated)	Toth *et al.,* 1999	*E. chrysanthemi* *E. carotovora* subsp. *carotovora* *E. carotovora* subsp. *atroseptica* Other genera amplified also. Banding patterns allow differentiation of *Pectobacterium* and restriction analysis improves discrimination.

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Genus *Erwinia*					
Species	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
E. amylovora	**A/B** Plasmid DNA (pEA29)	Conventional	Bacteria, plant (untreated)	Bereswill *et al.,* 1992; Brown *et al.,* 1996	
E. amylovora	**AMSbL/AMSbR** Chromosomal (*ams* genes) region **fD2/rP1** 16S rRNA gene	Conventional	Bacteria (untreated)	Bereswill *et al.,* 1995	Amplification also obtained for pathogenic strains that lack plasmid pEA29.
E. amylovora	**A/B (external)** Plasmid DNA (pEA29) + **AJ75/AJ76 (internal)** Plasmid DNA (pEA29)	Nested	Plant (GeneReleaser)	McManus and Jones, 1995	
E. amylovora	**Ea71** Chromosomal DNA (unknown)	Conventional	Bacteria (untreated), plant (enrichment followed by immuno-capture)	Guilford *et al.,* 1996	Amplifies also pathogenic strains that lack plasmid pEA29.
E. amylovora	**EaF/EaR** 23S rRNA gene	Conventional	Bacteria (proteinase K), plant (PVP and PVPP addition) lysates	Maes *et al.,* 1996a	Amplifies also *Erwinia piriflorinigrans* isolated from necrotic pear blossoms.
E. amylovora	**AJ75/AJ76 (external)** Plasmid DNA (pEA29) + **PEANT1/PEANT2 (internal)** Plasmid DNA (pEA29)	Nested	Bacteria, plant (DNA extraction)	Llop *et al.,* 2000	
E. amylovora	**PEA71** Chromosomal DNA	Conventional BIO	Bacteria (untreated), plant (DNA extraction, GeneReleaser™)	Taylor *et al.,* 2001	Amplifies also pathogenic strains that lack plasmid pEA29.
E. amylovora	See: Bereswill *et al.,* 1992; Llop *et al.,* 2000	Conventional Nested	Bacteria, plant (DNA extraction)	Anon., 2004a	Recommended in the EPPO protocol.
E. amylovora	**P29TF/P29TR (primers)** **P29TM (probe)** Plasmid DNA (pEA29)	Real-Time (TaqMan) (SBYR® Green Master Mix)	Bacteria (lysed), plant (untreated)	Salm and Geider, 2004	

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E. amylovora	**pEA29A/pEA29B** Plasmid DNA (pEA29) **AJ245/AJ246** Chromosomal *ams* region	Multiplex	Bacteria (DNA extraction)	Kabadjova-Hristova *et al.,* 2006	Amplification also obtained for pathogenic strains that lack plasmid pEA29.
E. amylovora	**A/B** Plasmid DNA (pEA29) **PEANT1/PEANT2** Plasmid DNA (pEA29) **AJ75/AJ76** Plasmid DNA (pEA29)	Conventional	Plant (DNA extraction)	Stöger *et al.,* 2006	
E. amylovora	**E3/E4** Plasmid DNA (pEA29) + **PEANT1/PEANT2** Plasmid DNA (pEA29)	Real-time (duplex format of Scorpion) Nested-Scorpion	Bacteria (DNA extraction)	De Bellis *et al.,* 2007	
E. amylovora	**FER 1-F/FER 1-R** Chromosomal DNA (unknown)	Conventional	Bacteria (boiled)	Obradovic *et al.,* 2007	Amplifies also pathogenic strains that lack plasmid pEA29.
E. pyrifoliae	**EP16A/EPIG2c** 16S rRNA/ITS region **CPS1/CPS2c** *cps* region	Conventional	Bacteria (DNA extraction), plant (untreated)	Kim *et al.,* 2001	
E. pyrifoliae	**EpSPF/EpSPR** Chromosomal DNA (unknown)	Conventional	Bacteria (DNA extraction)	Shrestha *et al.,* 2007	
Genus *Leifsonia*					
Species/subspecies	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
L. xyli subsp. *xyli*	**CxxITSf # 5/CxxITSr # 5** ITS region **CxFOR/CxxREV/ CxcREV** ITS region	Conventional Multiplex	Bacteria (untreated), vascular fluid (PVP)	Fegan *et al.,* 1998	*Clavibacter xyli* subsp. *xyli* Multiplex assay allows differentiation between *C. xyli* subsp. *xyli* and *C. xyli* subsp. *cynodontis.*
L. xyli subsp. *xyli*	**Cxx1/Cxx2** ITS region	Conventional	Bacteria (untreated), vascular sap (PVP and Ficoll)	Pan *et al.,* 1998	*C. xyli* subsp. *xyli*
L. xyli subsp. *xyli*	**RSD 33/RSD 297 (primary)** **+** **RST60/RST59 (secondary)** ITS region	Nested	Not indicated	Falloon *et al.,* 2006	

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L. xyli subsp. *xyli*	**Lxx82F/Lxx22R** **Lxx202F/Lxx331R** ITS region	Conventional Real-time (SBYR® Green Master Mix)	Plant (DNA extraction)	Grisham *et al.,* 2007	
Genus "*Candidatus* Liberibacter"					
Species/subspecies	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
"*Ca.* L. africanus subsp. capensis"	**OI1/OI2c** 16S rRNA gene **A2/J5** Ribosomal protein genes _-operon **CAL1/J5** 16S rRNA gene	Conventional	Plant (DNA extraction)	Garnier *et al.,* 2000	Amplification from *Calodendrum capense* but not from citrus hosts of huanglongbing disease.
"*Ca.* L. americanus"	**OI1/OI2c** 16S rRNA gene **LSg2f/LSg2r** 16S rRNA gene **A2/J5** Ribosomal protein genes β-operon	Conventional	Plant (DNA extraction)	Coletta-Filho *et al.,* 2005	"*Candidatus* Liberibacter americanus" was proposed in 2005 (Teixera *et al.,* 2005) and thus is not included in the ISPP list (updated to 2004).
"*Ca.* L. americanus"	**GB1/GB3** 16S rRNA gene4	Conventional	Plant (DNA extraction)	Teixera *et al.,* 2005	
"*Ca.* L. asiaticus"	**226-primer pair** Specific DNA fragment (unknown)	Conventional	Plant (DNA extraction)	Hung *et al.,* 1999	
"*Ca.* L. asiaticus"	**Rpl-FIP, Rpl-BIP, Rpl-F3, Rpl-B3** *nus*G-*rpl*KAJL-*rpo*B gene cluster	LAMP assay	Plant (DNA extraction)	Okuda *et al.,* 2005	
"*Ca.* L. africanus" "*Ca.* L. asiaticus"	**fD2/rD1** Universal 16S rRNA gene	Conventional	Plant (DNA extraction) (Immunocapture)	Jagoueix *et al.,* 1994	
"*Ca.* L. africanus" "*Ca.* L. asiaticus"	**fD1/rP1** Universal 16S rRNA gene **OI1/OI2c** **O12c/OA1** **O12c/OI1/OA1** 16S rRNA gene	Conventional	Plant (DNA extraction)	Jagoueix *et al.,* 1996	PrimersOI1/OI2c and O12c/OI1/OA1 amplify both *Ca.* L. species, whereas O12c/OA1primers amplify prefentially "*Ca.* L. africanus". Distinction of the two species requires restriction analysis.

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"*Ca.* L. africanus" "*Ca.* L. asiaticus"	**OI2/23S1** 16S-23S rDNA spacer region **TRN1/OI4** Isoleucine genes/ 16S rRNA gene	Conventional	Plant (DNA extraction)	Jagoueix *et al.,* 1997	
"*Ca.* L. africanus" "*Ca.* L. asiaticus"	**A2/J5** Ribosomal protein genes β -operon	Conventional	Plant (DNA extraction)	Hocquellet *et al.,* 1999	Direct distinction of the two species.
"*Ca.* L. africanus" "*Ca.* L. americanus" "*Ca.* L. asiaticus"	**HLBr (reverse)** (common) **HLBaf, HLBam, HLBas (forward)** (specific to each of the three species) 16S rRNA gene **COXf, COXr** Cytochrome oxidase gene **Probe** **COXfp** Cytochrome oxidase gene	Single Multiplex real-time (TaqMan)	Plant (DNA extraction)	Li *et al.,* 2006b	
"*Ca.* L. africanus" "*Ca.* L. americanus" "*Ca.* L. asiaticus"	See: Jagoueix *et al.,* 1996; Hocquellet *et al.,* 1999; Okuda *et al.,* 2005; Teixera *et al.,* 2005; Li *et al.,* 2006b	Conventional LAMP assay Real-time (TaqMan)	Plant (DNA extraction)	Li *et al.,* 2007	Comparison and validation of previously published protocols.
Genus *Pantoea*					
Species/subspecies	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
P. agglomerans	**pagF/pagR** 16S rRNA gene	Conventional	Grape phylloxera (*Daktulosphaira vitifoliae*) (DNA extraction)	Vorwek *et al.,* 2007	
P. agglomerans pv. *gypsophilae*	**iaaH** Acetamine hydrolase gene **etZI** Cytokinin biosynthesis gene **etZII** Cytokinin biosynthesis gene	Conventional Nested	Bacteria (untreated), plant	Manulis *et al.,* 1998	*Erwinia herbicola* pv. *gypsophilae*

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P. ananatis	**PanITS1/Gs4** ITS region	Immunomagnetic separation (IMS-PCR)	Bacteria (boiled), seed (IMS)	Walcott *et al.*, 2002	
P. stewartii subsp. *stewartii*	**16S-P5/16S-P3 (PCR)** 16S rRNA gene **Es1, Es2, Es3, Es4 (LCR)** 16S rRNA gene	PCR-coupled ligase reaction (LCR)	Bacteria, plant (DNA extraction and crude lysate)	Wilson *et al.*, 1994	*Erwinia stewartii*
P. stewartii subsp. *stewartii*	**ESIG1/ESIG2c** ITS region **ES16/ES1G2c** ITS region **HRP1d/HRP3r** *hrpS* region **CPSL1/CPSR2c** *cps*DE region	Conventional	Bacteria, plant (untreated)	Coplin and Majerczak, 2002	Faint bands obtained for *P. ananas* and *P. agglomerans* with ITS primers.
P. stewartii subsp. *stewartii*	**ES16/ES1G2c** 16S-23S rRNA/ITS region **HRP1d/HRP3r** *hrpS* ORF	Conventional	Bacteria (boiled or alkaline lysis)	Anon., 2006c	Rcommended in the EPPO protocol.
Pantoea agglomerans	**R 16-1/R 23-2R** 16S-23S rRNA/ITS region	Conventional	Bacteria (DNA extraction)	Kim and Song, 1996	*Erwinia herbicola* *Acidovorax avenae* (*Pseudomonas avenae*), *Burkholderia glumae* (*Pseudomonas glumae*), *Pseudomonas fuscovaginae, Pseudomonas syringae* pv. *syringae* and *Xanthomonas oryzae* (pathovars *oryzae* and *oryzicola*) also amplified and differentiated by primary and secondary fragments.
Genus *Pectobacterium*					
Species/subspecies	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
P. atrosepticum	**ECA1f/ECA2r** Chromosomal DNA (unknown)	Conventional	Bacteria, plant (DNA extraction) Tuber (immunomagnetic separation followed by alkaline lysis) Bacteria (boiled), enriched peel (DNA extraction) Bacteria, potato peel (enriched)	De Boer and Ward, 1995; van der Wolf *et al.*, 1996; Fraaije *et al.*, 1997; Hyman *et al.*, 1997	*Erwinia carotovora* subsp. *atroseptica*

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P. atrosepticum	**ECA1f/ECA2r** Chromosomal DNA (unknown) **ECA4r** Contains ECA2r sequence (competitor template)	Conventional	Bacteria (boiled), potato peel (DNA extraction)	Hyman *et al.,* 1997	
P. atrosepticum	**PEAF/PEAR** *Rbs* family gene	Conventional	Bacteria, potato tubers (DNA extraction)	Park *et al.,* 2006	
P. atrosepticum	**ERWFOR/ATROREV** **ERWFOR/CHRREV** **ERWFOR+ATROREV+ CHRREV** Metalloproteases coding genes	Conventional Multiplex	Bacteria (boiled), potato tubers (centrifugation and lysis buffer)	Smid *et al.,* 1995	*E. carotovora* subsp. *atroseptica* *Dickeya* sp. (*Erwinia chrysanthemi*) aso amplified. Lower specificity of multiplex PCR, undesirable band obtained with *P. carotovorum* subsp. *carotovorum.*
P. atrosepticum	**Dcd Forw/Dcd Rev** *pel*ADE gene fragments + **Pca For/Pca Rev** Chromosomal DNA (unknown)	Multiplex	Enriched potato tubers (microsphere immunoassay)	Peters *et al.,* 2007	*Dickeya dianthicola* also amplified.
P. carotovorum subsp. *carotovorum*	**EXPCCF/EXPCCR** Chromosomal DNA (unknown) **INPCCF/INPCCR** Nested to EXPCCF/EXPCCR	Competitive Nested	Bacteria (untreated), plant (DNA extraction)	Kang *et al.,* 2003	Amplification obtained with *P. carotovorum* subsp. *wasabiae* (distinction by restriction analysis).
P. atrosepticum, *P. carotovorum* subsp. *carotovorum*	**SR3F/SR1cR** 16S rRNA gene	Conventional and RFLP	Bacteria (untreated), microplant (enriched)	Toth *et al.,* 1999	*E. carotovora* subsp. *atroseptica* *E. carotovora* subsp. *carotovora* *Dickeya* sp. (*E. chrysanthemi*) also amplified. Amplification obtained for other genera. Banding patterns allow differentiation of *Pectobacterium* from other and restriction analysis improves discrimination.
P. atrosepticum, *P. carotovorum* subsp. *carotovorum*	**MpdEc-F/MpdEc-R** *mpd* gene	Real-time (iQ Supermix SBYR-Green)	Potato tubers (DNA extraction)	Atallah and Stevenson, 2006	*E. carotovora* subsp. *atroseptica* *P. wasabiae*, *P. betavasculorum*, as well as *Brenneria nigrifluens* and *B. quercina* also amplified. Primers for detection of four potato tubers pathogenic fungi are also described.

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P. atrosepticum *P. carotovorum* subsp. *carotovorum* *P. betavasculorum* *P. odoriferum* *P. wasabiae*	**Y1/Y2** *Y* family of pectate lyase (*pel*) genes	Conventional and RFLP	Bacteria (DNA extraction) Bacteria (boiled) after enrichment or immunomagnetic separation) Plant, soil and water (DNA extraction)	Darrasse *et al.,* 1994; Helias *et al.,* 1998	*E. carotovora* subsp. *atroseptica* *E. carotovora* subsp. *carotovora* *E. carotovora* subsp. *betavasculorum* *E. carotovora* subsp. *odorifera* *E. carotovora* subsp. *wasabiae* PCR reaction and restriction enzyme analysis do not clearly discriminate species.
Genus *Pseudomonas*					
Species/subspecies	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
Pseudomonas (*sensu stricto*)	**Ps-for/Ps-rev** *Pseudomonas* 16S rRNA gene	Conventional and RFLP	Bacteria or soil (DNA extraction)	Widmer *et al.,* 1998	
P. avellanae	**PAV 1/PAV 22** 16S rRNA gene	Conventional	Bacteria (boiled), plant (BLOTTO)	Scortichini and Marchesi, 2001; Scortichini *et al.,* 2002	
P. avellanae	**WA/WC** Harpin-encoding *hrpW* gene	Conventional	Bacteria, plant (DNA extraction)	Loreti and Gallelli, 2002	
P. corrugata	**PC1/1–PC1/2 (group I)** **PC5/1–PC5/2 (group II)** RAPD fragments	Conventional Multiplex	Bacteria, plant (alkaline extraction)	Catara *et al.,* 2000	
P. corrugata *P. mediterranea* (*P. corrugata* Type II)	**PC1/1–PC1/2** ***(P. corrugata)*** **PC5/1–PC5/2** ***(P. mediterranea)*** RAPD fragments	Conventional	Bacteria (DNA extraction)	Catara *et al.,* 2002	Protocol slightly modified from Bereswill *et al.* (1994). Differentiation between Type I (*P. corrugata*) and Type II (proposed new species, *P. mediterranea*).
P. savastanoi pv. *glycinea*	**Tn5-derived**	Random primer-dependent PCR	Bacteria (DNA extraction)	Ullrich *et al.,* 1993	*Pseudomonas syringae* pv. *glycinea*
P. savastanoi pv. *phaseolicola*	**HM6/HM13** Phaseolotoxin gene cluster	Conventional	Bacteria, seed (DNA extraction)	Prosen *et al.,* 1993	*Pseudomonas syringae* pv. *phaseolicola*
P. savastanoi pv. *phaseolicola*	**P 5.1/p 3.1 (external)** **P 5.2/P 3.2 (internal)** Phaseolotoxin gene cluster	Nested	Seed washes (untreated)	Schaad *et al.,* 1995	*P. syringae* pv. *phaseolicola*
P. savastanoi pv. *phaseolicola*	**HB14F/HB14R** Phaseolotoxin gene cluster	Conventional	Bacteria (boiled)	Borowicz *et al.,* 2002	*P. syringae* pv. *phaseolicola* Specificity improved by annealing temperature of 80°C.

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P. savastanoi pv. *phaseolicola*	**AVR1-F/AVR1-R** Locus *avrPphF* **PHTE-F/PHTE-R** Locus *pthE*	Conventional	Bacteria (DNA extraction)	González *et al.*, 2003	Toxigenic and nontaxigenic strains amplified.
P. savastanoi pv. *phaseolicola*	**PHA19/PHA95** *amtA* gene **P5.1/P3.1+P3004L/ P3004R** Locus *phtE*	BIO Multiplex	Seed washes (previously plated on semiselective medium MT)	Schaad *et al.*, 1995; Rico *et al.*, 2006	Toxigenic and nontaxigenic strains differentiated.
P. savastanoi pv. *phaseolicola*	**Real-time PsF-tox/PsR-tox Probe PsF-tox-286P** *tox-argK* chromosomal cluster	Real-time (TaqMan)	Bacteria, seed washes, plant (untreated)	Schaad *et al.*, 2007	*P. syringae* pv. *phaseolicola*
P. savastanoi pv. *savastanoi*	**IAALF/IAALR** *iaal* gene	Conventional	Bacteria, plant (DNA extraction)	Penyalver *et al.*, 2000	
P. savastanoi pv. *savastanoi*	**IAALF/IAALR (external) IAALN1/IAALN2 (internal)** *iaal* gene	Nested	Bacteria, pre-enriched plant (DNA extraction)	Bertolini *et al.*, 2003b	
P. savastanoi pv. *savastanoi*	**iaaMf/iaaMr** *iaaM* gene (IAA biosynthesis) **iaaHf/iaaHr** *iaaH* gene (IAA) (IAA biosynthesis) **ptzf/ptzr** *ptz* gene (cytokinin biosynthesis) **lscCf/lscCr** *lscC* gene (levan biosynthesis)	Conventional	Bacteria (DNA extraction)	Marchi *et al.*, 2005	
P. syringae pv. *actinidae*	Genomic DNA (unknown) RAPD-fragment	Conventional	Bacteria (alkaline lysis)	Koh and Nou, 2002	
P. syringae pv. *alisalensis*	**BOXA 1R** Repetitive DNA sequences	BOX-PCR	Bacteria (DNA extraction)	Cintas *et al.*, 2002, 2006	Bacterial identification.
P. syringae pv. *atropurpurea*	**P1/P2, P3/P4,P1-P4, P5/P8, P7/P8** Plasmid COR1 (coronatine synthesis)	Conventional	Bacteria, plant (untreated)	Takahashi *et al.*, 1996	

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P. syringae pv. *coryli*	**P0/P6** Entrire 16S rRNA gene **L7/L8** Full-length *hrpL* gene **L1/L2** Internal region hrpL gene **B1/B2** *SyrB* gene	Conventional Rep-PCR (BOX and ERIC)	Bacteria (DNA extraction)	Scortichini *et al.,* 2005	
P. syringae pv. *papulans*	**Pap1/Pap2** *hrpL* gene **Pap1/Pap3** *HrpL* gene	Conventional	Bacteria (boiled), plant (DNA extraction)	Kerkoud *et al.,* 2002	Pap1/Pap2 amplify only *P. syringae* pv. *papulans*, wehereas Pap1/Pap3 also amplify other *P. syringae* of genomospecies 1.
P. syringae pv. *papulans*	**PapHrp1/papHrp2** *Hrp*L gene	Conventional	Bacteria	Kerkoud *et al.,* 2002; Vanneste and Yu, 2006	
P. syringae pv. *pisi*	**AN3/1 Type I** **AN3/2 Type I** RAPD fragment **AN7/1 Type II** **AN7/2 Type II** RAPD fragment	Conventional Multiplex	Bacteria (untreated)	Arnold *et al.,* 1996	
P. syringae pv. *syringae* (strains producers of cyclic lipodepsinonapeptides)	**B1/B2** *syrB* gene **D1/D2** *SyrD* gene	Conventional	Bacteria (DNA extraction)	Sorensen *et al.,* 1998	
P. syringae pv. *tagetis*	**TAGTOX9** **FP1/TAGTOX9 RP1** *exbD* gene **TAGTOX10** **FP10/TAGTOX10 RP1** *Asnb* gene	Conventional	Bacteria (DNA extraction)	Kong *et al.,* 2004	*Pseudomonas syringae* pv. *helianthi* also amplified and considered as nontoxigenic form of *P. syringae* pv. *tagetis.*
P. syringae pv. *tomato*	**MM5F/MM5R** $hrpZ_{pst}$ gene	Conventional	Bacteria (boiled), plant (DNA extraction)	Zaccardelli *et al.,* 2005	
P. syringae pv. *tomato*	**RcalFor1/RTRev** RAPD fragment **27F/1492R+HSP1/HSP2** 16S rDNA+specific to *P. syringae* pv. *tomato* **Rtimefor/RTRev** **Probe** (molecular beacon)	Conventional Multiplex Real-time (molecular beacon)	Bacteria, plant (DNA extraction)	Fanelli *et al.,* 2007	

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P. tolaasii	**Pt-1A/Pt-1D1** **Pt-1A/Pt-1D1+Pt-PM/PtQM** Tolaasin biosynthesis genes	Conventional Nested and immunocapture-nested	Bacteria (untreated)	Lee *et al.,* 2002	
P. syringae (pathovars producers of toxic lipodepsipeptide)	***SyrD1ISyrD2*** *SyrD* gene	Conventional	Bacteria (DNA extraction)	Bultreys and Gheysen, 1999	
P. syringae (pathovars producers of tabtoxin)	**tblA1/tblA2** *tblA* (tabtoxin gene) **tabA1/tabA2** *tabA* (tabtoxin gene)	Conventional	Bacteria (untreated)	Lydon and Patterson, 2001	
P. savastanoi pv. *phaseolicola* *P. syringae* pv. *actinidae*	**OCTF/OCTR** *argK* gene (phaseolotoxin resistance)	Conventional	Bacteria (DNA extraction)	Sawada *et al.,* 1997	*P. syringae* pv. *phaseolicola*
P. savastanoi pv. *savastanoi* Four viruses: *Cucumber mosaic virus* (CMV) *Cherry leaf roll virus* (CLRV) *Strawberry latent ringspot virus* (SLRSV) *Arabis mosaic virus* (ArMV)	***P. savastanoi* pv. *savastanoi*** **IAALF/IAALR (external)** **IAALN1/IAALN2 (internal)** *iaal* gene **CMV1/CMV2+CMVi1/CMVi2** **CLRV1/CLRV2+CLRVi1/CLRVi2** **SLRV1/SLRV2+SLRVi1/SLRVi2** **ArMV1/ArMV2+ArMVi1/ArMVi2**	Nested Multiplex nested RT-PCR	Pre-enriched, plant (DNA extraction)	Bertolini *et al.,* 2003a, b; Penyalver *et al.,* 2000	Colorimetric detection of amplicons using digoxigenin marked internal probes.
P. syringae pv. *morsprunorum* *P. syringae* pv. *syringae*	**REP1R/REP2I** **ERIC1R/ERIC2** **BOXA1R**	Rep-PCR	Bacteria (DNA extraction)	Vicente and Roberts, 2007	Bacterial identification.
P. fuscovaginae *P. syringae* pv. *syringae*	**R16-1/R23-2R** 16S-23S rDNA spacer region	Conventional	Bacteria (DNA extraction)	Kim and Song, 1996	*Acidovorax avenae* (*Pseudomonas avenae*), *Burkholderia glumae* (*Pseudomonas glumae*), *Pantoea agglomerans* (*Erwinia herbicola*), *X. oryzae* (pathovars *oryzae* and *oryzicola*) also amplified and differentiated by primary and secondary fragments.
P. avellanae *P. syringae* pv. *theae* *P. syringae* pv. *actinidae*	**PAV 1/PAV 22** 16S rRNA gene	Conventional	Bacteria (DNA extraction)	Scortichini and Marchesi, 2001; Scortichini *et al.,* 2002	

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P. cannabina *P. savastanoi* pv. *glycinea* *P. savastanoi* pv. *phaseolicola* *P. syringae* pv. *sesami*	**ETH-1/ETH-2** Entire *efe* gene (ethylene-forming enzime) **ETH-1/ETH-3** Partial *efe* gene	Conventional	Bacteria (boiled)	Sato *et al.,* 1997	*P. syringae* pv. *cannabina* *P. syringae* pv. *glycinea* *P. syringae* pv. *phaseolicola*
P. syringae pv. *atropurpurea* *P. syringae* pv. *glycinea,* *P. syringae* pv. *maculicola* *P. syringae* pv. *morsprunorum* *P. syringae* pv. *tomato*	**cff primer 1/ cff primer 2** Coronatine biosynthesis gene cluster	Conventional and RFLP	Bacteria (DNA extraction)	Bereswill *et al.,* 1994	
P. savastanoi pv. *phaseolicola*	**HB 14F/HB 14R** **(*Pseudomonas*)** Phaseolotoxin gene cluster **X4c/X4e** **(*Xanthomonas*)** Plasmid DNA **HB 14F+HB 14R+ X4c+X4e** **(simultaneous detection)**	Conventional	Seeds (alkaline lysis)	Audy *et al.* 1996	*Pseudomonas syringae* pv. *phaseolicola* *Xanthomonas axonopodis* pv. *phaseoli* (*X. campestris* pv. *phaseoli*) also amplified.
P. syringae pv. *tomato*	**COR1/COR2** **(*Pseudomonas*)** Coronafacic acid *cfa7* gene **BSX1/BSX2** **(*Xanthomonas*)** Genomic DNA (unknown)	Conventional	Pure cultures or plant tissue (frozen-boiled method DNA extraction)	Cuppels *et al.,* 2006	Other coronatine-producing *P. sryringae* pathovars also amplified with COR primers. *X. axonopodis* pv. *vesicatoria* and *X. gardneri* are not valid names according to ISPP list. BSX primers also amplify *X. vesicatoria.*
Genus *Ralstonia*					
Species/ biovars	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
R. solanacearum	**PS96H/PS96I** Chromosomal DNA (unknown)	Conventional	Bacteria, plant (boiled)	Seal *et al.,* 1992a; Hartung *et al.,* 1998	*Pseudomonas solanacearum*
R. solanacearum	**T3A/T5A** tRNA consensus	Conventional	Bacteria (boiled)	Seal *et al.,* 1992b	*P. solanacearum*
R. solanacearum	**pehA # 3/ pehA # 6** *pehA* gene (polygalacturonase)	Conventional	Bacteria, plant (DNA extraction)	Gillings *et al.,* 1993	*P. solanacearum*
R. solanacearum	**759/760** Genomic DNA (unknown)	BIO	Soil suspensions plated on selective medium (DNA extraction)	Ito *et al.,* 1998	

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R. solanacearum	**D1/B** **D2/B** **OLI1/Z** 16S rRNA gene	Conventional	Bacteria, potato tuber (untreated)	Boudazin *et al.,* 1999; van der Wolf *et al.,* 2000	D1/B identify *R. solanacearum* division 1 strains. OLI/Z primers identify *R. solanacearum* division 2 strains.
R. solanacearum	**BP4-R/BP4-L** RAPD fragment	Conventional	Soil (DNA extraction)	Lee and Wang, 2000	
R. solanacearum	**PS-1/PS-2** 16S rRNA gene	Conventional	Potato tubers (DNA extraction)	Pastrik and Maiss, 2000	
R. solanacearum	**OLI-1/Y-2** 16S rRNA gene **OLI-1/OLI-2 + JE2/Y2** 16S rRNA gene	Conventional Nested	Soil suspensions (previously enriched and boiled)	Pradhanang *et al.,* 2000	
R. solanacearum	**Multiplex (generic)** **RS-I/RS-II** (primers) **RS-P** (probe) 16S rRNA gene **Multiplex (biovar 2A)** **B2-1/B2-II** (primers) **B2-P** (probe) 16S rRNA gene **Multiplex (internal control, host)** **RS or B2** **+** **COX-F/COX-R** (primers) **COX-P** (probes) Potato cytochrome oxidase gene	Multiplex real-time (TaqMan) Real-time (TaqMan)	Bacteria, potato tubers extract (boiled)	Weller *et al.,* 2000	
R. solanacearum	**PS-IS-F/PS-IS-R** Insertion sequence (IS*1405*) **PS-IS RA1** **PS-IS-RB1** Flanking regions of IS*1405*b and IS*1405*d	Conventional	Bacteria (untreated)	Lee *et al.,* 2001	Specific detection of *R. solanacearum* race 1.
R. solanacearum	**Rs-1-F/Rs-3-R** 16S-23S rDNA spacer region/ *R. solanacearum* division I **Rs-1-F/Rs-1-R** 16S-23S rDNA spacer region/ *R. solanacearum* division II	Conventional	Bacteria, potato tubers (DNA extraction)	Pastrik *et al.,* 2002	

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	NS-5-F/NS-6-R 18S rDNA (host internal control) **Rs-1-F/Rs-1-R+NS-5-F/NS-6-R**	Multiplex			
R. solanacearum	**OLI1/Y2** **OLI1/Z** **OLI1/OLI2** **OLI1/OLI2/JE-2 (Co-PCR)** 16S rRNA gene	Conventional Co-operative	Bacteria (boiled), water	Caruso *et al.,* 2003	
R. solanacearum	**RSC-F/RSC-R (primers)** **RSC-P (probe)** DNA fragment specific to biovar 2	Real-time (TaqMan)-BIO	Potato tuber extract (boiled)	Ozakman and Schaad, 2003	Race 3, biovar 2 strains are specifically amplified.
R. solanacearum	**RsoLfliC** *fliC* gene (flagellar subunit protein)	Conventional	Bacteria, soil (DNA extraction)	Schönfeld *et al.,* 2003	
R. solanacearum	**OLI-1/Y-2** 16S rRNA gene	Conventional	Pure culture (DNA extraction)	Seal *et al.,* 1993	*P. solanacearum* *Ralstonia syzygii* (*Pseudomonas syzygii*) and Blood Disease Bacterium also amplified.
R. solanacearum	**DIV1F/DIV1R** **DIV2F/DIV2R** **OLI1/BV345** **DIV2F/ITRS** 16S rRNA gene and 16S-23S rRNA region **OLI1+Y2+BV345**	Conventional Multiplex	Bacteria (boiled)	Seal *et al.,* 1993, 1999	Division I and II of *Ralstonia solanacearum* differentiated. *Ralstonia syzygii* (*Pseudomonas syzygii*) and Blood Disease Bacterium also amplified.
R. solanacearum	**RS30/RS31 (external)** **+** **RS30a/RS31a/RS30b/RS31b (internal)** *hrp* genes cluster	Nested	Bacteria (boiled), plant, water and soil (DNA extraction)	Poussier and Luisetti, 2000	*Ralstonia syzygii* and Blood Disease Bacterium also amplified.
R. solanacearum	**RS3/Rs4** *R. solanacearum pehB* gene **XcpM1/XcpM2** *X. c.* pv. *pelargonii* DNA (ERIC) **DG1/DG2** 18S rRNA gene (host internal control)	Multiplex	Bacteria or plant (DNA extraction)	Glick *et al.,* 2002	*Xanthomonas hortorum* pv. *pelargonii* (*X. campestris* pv. *pelargonii*) also amplified.

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Genus *Rhodococcus*					
Species	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
R. fascians	**JRERIGHT/JRELEFT** *fas-1* gene (cytokinin biosynthesis)	Conventional	Plant (DNA extraction)	Stange *et al.*, 1996	
Genus *Streptomyces*					
Species	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
S. acidiscabies *S. scabiei* *S. turgidiscabies*	**Nf/Nr** *nec1* gene	Conventional	Mycelium (boiled)	Burkhalid *et al.*, 1998	*S. scabies*
S. scabiei *S. turgidiscabies* *S. aureofaciens*	Universal for *Streptomyces* **pA/pH´** Specific for *S. scabies* **ScabI/SacbII** Specific for *S. turgidiscabies* **TurgI/TurgII** Specific for *S. aureofaciens* **AurI/AurII** 16S rRNA gene	Conventional	Bacteria, potato tubers (DNA extraction)	Lehtonen *et al.*, 2004	*S. scabies*
S. acidiscabies *S.scabiei* *S.scabies* var. *achromogenes*	**16S-1F/16S-1R** 16S rRNA gene **Nec1F/Nec1R** *nec1* gene **TxtA1/TxtA2** *txtA* gene (thaxtomin biosynthesis)	Conventional	Bacteria (DNA extraction)	Wanner, 2004	*S.scabies* var. *achromogenes* is not included in the ISPP list.
Streptomyces spp.	**NEC-F2/NEC-R2** (primers) **Probe T** *nec1* gene **Probe IS** Internal standard DNA	Quantitative competitive quenching probe (QCQP)	Potato tubers, soil (DNA extraction)	Koyama *et al.*, 2006	
S. acidiscabies *S. aureofaciens* *S. bottropensis* *S. europaeiscabiei* *S. scabiei* *S. stelliscabiei* *S. turgidiscabies* New *Streptomyces* group	Species and strain-specific 16S rDNA sequences **scab1m/scab2m** **scab1/scab2m** **ASE3/scab2m** *S. scabies* and *S. europaeiscabiei* **Stel3/ T2st2**	Conventional	Bacteria (DNA extraction)	Wanner, 2006	

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	S. stelliscabiei **ASE3/ Aci2** *Streptomyces* newly identified group **Stel3/ Aci2** *S. bottropensis* **Aci1/ Aci2** *S. acidiscabies* **Turg1m/ Turg2m** *S. turgidiscabies* **Aur1/ Aur2** *S. aureofaciens*				
Streptomyces spp.	**NecF1/NecR1 (external)** **NecNF1/NecNR2 (internal)** **NecTqF1/NecTqR1 (primers)** **NecTqP1 (probe)** *nec1* gene	Nested Real-time (TaqMan)	Bacteria, potato tubers and soil (DNA extraction)	Cullen and Lees, 2007	
S. acidiscabies *S. aureofaciens* *S.europaeiscabiei* *S. scabiei* *S. stelliscabiei* *S. turgidiscabies*	**16S-1F/16S 455-435** 16S rDNA **Nf/Nr** *nec1* gene **TxtAB TxtAB1/ TxtAB2** *TxtAB* gene **Tom3/Tom4** *TomA* gene **Species-specific 16s rDNA ASE3/Scab2m** (*S. scabies* and *S. europaeiscabiei*) **ASE3/ Aci2** (Newly identified *Streptomyces* group) **Aci1/ Aci2** (*S. acidiscabies*)	Conventional	Bacteria (DNA extraction)	Wanner, 2007	
Genus *Xanthomonas*					
Species/pathovars	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
Xanthomonas (genus)	**8/27** **461/477** 16S rRNA gene	Conventional	Bacteria (boiled) or seed extract	Maes, 1993	

X. albilineans	**Ala4/IIe2** Inter tRNA region **16S+IIe1 or Ala1+23S** Region between 16S rRNA gene and $tRNA^{ala}$ or $tRNA^{ile}$ and 23S rRNA	Conventional Nested	Bacteria (boiled) or leaf (DNA extraction)	Honeycut *et al.,* 1995	
X. albilineans	**Ala4/L1** Inter tRNA region	Conventional	Bacteria, sap, leaf (untreated)	Pan *et al.,* 1997	
X. albilineans	**XAF1/XAR1** Genomic DNA (unknown)	Conventional BIO	Bacteria, sap, leaf (boiled)	Wang *et al.,* 1999	
X. arboricola pv. *pruni*	**Y17CoF/Y17CoR** RAPD fragment	Conventional	Bacteria, plant (untreated)	Pagani, 2004	
X. axonopodis pv. *dieffenbachiae*	**$KJM11^{f}$/$KJM12^{r}$** **+** **$KJM34^{f}$/$KJM36^{r}$** **+** **$KJM74^{f}$/$KJM73^{r}$** RAPD fragment	Conventional Multiplex Previous immunocapture	Bacteria, plant (DNA extraction or immunocapture)	Khoodoo *et al.,* 2005	
X. axonopodis pv. *dieffenbachiae*	**PXadU/PXadL** (external) **NXadU/NXadL** (internal) RAPD fragment	Nested	Bacteria (boiled), plant (PP buffer with 5% PVP)	Robène-Soustrade *et al.,* 2006	
X. axonopodis pv. *manihotis*	Plasmid fragment (unknown)	Conventional	Plant extracts (without DNA extraction)	Verdier *et al.,* 1998	
X. axonopodis pv. *phaseoli* *X. campestris phaseoli* var. *fuscans*	**X4c/X4e** Plasmid DNA	Conventional	Bacteria, leaf (DNA extraction)	Audy *et al.,* 1994	*Xanthomonas campestris* pv. *phaseoli* *X. campestris phaseoli* var. *fuscans* is not a valid name according to the ISPP list.
X. axonopodis pv. *phaseoli* *X. campestris phaseoli* var. *fuscans*	**OP-G11** Random primer	RAPD	Bacteria (DNA extraction)	Birch *et al.,* 1997	*X. campestris* pv. *phaseoli*
X. axonopodis pv. *phaseoli* *X. campestris phaseoli* var. *fuscans*	**Xf1/Xf2** RAPD fragment + **X4c/X4e** Plasmid DNA	Conventional Multiplex	Bacteria, plant (DNA extraction)	Toth *et al.,* 1998	*X. campestris* pv. *phaseoli* Xf1/Xf2 specific for *Xanthomonas campestris* pv. *phaseoli* var. *fuscans.* X4c/X4e amplify both *X. arboricola* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*.
X. axonopodis pv. *phaseoli*	**X4c/X4e (Xanthomonas)** Plasmid DNA	Conventional	Seeds (alkaline treatment)	Audy *et al.,* 1996	*X. campestris* pv. *phaseoli* *Pseudomonas savastanoi* pv. *phaseolicola* (*P. syringae* pv. *phaseolicola*) also amplified.

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	HB 14F/HB 14R (*Pseudomonas*) Phaseolotoxin gene cluster Simultaneous detection **HB 14F+HB 14R+ X4c+X4e**	Concurrent detection			
X. campestris pv. *campestris*	**HrcCF2/HrcCR2** *hrcC* gene (pathogenicity-associated)	Conventional	Bacteria, plant and seeds (DNA extraction)	Zaccardelli *et al.,* 2007	
X. citri subsp. *citri*	**2/3** Pathotype A strains **4/5; 6/7; 1/5** Pathotype A strains (variable for pathotypes B and C) Plasmid DNA	Conventional	Bacteria, plant (DNA extraction)	Hartung *et al.,* 1993	*X. campestris* pv. *citri*
X.citri subsp. *citri*	(first round) + **94-3 bio/94-4 lac** (second round) Plasmid DNA	Nested	Plant (immunocapture)	Hartung *et al.,* 1996	*X. axonopodis* pv. *citri*
X.citri subsp. *citri*	**CiH2/CiH3** Contains 5´termini for a plasmid DNA of *X. axonopodis* pv. *citri* and 3´termini homologous to Figwort mosaic virus (FMV)	Competitive (Internal standard)	Plant (DNA extraction)	Cubero *et al.,* 2001	*X.axonopodis* pv. *citri*
X. citri subsp. *citri*	**Xac01/Xac02** *rpf* gene cluster	Conventional	Bacteria, plant (DNA extraction)	Coletta-Filho *et al.,* 2006	*X. axonopodis* pv. *citri*
X. citri subsp. *citri*	**A5, C5, A2, D2, A3, D7, A9, A10** Genomic and plasmid DNA (unknown)	Conventional	Plant (DNA extraction)	Li *et al.,* 2006a	*X. axonopodis* pv. *citri*
X. citri subsp. *citri* (Pathotypes A, B and C)	**J-pth1/J-pth2** Pathotypes A, B and C strains *pthA* gene (involved in virulence) **J-RXg/J-RXc2** Pathotype A strains ITS region	Conventional	Bacteria (DNA extraction)	Cubero and Graham, 2002	*X. axonopodis* pv. *citri*

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X. citri subsp. *citri* (Pathotypes A, B and C)	**2/3** Pathotype A strains Plasmid DNA **J-pth1/J-pth2** Pathotype A, B and C strains *pthA* gene (involved in virulence) **J-RXg/J-RXc2** Pathotype A strains ITS region	Conventional	Plant (DNA extraction)	Hartung *et al.,* 1993, 1996; Cubero *et al.,* 2001; Cubero and Graham, 2002; Anon., 2005b	*X. axonopodis* pv. *citri* Recommended in the EPPO protocol.
X .citri subsp. *citri* *X. citri* pv. *aurantifolii*	**VM1/VM2** **VM3/VM4** **VM5/VM6** *pthA* gene family **Kingsley forward/reverse** *X. citri* pv. *citri* A chromosome	Real-time (SBYR® Green Master Mix)	Bacteria, plant (DNA extraction)	Mavrodieva *et al.,* 2004	*X. citri* pv. *citri* *X. citri* pv. *aurantifolii* is not included in the ISPP List.
X. citri subsp. *citri* *X. axonopodis* pv. *citrumelo*	**J-RT pth3/J-RT pth4** *pth* gene, citrus bacterial canker strains **J-RTRib 16Sup/J-RTRib downXC2** Ribosomal sequence, *X. axonopodis* pv. *citrumelo* **J-AdlrpU1J-AdlrpU2** *lrp* gene, *Xanthomonas* spp. **J-Taqpth2** *pth* gene, citrus bacterial canker strains **J-Taq16S-1** Ribosomal sequence, *X. axonopodis* pv. *citrumelo* **J-Alrpallelic1** *lrp* gene, *X. citri* pv. *citri* wide host range strains **J-Awlrpallelic1** *lrp* gene, *X. citri* pv. *citri* restricted host range strains	Real-time (TaqMan)	Bacteria, plant (DNA extraction)	Cubero and Graham, 2005	*X. axonopodis* pv. *citri* *X. axonopodis* pv. *citrumelo* is not included in the ISPP list. Allelic discrimination of citrus *Xanthomonas* strains allowed and a single nucleotide difference detected.

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X. fragariae	**REP1R-I, REP2-I, ERIC1R, ERIC2**	rep	Bacteria (untreated)	Opgenorth *et al.,* 1996	Bacterial identification.
X. fragariae	**241A/241B** **245A/245B** **295A/295B** **Multiplex** (different primer pairs combinations) **241+245, 241+295, 245+295, 241+245+295** RAPD fragment	Conventional Multiplex	Bacteria (DNA extraction)	Pooler *et al.,* 1996	
X. fragariae	**XF9/XF11** (first round) **+** **XF9/XF12** (second round) *hrp* gene	Nested	Bacteria, plant (DNA extraction)	Roberts *et al.,* 1996; Mahuku and Goodwin, 1997	
X. fragariae	**JJ9/JJ12** *hrp* gene	Conventional	Bacteria, plant (DNA extraction)	Zhang and Goodwing, 1997	
X. fragariae	**XF10/XF12** *hrp* gene	Conventional	Plant (DNA extraction)	Stöger and Ruppitsch, 2004	
X. fragariae	**245A/245B** (first round) RAPD fragment **245.5/245.267** (second round) 245A-245B fragment	Conventional Nested	Bacteria, plant (DNA extraction)	Pooler *et al.,* 1996; Zimmermann *et al.,* 2004	Both pairs of primers can be used in conventional or nested PCR.
X. fragariae	**245A/245B** (first round) RAPD fragment **245.5/245.267** (second round) 245A-245B fragment **XF9/XF11** (first round) **+** **XF9/XF12** (second round) *hrp* gene	Conventional Nested	Plant (DNA extraction)	Roberts *et al.,* 1996; Zimmermann *et al.,* 2004; Moltmann and Zimmermann, 2005	Primers pair 245A/245B and 245.5/245.267 can be used in both conventional and nested PCR.
X. fragariae	**REP1R-I, REP2-I, ERIC1R, ERIC2** **241A, 241B, 245A, 245B, 29 A, 295B** RAPD fragments	rep Multiplex	Plant (with or without DNA extraction) (enrichment)	Opgenorth *et al.,* 1996; Anon., 2006d Pooler *et al.,* 1996; Stöger and Ruppitsch, 2004; Anon., 2006d	Recommended in the EPPO protocol.

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	XF9/XF11 *hrp* gene	Conventional		Roberts *et al.,* 1996; Anon., 2006d	
X. hortorum pv. *carotae*	**3SF/3SR** RAPD fragment	Conventional	Bacteria (boiled), plant, seeds (DNA extraction)	Meng *et al.,* 2004	*X.campestris* pv. *carotae*
X. hortorum pv. *pelargonii*	RAPD fragment	Conventional	Bacteria (DNA extraction)	Manulis *et al.,* 1994	*X. campestris* pv. *pelargonii*
X. hortorum pv. *pelargonii*	**XcpM1/XcpM2** *X. c.* pv. *pelargonii* DNA (ERIC)	Conventional	Bacteria, plant (DNA extraction)	Sulzinski *et al.,* 1996, 1997, 1998	*X. campestris* pv. *pelargonii*
X. hortorum pv. *pelargonii*	RAPD fragment	Conventional	Bacteria, plant (DNA extraction)	Chittaranjan and De Boer, 1997; Manulis *et al.,* 1994	*X. campestris* pv. *pelargonii*
X. hyacinthi	**JAAN/JARA** *fimA* gene (type IV structural fimbrial-subunit)	Conventional	Bacteria, plant (untreated)	van Doorn *et al.,* 2001	
X. oryzae pv. *oryzae*	**XOR-F/XOR-R2** ITS region	Conventional	Bacteria, plant (boiled)	Adachi and Oku, 2000	A fragment of the same size also obtained from *X. campestris* pathovars *citri, incanae* and *zinniae*.
X. oryzae pv. *oryzae*	**TXT/TXT4R** *IS1113* insertion element	Conventional BIO	Pure cultures, and plant tissue (DNA extraction) or BIO-PCR from seeds (without DNA extraction)	Sakthivel *et al.,* 2001	
X. oryzae pv. *oryzae*	**PF/PR** Putative siderophore receptor gene *cds*	Real-time (TaqMan)	Rice seeds washes (untreated)	Zhao *et al.,* 2007	At an annealing of 60ºC both pv. *oryzae* and pv. *oryzicola* and *oryzicola* are amplified, whereas at 68ºC only *X. oryzae* pv. *oryzae* results in a fluorescent signal.
X. oryzae pv. *oryzae* *X. oryzae* pv. *oryzicola*	**XOR-F/XOR-R2** ITS region **TXT/TXT4R** *IS1113* insertion element Differentiation of pathovars *oryzae* and *oryzicola* **R16-1/R23-2R** ITS region	Conventional BIO	Bacteria, plant (DNA extraction or BIO-PCR from seeds without DNA extraction)	Kim and Song, 1996; Adachi and Oku, 2000; Sakthivel *et al.,* 2001; Anon., 2007	
X. translucens	**PAf/PBf/PABr** ITS region	Multiplex	Bacteria (DNA extraction) or plant (PVP addition)	Marefat *et al.,* 2006	Groups A and B of *X. translucens* from pistachio differentiated. *X. translucens* pv. *cerealis* also amplified.

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X. translucens pv. *poae*	**XAN1/XAN2** **XAN3/XAN4** **XAN5/XAN7** Encompassing 16S rRNA, ITS, 23S rRNA	Conventional	Bacteria (DNA extraction)	Mitkowski *et al.,* 2005	
X. vesicatoria	**RST2/RST3** **RST9/RST10** *hrpB* (hypersensitive reaction and pathogenicity gen cluster)	Conventional	Seed washes (DNA extraction) (sodium ascorbate and PVPP)	Leite *et al.,* 1995	*X. campestris* pv. *vesicatoria*
X. campestris pathovars: *aberrans, armoriaceae, barbarae, campestris, incanae, raphani*	**DLH120/DLH125** *hrpF* gene (Specific for *X. campestris*) + **DLH138/DLH139** ITS region from *Brassica* spp. (host internal control) **DHL153/DHL154** *hrpF* gene (Specific for *X. campestris*) **+** **DHL155/DHL156** ITS region and 5.8S rRNA gene from *Brassica* spp.	Multiplex-Conventional Multiplex-Real-time (SBYR® Green Master Mix) (fluorescently labeled probes)	Bacteria, seed-washes (DNA extraction)	Berg *et al.,* 2005, 2006	
Xanthomonas Numerous pathovars (not *translucens* group)	**RST2/RST3** **RST9/RST10** *hrpB* (hypersensitive reaction and pathogenicity gen cluster) **RST21RST22** *hrpC, hrpD* groups	Conventional and RFLP	Bacteria (DNA extraction)	Leite *et al.,* 1994	
X, campestris hordei *X. translucens* pathovars: *arrhenatheri, cerealis, graminis, phlei, phleipratensis, poae secalis, translucens* and *undulosa*	**T1/T2** ITS region	Conventional	Bacteria, seeds (boiled)	Maes *et al.,* 1996b	*X. campestris* pathovars: *cerealis, secalis, translucens, undulosa, arrhenatheri, graminis, phlei, phleipratensis, poae* No distinction of the five cereal leaf streak pathovars from the other five pathovars.

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X. fragariae *X. arboricola* pv. *fragariae*	*X. fragariae* **Xf gyrB-F/ Xf gyrB-R** (primers) **Xf gyrB-P** (probe) *GyraseB* gene *X. arboricola* pv. *fragariae* **Xaf pep-F/ Xaf pep-R** (primers) **Xaf pep-P** (probe) *pep* propyl endopeptidase gene	Real-time (TaqMan)	Bacteria (boiled) and plant (DNA extraction)	Weller *et al.*, 2007	*Xanthomonas arboricola* pv. *fragariae* not included in the ISPP List. Primers *Xf gyrB* specific for *X. fragariae*. *Xaf pep* primers detect other *X. arboricola* pathovars assayed also, but not *X. fragariae*.
X. hortorum pv. *pelargonii*	**XcpM1/XcpM2** DNA (ERIC) **RS3/Rs4** *R. solanacearum pehB* gene **DG1/DG2** 18S rRNA gene (host internal control)	Multiplex	Bacteria (DNA extraction), plant (GeneReleaser)	Glick *et al.*, 2002	*X. campestris* pv. *pelargonii* *Ralstonia solanacearum* also amplified.
X. oryzae (pathovars *oryzae* and *oryzicola*)	**R16-1/R23-2R** 16S-23S rDNA spacer region	Conventional	Bacteria (DNA extraction)	Kim and Song, 1996	Differentiation between *X. oryzae* pathovars *oryzae* and *oryzicola*. *Acidovorax avenae* (*Pseudomonas avenae*), *Burkholderia glumae* (*Pseudomonas glumae*), *Pantoea agglomerans* (*Erwinia herbicola*), *Pseudomonas fuscovaginae* and *Pseudomonas syringae* pv. *syringae* also amplified and differentiated by primary and secondary fragments.
X. vesicatoria *Pseudomonas syringae* pv. *tomato*	**BSX1/BSX2** **(Xanthomonas)** Genomic DNA (unknown) **COR1/COR2** **(Pseudomonas)** Coronafacic acid *cfa7* gene	Conventional	Bacteria, plant (freeze-boiled method DNA extraction)	Cuppels *et al.*, 2006	*X. axonopodis* pv. *vesicatoria* and *X. gardneri* are not valid names according to the ISPP List. BSX primers amplify *X. vesicatoria.* Other coronatine-producing *P. sryringae* pathovars also amplified with COR primers.
Genus *Xylella*					
Species / hosts	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
X. fastidiosa / citrus	*X. fastidiosa* citrus strains specific **CVC-1/272-2-int** RAPD fragment *X. fastidiosa* strains (general) **272-1-int/272-2-int**	Conventional	Bacteria, sap (DNA extraction)	Pooler and Hartung, 1995	

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X. fastidiosa / citrus	Primers **CVC-1/CCSM-1** Probe **TAQCVC 5´6FAM-labeled 3´TAMRA labeled** Genomic DNA (unknown)	Real-time (TaqMan)	Bacteria, leaf (DNA extraction)	Oliveira *et al.,* 2002	
X. fastidiosa / grapevine	**RST31/RST33** Genomic DNA (unknown)	Conventional	Bacteria, leaf (DNA extraction)	Berisha *et al.,* 1998	
X. fastidiosa / grapevine	**XfF1/XfR1** ITS region **XfF2/XfR2** 16S rRNA gene Probes **5´6FAM-labeled 3´TAMRA labeled** (ITS) **5´6FAM-labeled 3´TAMRA labeled** (16S)	Real-time (TaqMan) Multiplex-Real-time (TaqMan)	Sap and macerated chips of secondary trunks of vines xylem (untreated)	Schaad *et al.,* 2002	
X. fastidiosa / grapevine	**XfF1/XfR1** ITS region **Probe 5´6FAM-labeled 3´TAMRA labeled ITS**	Real-time (TaqMan) BIO (Agar absorption)	Xylem sap (DNA extraction) Leaf and petiole (directly or previous plating)	Baumgartner and Warren, 2005 Fatmi *et al.,* 2005	
X. fastidiosa / citrus and grapevine	**RST31/RST33** Genomic DNA (unknown)	Conventional	Plant and xylem fluid (PVPP and sodium ascorbate addition)	Minsavage *et al.,* 1994	
X. fastidiosa / citrus and grapevine	*X. fastidiosa* grapevine strains **RST31/RST33** Genomic DNA (unknown) *X. fastidiosa* citrus strains **CVC-1/272-2-int** RAPD fragment	Conventional	Plant and xylem fluid (DNA extraction)	Minsavage *et al.,* 1994; Pooler and Hartung, 1995; Anon., 2004b	Recommended in the EPPO protocol.
X. fastidiosa / citrus and coffe	**JB-1/JB-2** RAPD fragment	Conventional	Plant tissue (DNA extraction)	Ferreira *et al.,* 2000	Strains from various hosts amplified at annealing 64ºC. Only citrus and coffe related strains amplify at 68ºC.
X. fastidiosa / grapevine and oleander	**RST31/RST33** Genomic DNA (unknown)	Conventional	Bacteria, xylem sap or plant (DNA extraction)	Bextine and Miller, 2004	
X. fastidiosa / grapevine, almond, oleander	**XF1968-L/1968-R** XF1968 methyltransferase gene	Conventional Multiprimer	Bacteria, plant tissue (DNA extraction)	Hernandez-Martinez *et al.,* 2006	

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	XF2542-L/XF2542 XF2542 fimbrial protein gene **ALM1/ALM2** Genomic DNA (unknown)				
X. fastidiosa	**272-1/272-2** (external) **272-1-int/272-2-int** (internal) RAPD fragment	Nested + Immunomagnetic separation (IMS)	Immunomagnetic separation of insects tissue extracts	Pooler *et al.*, 1997	
X. fastidiosa	**S-S-X.fas (sets A, B, C)** 16S rRNA gene **FXYgyr499/RXYgyr907** *gyrB* gene	Multiplex	Plant tissue, vector insects (DNA extraction)	Rodrigues *et al.*, 2003	
X. fastidiosa	**RST31/RST33** Genomic DNA (unknown) **G1/L1** ITS region	Conventional Immunocapture and conventional	Plant (DNA extraction)	Costa *et al.*, 2004	
X. fastidiosa	**RST31/RST33** Genomic DNA (unknown) **HL5/HL6** Genomic DNA (unknown) Probe **5´6FAM-labeled** **3´BHQ1™ labeled**	Conventional Real-time (TaqMan)	Plant and insect vectors (DNA extraction)	Francis *et al.*, 2006	
Genus *Xylophylus*					
Species	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
X. ampelinus	**Xamp 1.27A/Xamp 1.27B** **Xamp 1.27A/Xamp 1.27C** **Xamp 1.3A/Xamp 1.3B** Subtractive hybridization	Conventional	Bacteria (DNA extraction)	Manceau *et al.*, 2000	
X. ampelinus	**A1/B1** **S3/S4** **A1/B1** (external primers) **S3/S4** (internal primers) ITS region	Conventional Nested	Bacteria and stem sap (DNA extraction)	Botha *et al.*, 2001	
X. ampelinus	**XATS1/XATS2-Biotin** ITS region	PCR-ELISA	Bacteria (boiled) and bleeding sap (DNA extraction)	Grall *et al.*, 2005	
X. ampelinus	**Xamp 1.27A/Xamp 1.27C** Subtractive hybridization	Conventional	Plant, sap (DNA extraction)	Manceau *et al.*, 2005	

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	S3/S4 ITS region **XATS1/XATS2**-Biotin ITS region	PCR-ELISA			
X. ampelinus	**A1/B1** (external primers) **S3/S4** (internal primers) ITS region **Xamp 14F/Xamp 104R (primers)** **Xamp 14F/104 MGB (probe)** Subtractive hybridization	Nested Real-time (TaqMan)	Plant (DNA extraction)	Botha *et al.,* 2001 Dreo *et al.,* 2007	
Other bacteria "Bacteria-like Organisms" (BLOs)					
"Candidatus Phlomobacter fragariae"					
Species	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
Ca. Phlomobacter fragariae	**Fra4/Fra5** 16S rRNA gene	Conventional	Plant (DNA extraction)	Zreik *et al.,* 1998	Bacterium within group 3 of the gamma subclass of *Proteobacteria.*
Papaya bunchy top disease of Cucurbita (PBT)					
Organism	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
PBT (Gamma-3 proteobacterium associated with BLO disease)	**YV1/YV2** **YV1/YV3** 16S rRNA gene	Conventional	Phloem tissue (DNA extraction)	Davis *et al.,* 1998	
Yellow vine disease (YVD)					
Organism	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/bbservations
YVD (Gamma-3 proteobacterium associated with BLO disease)	**YV1/YV2** **YV1/Yv3** 16S rRNA gene	Conventional	Phloem tissue (DNA extraction)	Avila *et al.,* 1998	
Blood Disease Bacterium (BDB)					
Organism	Primer name Target DNA	Variant of PCR protocol	Sample (treatment)	Reference	Synonyms/observations
Blood Disease Bacterium (remains unclassified)	**OLI1/Y2** 16S rRNA gene	Conventional	Bacteria (boiled)	Seal *et al.,* 1993	*Ralstonia solanacearum* and *R. syzygii* also amplified.
Blood Disease Bacterium (remains unclassified)	**D2/B**[1] **OLI1/Z** 16S rRNA gene	Conventional	Bacteria (untreated)	Boudazin *et al.,* 1999	

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