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Specific DNA markers for detection of bacterial canker of kiwifruit in Sichuan, China

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Bacterial canker of kiwifruit (*Actinidia chinensis*) has become one of the most serious factors that limit the cultivation of kiwifruit in Sichuan, China. Therefore, it is urgent to develop a rapid and precise method to detect the disease at its early infection stage. A 1300 bp specific fragment of pathogenic bacteria was obtained by random amplified polymorphic DNA (RAPD) analysis. This fragment was cloned and sequenced. Based on the sequences of the specific fragment, a pair of specific primers（F7'/R7'）that generated 994 bp single fragment only from the strains of *Pseudomonas syringae* pv. *actinidiae* were designed and synthesized. Then, for the detection of diseased kiwifruit trunk tissues collected from orchard and inoculated kiwifruit trunk tissues, the result indicated that the genomic DNA of *P. syringae* pv. *actinidiae* can be detected by the specific primers. The sensitivity of the primer set for genomic DNA of *P. syringae* pv. *actinidiae* was 100 fg μl^{-1}. Compared with the designed primers with four pairs of published primers. The comparison concluded that only the primers designed in this study was specific to detect bacterial canker of kiwifruit in Sichuan. The result of this experiment showed that the designed and synthesized specific primers F7'/R7' made it possible to diagnose the kiwifruit canker rapidly and precisely at its early infection stage.

Key words: Bacterial canker, kiwifruit, primer, RAPD, SCAR, *P. syringae pv. Actinidiae*.

INTRODUCTION

Kiwifruit (*Actinidia chinensis*) is the fruit of high economic value. So many countries, such as Italy, New Zealand, Chile, Japan, Iran and China, have actively developed their planting industries. However, the occurrence of bacterial canker of kiwifruit has become one of the most serious factors that limit the cultivation of kiwifruit in these countries. The disease was first reported in Japan and USA in early 1980s (Opgcnorth et al., 1983; 1984; Serizawa et al., 1989). Subsequently, it was also observed in Korea (Koh and Lee, 1992), Iran (Mazarei and Mostofipour, 1994) and Italy (Scortichini, 1994). In China, the disease was first found in Hunan province in 1984 (Fang et al., 1990). Then within a few years, it rapidly spread to the provinces of Sichuan (Liu et al.,1996), Anhui (Cheng et al., 1995), Fujian (Lin et al., 1995) and so on. Since the disease has caused serious

damages to the production of kiwifruit which is strong enough to destroy some of the orchards, it is very essential to establish efficient control strategies for the disease. Some traditional methods, such as spraying antibiotics, copper compounds or trunk injection of antibiotics, have been proved to achieve partial success in controlling the disease (Koh et al., 1996, 1999; Serizawa et al., 1989; Ushiyama, 1993). Nevertheless, the disease shows the typical symptoms two or three years after latent infection, which makes it difficult to diagnose the disease with the naked eyes. Meanwhile, chemical control is considered to be less effective after the symptoms appear. Therefore, it is very important to develop a method that can detect the disease in its early infection stage. The casual agent of bacterial canker of kiwifruit was identified as *Pseudomonas syringae pv. actinidiae*, which was first reported in Japan (Takikawa et al., 1989) and then in Italy (Scortichini, 1994). It was also demonstrated in China by many scholars (Wang et al., 1992; Zhu et al., 1993; Cheng et al., 1995). However,

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Table 1. Strains of *Pseudomonas syringae* pathovars or similar strains of *Pseudomonas*, or other genuses used in this study.

Strain	Specie or pathovars	Source
DY10	*Pseudomonas syringae pv. actinidiae*	Deyang, Sichuan, China
QL14	*Pseudomonas syringae pv. actinidiae*	Qionglai, Sichuan, China
DJY08	*Pseudomonas syringae pv. actinidiae*	Du Jiangyan, Sichuan, China
CZ20	*Pseudomonas syringae pv. actinidiae*	Chongzhou, Sichuan, China
P2105	*Pseudomonas syringae pv. actinidiae*	Chinese Academy of Forestry, Beijing,China
P1336	*Pseudomonas syringae pv. syringae*	Chinese Academy of Forestry, Beijing,China
DY32	*Pseudomonas fluorescens*	Deyang, Sichuan, China
P1368	*Pseudomonas syringae pv. morsprunorum*	Chinese Academy of Forestry, Beijing,China
QL04	*Erwina amylovora*	Qionglai, Sichuan, China
P2104	*Bacillus pumilus*	Chinese Academy of Forestry, Beijing,China
P2726	*Pseudomonas eruginosa*	Chinese Academy of Forestry, Beijing,China
P1481	*Xanthomonas oryzae*	Chinese Academy of Forestry, Beijing,China
P2090	*Pseudomonas syringae*	Chinese Academy of Forestry, Beijing,China
P2430	*Pseudomonas syringae pv. tomato*	Chinese Academy of Forestry, Beijing,China

there are several similar pathovars or species, such as *Pseudomonas syringae pv. syringe* that causes blossom blight and *Pseudomonas syringae pv. morsprunorum*, to be repeatedly isolated from kiwifruit trees, which are not easy to distinguish from the pathogenic bacteria (Koh et al., 1993; Young et al., 1988; Opgcnorth et al., 1983; Koh and Lee, 1992; Takikawa et al., 1989). The traditional physiological and biochemical methods to identifying the pathovars of plant pathogenic bacteria are considered tedious and time-consuming (Schaad, 1988). Recently, the development of molecular marker technology has provided new tools for detecting the pathovars of plant pathogenic bacteria by looking directly at the level of DNA sequence and structure. Random amplified polymorphic DNA (RAPD; Williams et al., 1990) is a PCR-based technique and extremely efficient for DNA analysis in complex genomes as it is relatively inexpensive and yields information on a large number of loci without having to obtain sequence data for primer design (De Wolf et al., 2004). This technology has been widely used to identify and diagnose the plant pathogenic bacteria (Audy et al., 1996; Parry Nicholson, 1996; Sorensen et al., 1998; Koh and Nou, 2002). However, RAPD markers lack reproducibility due to mismatch annealing (Demeke et al., 1997; Karp et al., 1997; Neale and Harry, 1994). To overcome this problem associated with the RAPD analysis, RAPD markers have been converted into sequence characterized amplified region markers (SCAR; Paran and Michelmore, 1993). Several reports are available of the development of SCAR marker in many plants (Hernandez et al., 1999; Evans and James, 2003).

Although several countries have developed the specific DNA markers to detect the bacterial canker of kiwifruit (Koh and Nou, 2002; Templeton et al., 2005; George et al., 2010), there are no appropriate markers among them for the disease in China especially in Sichuan province, probably because of the geographical differences. The objective of this study was to develop an early diagnosis method which would be able to detect a latent infection of *P. syringae pv. actinidiae* on kiwifruit, especially for the disease which occurred in Sichuan province in China. The pathovar-specific RAPD and SCAR markers were selected in this experiment for the rapid and precise identification of *P. syringae pv. actinidiae* among similar pathovars or species that are isolated from kiwifruits.

MATERIALS AND METHODS

Strains used and cultivating condition

Five strains of *P. syringae pv. actinidiae* and nine similar pathovars or species were used in this study (Table 1). The NO.1-NO.4 strains of *P. syringae pv. actinidiae* were isolated from the infected trunks of kiwifruit trees which were collected in Deyang, Qionglai, Du Jiangyan, and Chongzhou respectively, and identified as the agent of bacterial canker of kiwifruit with the pathogenicity, bacteriological study and molecular identification based on the 16S rDNA sequences. The NO.7 and NO.9 strains were also isolated from the infected trunks, but identified as the other species rather than the pathogenic bacteria. The other eight strains were all kindly provided by the Research Institute of Forestry Ecology, Environment and Conservation, Chinese Academy of Forestry, China. A single colony of each strain was isolated on the peptone-sucrose-agar (PSA; peptone 20%, sucrose 20%, agar 15%, pH 6.8) medium for the experiment.

P. syringae pv. actinidiae and all of the other strains were transferred onto fresh PSA medium, and cultivated at 25°C.

Extraction of genomic DNA

Cultures for DNA extraction were grown overnight in Luria-Bertani (LB; tryptone 10 g/L, Yeast extract 5 g/L, NaCl 10 g/L, pH 7.0) medium at 26°C on a rotating shaker at a speed of 250 rpm. DNA

was extracted by TIANamp Bacteria DNA Kit (Tiangen, China). The concentration and quality of DNA samples were estimated by comparing band intensities on agarose gel and by measuring the 260 nm/280 nm absorbance ratio.

Extraction of kiwifruit tissue DNA

All the kiwifruit tissue DNA was extracted by TIANamp Plant Genomic DNA Kit (Tiangen, China). Then, the samples were tested by the same method as the genomic DNA of strains.

RAPD analysis

One hundred and twenty random primers, including 40 commercial 10-mer oligonucleotide primers and 80 commercial 12-mer oligonucleotide primers (Sangon Biotech, Shanghai, China), were used to select primers for screening the specific fragment of *P. syringae pv. actinidiae*. Amplification was performed in a total volume of a 25 µl reaction mixture with 50 pmol of primer, 100 ng of genomic DNA, 1 Unit of Taq DNA polymerase, 10× reaction buffer (500 mM KCl, 100 mM of Tris-HCl pH 8.3, 25 mM of MgCl$_2$, 0.01% of gelatin), 10mM of dNTP (dCTP, dGTP, dATP, dTTP), and extra sterilized distilled water. The conditions of DNA amplification were optimized and followed the procedure of Williams et al. (1990) with few modifications. The PCR program consisted of an initial denaturation of 5 min at 94°C, followed by 40 cycles at 94°C for 60 s (denaturation), 36°C for 60 s (annealing) and 72°C for 2 min (extension), with an additional extension period of 10 min at 72°C. A negative control, without genomic DNA, was run with every set of samples to confirm that no contaminating DNA was present in the reaction. Amplifications were carried out in a Mastercycler (Mastercycler Gradient, Eppendorf, Germany). The amplified PCR products were separated by electrophoresis on 1.5% agarose gels in 1×TAE buffer, visualized and imaged by gel documentation system (GelDoc 2000, Bio-Rad, USA) after staining with ethidium bromide.

Cloning and sequencing of the specific DNA fragment

The selected specific DNA fragment was isolated from the gel by TIANgel Midi Purification Kit (Tiangen, China) and cloned into PMD18-T Vector (TaKaRa, Dalian, China). Plasmid DNA was extracted by the alkaline lysis method and harvested by centrifugation at 12,000 rpm for 20 min at 4°C after incubation on ice for 20 min with the PEG solution (20% of polyethylene glycol, 2.5 M of NaCl). For determining the sequences of specific DNA fragment, the purified plasmid DNA was used as a template to amplify the inserted DNA. The nucleotide sequence of the cloned DNA was sequenced by the Sangon Biotech (Shanghai, China).

Establish of sequence characterized amplified regions (SCAR) markers

Based on the sequence of a specific DNA fragment (1300 bp) of *P. syringae pv. actinidiae*, the forward and reverse primers were designed by extending the original twelve bases of RAPD primer. The designed SCAR primers (F7'/R7') were synthesized by Sangon Biotech (Shanghai, China).

Exploration of the optimal annealing temperature

PCR conditions for genomic DNA amplification with SCAR primers were the same as for RAPD analysis, except for the annealing temperatures. Twelve annealing temperature gradient have been set by the Gradient PCR instrument from 55 to 65°C automatically, that is 55, 55.2, 55.7, 56.8, 57.8, 59.1, 60.5, 61.8, 63.1, 64.2, 65, 65.5°C. Then, PCR amplifications were conducted with the specific primers at the different annealing temperature in order to explore the optimal annealing temperature.

Specificity detection of the SCAR markers

The genomic DNA of strains listed in Table 1 were amplified by the specific primers (F7'/R7') respectively, with sterilized distilled water as the control. The PCR reactions were conducted at the explored optimal annealing temperature and the other PCR conditions for the test were the same as for RAPD analysis.

Specificity detection of pathogenic bacteria in kiwifruit trunk tissues

The genomic DNA of *P. syringae pv. actinidiae* and the genomic DNA of trunk tissues of diseased kiwifruit collected from orchard and inoculated kiwifruit were amplified by the specific primers (F7'/R7') respectively, with healthy trunk tissues and sterilized distilled water as the control. The PCR reactions were conducted according to the above descriptions. Then, isolated the pathogenic bacteria from the tissues which have been detected pathogens by molecular method, and identified the isolates with the pathogenicity, bacteriological study and molecular identification based on the 16S rDNA sequences to see whether the results are consistent with the molecular detection.

Sensitivity test of the SCAR markers

The concentration of the extracted genomic DNA of *P. syringae pv. actinidiae* have been determined by the nucleic acid concentration detector, and then diluted to 6 concentration gradient, that is 1 ng µl^{-1}, 100 pg µl^{-1}, 10 pg µl^{-1}, 1 pg µl^{-1}, 100 fg µl^{-1}, 10 fg µl^{-1}. The different concentrations of genomic DNA were amplified by the specific primers with sterilized distilled water as control. The other PCR conditions were the same as the above descriptions.

Testing other published primers

Using four pairs of published primers (Table 2) amplified the genomic DNA of *P. syringae pv. actinidiae* and other two similar pathovars or species (*P. syringae pv. syringae* and *P. syringae*) respectively. PCR conditions were the same as for SCAR primers. The PCR products were also screened by electrophoresis on 1.5% agarose gels in 1×TAE buffer, visualized and imaged by gel documentation system (GelDoc 2000, Bio-Rad, USA) after staining with ethidium bromide.
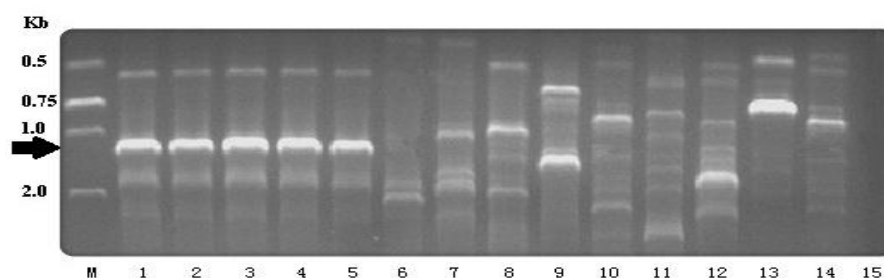
RESULTS

Screening of specific RAPD primers for the pathogenic bacteria

According to the RAPD profile (Figure 1), only the primer P7 (5'-CGCAGCCGAGAT-3') repeatedly produced a specific, stable and distinct DNA fragment, which was produced only from the strains of *P. syringae pv. actinidiae*, but not from the other strains. The size of the

Table 2. The sequences of four pairs of published primers.

Primer pair	Sense	Product size (bp)	Sequences (5'-3')	Reference
KNF	Forward	492	CACGATACATGGGCTTATGC	Koh and Nou (2002)
KNR	Reverse		CTTTTCATCCACACACTCCG	
PsaF1	Forward	280	TTTTGCTTTGCACACCCGATTTT	George et al. (2010)
PsaR2	Reverse		CACGCACCCTTCAATCAGGATG	
ArgKF3	Forward	800	TCCCCCCGGGAGGAAATTCAATGAAGATTA	Templeton et al. (2005)
ArgKR4	Reverse		AACTGCAGTCAGGGGACGACTGTCTC	
Ps.ITSF1	Forward	880	GTGATTCATGACTGGGGTGA	George et al. (2010)
Ps.ITSR2	Reverse		ATAACCCCAAGCAATCTGGT	

Figure 1. RAPD profiles are generated with primer P7. Lane M, 2 kb DNA ladder; lanes 1-14, strains of *P. syringae* pathovars or similar strains of *Pseudomonas* or other species listed in Table 1; lane15, CK (ddH$_2$O).

specific fragment is about 1300 bp that is suitable for the development of a primer to detect the pathovar-specific DNA fragment of *P. syringae pv. actinidiae*.

Cloning and sequencing of the pathovar-specific DNA fragment

The specific fragment generated by primer P7 was isolated from the gel, and cloned into a T-vector, followed by transforming in *E.coli* (JM109). Plasmid DNA was extracted from the transformations, and the target fragment was obtained and cloned. The purified plasmid DNA was sent to Sangon Biotech to determine the sequence of the specific fragment. The result of the sequencing is shown in Figure 2, which confirmed the genome size of the specific fragment is 1300 bp.

Establish of SCAR markers

Based on the sequence of the 1300 bp specific fragment, a pair of distinctive primers were designed and synthesized to examine the pathogenic bacteria of

bacterial canker on kiwifruit trees. The sequences of the forward primer were found to be 5'-CGATTCGCAGCCGAGATG-3' from 27 to 44 bp, by extending the original twelve bases of RAPD primer. The reverse primer was designed as 5'-CTACGAGGTTAGGTTCAGAGT-3' from 1000 to 1020 bp (Figure 2). The G+C content of forward and reverse primer were 61.1 and 47.6% respectively.

Exploration of the optimal annealing temperature

According to the PCR profiles (Figure 3), all the 12 annealing temperature gradient could produced ideal band. But the third one is relatively more distinct and stable. Therefore, the optimal annealing temperature was determined as 56°C.

Specificity detection of the SCAR markers

Using the synthesized primer pair amplified, the genomic

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  1 - TGCCAGCTTG CATGCCTGCA GGTCGACGAT TCGCAGCCGA GATGCAATCC ATGCTCATCA - 60
 61 - TGAAGGGCAA TCATTTCGCC AGACGCCCAG AATCGCACTC CCTCACGGGC TATATGCCAG - 120
121 - CGCTCGTCCA TGGCACGTCC ATCGCGTAAA GCGACGTTCA TTTCATAAGC TACGCGTCCT - 180
181 - GCAGCACGGT CTTCGGGCGT AAAGAATCTG GACGCGTCCT GACCGCACAT TTCCTCAGAG - 240
241 - GTCCACCCCA TAACCAGTTG AGCGCCTGCG TTCCAAGCTG AAATTGTTCC ATTGGGATCG - 300
301 - GTGACCACCA TGGCGAACTC GGTTGCACTG TCGAATATTG CATCCTGTCG AACCCTGTTC - 360
361 - ATTTTTTGAT CAAAGGATGT TCTGCGCAGT TGCCGCACTT CGTTTTCAAG CGCCTCACGG - 420
421 - GACATTAGGC TTAGATCTTT GTCGGACAAT TTTCAATTCC TCAACGGCAG TAACACGCCG - 480
481 - CGCCTCACGG TATGGCACCC GCCCATCGAG ATAACGAGAG CTGAGGTGTA TCTTTTTGAA - 540
541 - ACAAAAGCAA TCCACCTTAT CTCATATAGG GCATATGACA CTGAAGATAG GTCTATGGAC - 600
601 - GCAACAATTT TACTCGTAGA AGATGATGAC ACGCTGCGAA TGGTGACGGC AGATGCGCTA - 660
661 - TCTTTGCTTG AAGCCAATGT CATAGAGTGC AACAGTGCCG ACCAGGCTGT AGCCGTGCTT - 720
721 - GAGTCAGGCG CTCTTATCCA GTTGCTTTTC ACTGACATAT GCATGCCCGG TTCGATGGAT - 780
781 - GGCTTGCAGC TTGCTGGGTT GGTAGCATCT CGCTGGCCAA GCTTAGAGAT TATCGTCACA - 840
841 - TCAGGGAACC GGTTGCCAAC CGAGGTCTTA CCTGAAAAGG TTCTGTTCAT CGCAAAACCT - 900
901 - TGGACGCTTT CGGAGCTTTT GCATAGAGTC CGACCGCTGC TGTCGCACAC TCATCCAACT - 960
961 - GACTAGCAGC CAACTCCAAT TTTCCTGTCT ACATAGCACA CTCTGAACCT AACCTCGTAG - 1020
1021- TATGTACTCC TGCACTGCGT GAAGCTCACA GAGGTACTCG ACCGCCAAAT CGAGCAATTA - 1080
1081- GCGTCATTAC CGTACTCTAA CTTTTTTTGG AATTGCAAAT GCCCATGCTT AACCAATCGC - 1140
1141- AGCAAGAGGT TAAAATATCT GGAAAAGTAG TGGTCGTAGA AGATGATGAG TTGATCGCG - 1200
1201- GCCTGATTGT AGAGATTTTC ACAGACCTCG GCGCTAGCTG CATATCTTTT ATCTCGGCTG - 1260
1261- CGAATCTCTA GAGGATCCCC GGGTACCGAG CTCGAATTCG - 1300
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Figure 2. Nucleotide sequences of *P. syringae pv. actinidiae* specific DNA fragment. Underlined sequences are forward and reverse primers for the detection of a specific DNA fragment in *P. syringar pv. actinidiae*; Shadow sequences are the primer P7 that is used in RAPD analysis.

DNAs of all the strains are listed in Table 1. A single, distinct and brightly resolved band of 994 bp was obtained only from the strains of *P. syringae pv. actinidiae*, but not from the other strains (Figure 4). It indicated that the designed primers had certain specificity which could detect the *P. syringae pv. actinidiae* from other similar strains.

Specificity detection of pathogenic bacteria in kiwifruit trunk tissues

According to Figure 5, after artificial inoculation, no matter what stage the trunk tissue was, the genomic DNA of the trunk tissues could produce a 994 bp specific band. Nevertheless, the healthy trunk tissue and sterilized distilled water could not amplify a certain band, which illustrated that the specific primers could detect the pathogenic bacteria from the host tissues and even diagnose the disease before any symptoms appears. Furthermore, the detections for the pathogenic bacteria which was isolated from the same tissues with classic method turned out to be consistent with the result of the molecular dectections.

Sensitivity test of the SCAR markers

According to Figure 6, the specific primers could amplify a distinct and stable band at the concentration of 100 fg μl^{-1}, whereas it could not produce a fragment at or under the concentration of 10 fg μl^{-1} as well as the sterilized
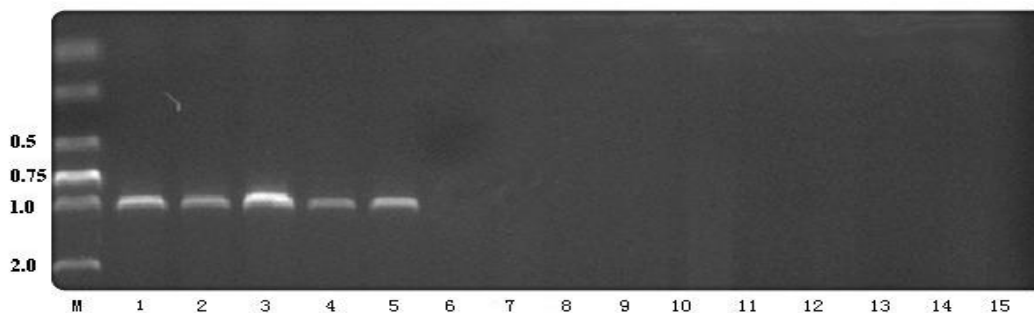
Figure 4. PCR profiles of all the strains amplified with the designed specific primer F7'/R7'. Lane M, 2 kb DNA ladder; lanes1-14, strains of *P. syringae* pathovars or similar strains of *Pseudomonas* or other species listed in Table 1; lane15, CK (ddH$_2$O).
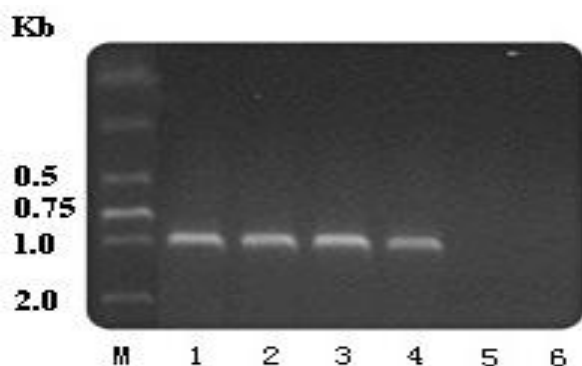

Figure 5. PCR profiles of different samples amplified with the specific primer F7'/R7'. Lane M, 2 kb DNA ladder; lane 1, genomic DNA of *P. syringae pv. actinidiae*; lane 2, DNA of early days of artificial inoculation (not show symptoms); lane 3, DNA of heavy period of artificial inoculation (has shown symptoms); lane 4, DNA of diseased kiwifruit tissues which collected from orchard; lane 5, healthy trunk tissues; lane 6, CK (ddH$_2$O).
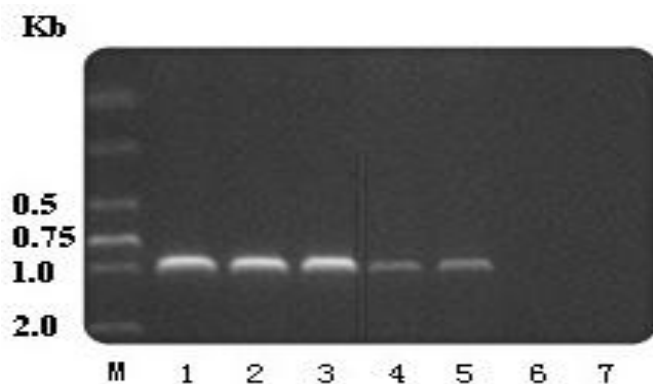

Figure 6. Sensitivity of PCR with specific primer F7'/R7' at different concentrations of DNA. Lane M, 2 kb DNA ladder; lanes 1-6 amplified products using DNA at concentration of 1 ng µl^{-1}, 100 pg µl^{-1}, 10 pg µl^{-1}, 1 pg µl^{-1}, 100 fg µl^{-1}, 10 fg µl^{-1}, respectively; lane 7, CK (ddH$_2$O).

distilled water.

Detection of *P. syringae pv. actinidiae* by other published primers

The extracted genomic DNAs of *P. syringae pv. actinidiae* and other two similar pathovars or species (*P. syringae pv. syringae* and *P. syringae*) were amplified by four pairs of published primers respectively. The PCR profile showed that all of the four pairs of primers were not specific for detecting the pathogenic bacteria from the three tested strains (Figure 7). Primer KNF/KNR and Ps.ITSF1/Ps.ITSR2 could not only produce the fragment from the strains of *P. syringae pv. actinidiae*, but also from the other strains. And primer PsaF1/PsaR2 could hardly amplify the bands as well as primer ArgKF3/ArgKR4.

DISCUSSION

In this study, a 1300 bp fragment that was amplified with random primer P7 by RAPD analysis was only screened from *P. syringae pv. actinidiae* among all the tested strains that are frequently isolated from kiwifruit trees. It is expected that the pathogenic bacteria could be identified quickly and precisely by the RAPD experiment using the random primer P7. However, the sensitivity of the RAPD reaction to a number of reaction parameters at a low annealing temperature has made it hard to generate consistent profiles even under laboratory conditions, which necessitates converting the RAPD marker into a more stable SCAR marker. But the transformation is not easy to succeed for many reasons, such as inappropriate primer design, multi-copy sequences of obtained RAPD fragment, DNA methylation and many other reasons (Sun et al., 2010). To overcome these problems, we need to design pairs of primers and explore the optimal annealing temperature and reaction conditions. There have been many cases of successful conversions (Meetu and
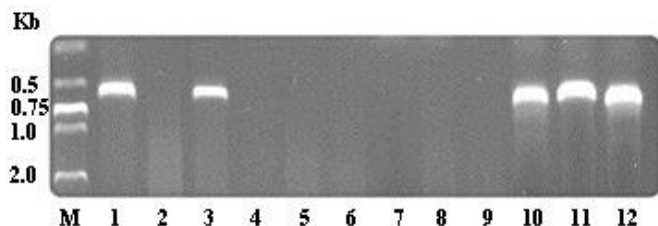
Figure 7. PCR profiles of screening of *Pseudomonas syringae pv. actinidiae* by four pairs of published primers. Lane M, 2 kb DNA ladder; lane 1-3, strains of *P. syringae pv. actinidiae, P. syringae pv. syringae* and *P. syringae* listed in Table 1 amplified with primer KNF/KNR, respectively; lane 4-6, lane7-9,lane 10-12, the same tested strains as lane 1-3 amplified with primer PsaF1/PsaR2,   ArgKF3/ArgKR4   and   Ps.ITSF1/Ps.ITSR2, respectively.

Neera, 2009; Masuzaki et al., 2008; Kim et al., 2008; Mikolajczyk et al., 2008). In this experiment, the specific 1300 bp fragment that was amplified by RAPD reactions was cloned and sequenced for designing the SCAR primers. Based on the sequences of the 1300 bp fragment, a pair of SCAR primers (F7'/R7') was selected from the several pairs of designed primers to generate the 994 bp single fragment only in the strains of *P. syringae pv. actinidiae*, but not in other strains and sterilized distilled water . The optimal annealing temperature of this pair of synthesized primers was found to be 56°C. Then, for detection of inoculated kiwifruit trunk tissues, the result indicated that the genomic DNA of *Pseudomonas syringae* pv. *actinidiae* could be detected by the specific primers, no matter what stage the inoculated kiwifruit trunk tissues was. This result confirmed that the specific primers could detect the pathogenic bacteria from the host tissues and even diagnose the disease before any symptoms appears. Meanwhile, through the sensitivity test, the specific primers could successfully detect the pathogen genome DNA at the concentration of 100fg/µl. That means the specific primers could detect the pathogen at a very low concentration of the genomic DNA, which makes it possible to detect the disease at its early latent period. In addition, this experiment also used four pairs of published primers to amplify the strains of *P. syringae pv. actinidiae*, *P. syringae pv. syringae* and *P.syringae*, in order to compare the primers designed in this study with the published primers and prove the specificity of the designed primers to detect the bacterial canker of kiwifruit in Sichuan. The result of comparison showed that the published primers were not specific to *P. syringe pv. actinidiae* that was isolated from kiwifruit trees in Sichuan.   Primer   KNF/KNR   and   primer Ps.ITSF1/Ps.ITSR2 detected other Pseudomonad's common as well as *P. syringae pv. actinidiae*. And primer PsaF1/PsaR2 could hardly detect a fragment from the three tested strains as well as primer ArgKF3/ArgKR4.

Only the primers designed in this study were able to distinguish *P. syringae pv. actinidiae* from other similar pathovars or species that frequently isolated from the kiwifruit trees in Sichuan. The reason for the result may be the geographical differences the pathogenic bacteria's genomic DNA mutates so that those published primers cannot perfectly detect the bacterial canker of kiwifruit in Sichuan.

Compared with the traditional detection methods, the specific primers (F7'/R7') can greatly shorten the time of diagnosis and has higher accuracy and sensitivity. Moreover, the method is relatively simple, cheap and flexible, and has less intermediate procedures so that it can avoid unnecessary interference. Besides, it can detect the pathogen in the early latent period of the disease. Therefore, it can be used to inspect and quarantine the plants and plant products between the endemic area and non endemic areas in order to control the further spread of the kiwifruit canker. To some extent, the development of the primers for detection the pathogen of the kiwifruit canker also fills the gap of molecular detection of *Pseudomonas syringae* pv. *actinidiae* in China. Since the use of the primers designed in this study would make it possible to rapidly and precisely detect the *P. syringae pv. actinidiae* among the various germs that are similar to the pathogenic bacteria or frequently isolated from the kiwifruit trees, the development of the primers is expected to greatly contribute to the early diagnosis and control of the bacterial canker disease on kiwifruit.

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