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Development of a Species-specific PCR Assay for Three *Xanthomonas* Species, Causing Bulb and Flower Diseases, Based on Their Genome Sequences

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In this study, we developed a species-specific PCR assay for rapid and accurate detection of three *Xanthomonas* species, *X. axonopodis* pv. *poinsettiicola* (XAP), *X. hyacinthi* (XH) and *X. campestris* pv. *zantedeschiae* (XCZ), based on their draft genome sequences. XAP, XH and XCZ genomes consist of single chromosomes that contain 5,221, 4,395 and 7,986 protein coding genes, respectively. Species-specific primers were designed from variable regions of the draft genome sequence data and assessed by a PCR-based detection method. These primers were also tested for specificity against 17 allied *Xanthomonas* species as well as against the host DNA and the microbial community of the host surface. Three primer sets were found to be very specific and no amplification product was obtained with the host DNA and the microbial community of the host surface. In addition, a detection limit of 1 pg/μl per PCR reaction was detected when these primer sets were used to amplify corresponding bacterial DNAs. Therefore, these primer sets and the developed species-specific PCR assay represent a valuable, sensitive, and rapid diagnostic tool that can be used to detect three specific pathogens at early stages of infection and may help control diseases.

Keywords **:** bulb and flower disease, detection, draft genome sequence, species-specific primer, *Xanthomonas* spp.

Numerous species and pathovars of *Xanthomonas* cause diseases in a variety of economically important plants. Symptoms of these diseases include cankers, spots, blights, and necrosis (Rockey, 2012). Three *Xanthomonas* species, *X. axonopodis* pv. *poinsettiicola* (XAP), *X. hyacinthi* (XH) and *X. campestris* pv. *zantedeschiae* (XCZ), cause severe bulb and flower diseases in different ornamental plants and agriculturally important crops, like poinsettia, calla lily, hyacinth and some closely related bulb plants (Janse and Miller, 1983; Joubert and Truter, 1972; Lee et al., 2005b). Diseases caused by these pathogens are easily spread in the field by wind and rain. The unpredictable and severe nature of diseases caused by XAP, XH and XCZ to economically important plants makes them a constant threat. Thus, the development of a fast and specific method to ascertain whether symptoms are caused by these bacteria is the utmost importance to hyacinth and poinsettia plant growers (Van Doorn, 2001). Furthermore, bulb and flower production is no longer limited to specific countries; its production and transportation have significantly increased in Asia during the last decade. Therefore, researchers provided diagnostic tools to prevent infection (Benschop, 2010). The previous bacterial detection certified techniques relied mainly upon plant bioassays and culture based approaches (Smith et al., 1997). These methods are expensive for routine usage, often exceedingly time-consuming, and only allow for a presumptive identification, but does not discriminate pathogens at the species or pathovar level (Lopez et al., 2003). To overcome these limitations, more accurate and efficient molecular-based detection methods have been proposed as alternatives. Particularly, DNA based techniques, which have already been validated, showed their potential in routine surveys (Lopez et al., 2009).

The genome sequence of a pathogen is an important

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source to study the pathogenesis mechanism and develop specific detection methods for pathogen in particular having narrow host range (Lee et al., 2005a). Furthermore, the genome sequencing of *X. citri* pv. *malvacearum* has enabled researchers to develop a specific PCR assay for the detection of pathogens from plant materials based on draft genome sequences (Showmaker et al., 2014). Furthermore, DNA assays used for the detection of these pathogens are mainly based on 16S ribosomal DNA amplification and analysis of DNA restriction fragment length polymorphism, which does not allow the discrimination of these pathogens at the species level (Van Doorn, 2001).

The present study was designed to analyze the genome of XAP, XH and XCZ and develop a rapid, sensitive, and species-specific PCR assay for their detection based on their genomic analysis. Various primer sets were designed using bioinformatics methods based on the draft genome sequence of XAP, XH and XCZ. The sensitivity and specificity of the primers to these species in comparison with other *Xanthomonas* species as well as the host DNA and the microbial community of the host surface were also tested.

Materials and Methods

Bacterial strains and DNA extraction. In this study, three *Xanthomonas* species, XAP (NCPPB 581), XH (NCPPB 205) and XCZ (NCPPB 4326) were obtained from the National Collection of Plant Pathogenic Bacteria (NCPPB). All *Xanthomonas* species were cultured on LB agar (Difco, Detroit, MI, USA) and incubated at 28°C for 48 h. Yellow colonies of *Xanthomonas* were subcultured until pure colonies were obtained. Genomic DNA was extracted from bacterial cells using HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea) according to the manufacturer's instructions. The extracted DNA was confirmed by agarose gel (1%) electrophoresis and genomic DNA was then quantified by using PicoDrop™ Pico100 (PicoDrop Technologies, Cambridge, UK).

Genome sequencing and annotation. XAP, XH and XCZ genome sequences were determined on the Ion Torrent personal genome machine (Life Technologies, Korea). Libraries were produced using 1 µg of total genomic DNA and an Ion Xpress Plus fragment library kit comprising of the Ion Shear chemistry. During the emulsion PCR, each diluted library was used as the template for clonal amplification on Ion Sphere particles and the enriched amplification products were then loaded onto an Ion 316 chip and sequenced using 500 sequencing cycles. Five hundred sequencing cycles result in an average reading length of approximately 200 nucleotides. The MIRA program (version 4.0.2.1) was then used for *de novo* assembly. The genome projects for XAP, XH and XCZ are listed in the Genome OnLine Database (GOLD) as projects Gi0074998, Gi0075001 and Gi0074999, respectively. The JGI microbial genome automated annotation pipeline (Mavromatis et al., 2009) was used for the annotation of the selected genome followed by manual curation using the GenePRIMP software program. The predicted coding sequences (CDSs) were then translated and used to search the NCBI non redundant database and the TIGRFam, Uni-Prot, PRIAM, Pfam, COG, KEGG and InterPro databases. Additional functional annotations and gene predictions were performed by using the IMG-ER platform.

Primer design. The bioinformatics approach was used to select target DNA sequences for *Xanthomonas* species-specific primer designing from the draft genome sequence DNA sequences were selected randomly and a standard nucleotide BLAST search was performed on the NCBI database (http://www.ncbi.nlm.nih.gov). Through BLAST analysis, unique regions in XAP, XH and XCZ genome were chosen to obtain specific target sequences for primer design. The ClustalW software (http://clustalw.ddbj.nig.ac.jp/top-e.html) was used to design specific primers from the obtained consensus sequences, following multiple alignments of several *Xanthomonas* sequences.

Primer specificity. A total of 36 primer sets (12 primer sets for each species) were tested for species specificity against XAP, XH and XCZ (data not shown). Among them, three primer pairs, XPN: XPN-F (5′-TGT TGG TGT CGA CGT ACA GC-3′)/XPN-R (5′-TGA CAC ACG CAG CGT AGG AG-3′), XHG:XHG-F (5′-ACC ACC ACG TCG CTG GGT TCC-3′)/XHG-R (5′-CGG CGC GCT GGC GAC CCA CCG-3′) and XZRN: XZRN-F(5′-TTC TTC TCC GTC CTC CCT CA-3′)/XZRN-R (5′-ACA GGT GCC CAC CGG TTA CT-3′) were then selected on the basis of their amplification results and specificity to respective strains. The specificity of these primers was assessed on genomic DNA extracted from pure bacterial cultures: 9 strains of XAP (NCPPB 581, 1939 and 3948), XH (NCPPB 205, 1622 and MAFF 301390), XCZ (NCPPB 4326, 2978 and MAFF 301641) from different origins, and 17 strains of allied *Xanthomonas* species (Table 1). Furthermore, these primers were also tested for specificity against the host DNA and the microbial community of the host surface. PCR amplification was performed in a total volume of 20 µl containing 1× reaction buffer, 0.4 µl dNTP

Table 1. Summary of *Xanthomonas axonopodis* pv. *poinsettiicola* (XAP), *Xanthomonas hyacinthi* (XH) and *Xanthomonas campestris* pv. *zantedeschiae* (XCZ) strains and allied bacterial strains

No	Bacterium	Condition	Provided by
1	*Xanthomonas axonopodis* pv. *poinsettiicola*	Cultures	NCPPB[1] (581)
2	*X. axonopodis* pv. *poinsettiicola*	Cultures	NCPPB[1] (1939)
3	*X. axonopodis* pv. *poinsettiicola*	Cultures	NCPPB[1] (3948)
4	*X. hyacinthi*	Cultures	NCPPB[1] (205)
5	*X. hyacinthi*	Cultures	NCPPB[1] (1622)
6	*X. hyacinthi*	Cultures	MAFF[2] (301390)
7	*X. campestris* pv. *zantedeschiae*	Cultures	NCPPB[1] (4326)
8	*X. campestris* pv. *zantedeschiae*	Cultures	NCPPB[1] (2978)
9	*X. campestris* pv. *zantedeschiae*	Cultures	MAFF[2] (301641)
10	*X. campestris* pv. *campestris*	DNA	Chungbuk National University[3]
11	*X. citri* pv. *glycines*	DNA	Chungbuk National University[3]
12	*X. arboricola* pv. *fragariae*	DNA	Chungbuk National University[3]
13	*X. arboricola* pv. *juglandis*	DNA	Chungbuk National University[3]
14	*X. translucens*	DNA	Chungbuk National University[3]
15	*X. arboricola* pv. *populi*	DNA	Chungbuk National University[3]
16	*X. axonopodis* pv. *allii*	DNA	Animal and Plant Quarantine Agency
17	*X. axonopodis* pv. *citri*	DNA	Animal and Plant Quarantine Agency
18	*X. axonopodis* pv. *phaseoli*	DNA	Animal and Plant Quarantine Agency
19	*X. campestris* pv. *armoraciae*	DNA	Animal and Plant Quarantine Agency
20	*X. campestris* pv. *diffenbachiae*	DNA	Animal and Plant Quarantine Agency
21	*X. campestris* pv. *glycines*	DNA	Animal and Plant Quarantine Agency
22	*X. campestris* pv. *malvacearum*	DNA	Animal and Plant Quarantine Agency
23	*X. campestris* pv. *poinsettiicola*	DNA	Animal and Plant Quarantine Agency
24	*X. campestris* pv. *vesicatoria*	DNA	Animal and Plant Quarantine Agency
25	*X. cucurbitae*	DNA	Animal and Plant Quarantine Agency
26	*X. oryzae* pv. *oryzae*	DNA	Animal and Plant Quarantine Agency

[1]NCPPB, National Collection of Plant Pathogenic Bacteria; [2]MAFF, Ministry of Agriculture, Forestry and Fisheries; [3]These DNA samples were provided by Professor Jae-Soon Cha, Chungbuk National University, Cheongju, South Korea.

(10 mM), 0.1 µl Taq (Solg™ h-Taq DNA Polymerase), 1 µl (10 pm/µl) primers, and 1 µl (10 ng/µl) of DNA. The PCR program for XHG and XPN primer sets consisted of an initial denaturation at 95°C for 5 min followed by 35 cycles at 95°C for 30 s, 65°C for 20 s and 72°C for 30 s with a final extension step at 72°C for 5 min. For the primer set XZRN, the amplification conditions were as follows: initial denaturation at 95°C for 5 min followed by 35 cycles at 95°C for 30 s, 60°C for 20 s and 72°C for 30 s with a final extension step at 72°C for 5 min. The amplified PCR products were separated by gel electrophoresis using 1.5% agarose. The gel was stained with ethidium bromide and visualized with a UV transilluminator.

Primer sensitivity and PCR efficiency. Three primer sets XPN, XHG and XZRN were selected based on their high specificity to XAP, XH and XCZ, respectively. To check their sensitivity, a test was performed using a 10 fold dilutions of purified genomic DNA from XAP, XH and XCZ strains. The concentration of the samples used to test the specificity of the primers was 10 ng/µl. The XAP, XH and XCZ genomic DNA was then diluted to 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} ng/µl with DDW to test primer sensitivity. Furthermore, to check the efficiency of the primers to amplify XAP, XH and XCZ from four symptomless plants (*E. pulcherrima*, *H. orientalis*, *M. armeniacum*, and *Z. aethiopica*), plant tissues (0.2 g) from four host plants, and their respective pathogenic bacterial cells (1×10^{9} cfu/µl) were mixed and subjected to grinding. Genomic DNA was extracted from these mixtures by using HiGene Genomic DNA Prep Kit (BIOFACT) following the manufacturer's instruction. The PCR efficiency of the three primer sets,

XHG, XPN and XZRN, was checked on these DNA mixtures.

Results and Discussion

Genome properties. The properties and statistical analyses for the genome of the three *Xanthomonas* species, XAP, XH and XCZ, are summarized in Table 2. The genes distribution into COG functional categories for each genome is detailed in Table 3 and Fig. 1. The genome of XAP, XH and XCZ consists of single chromosomes and contains 5,221, 4,395 and 7,986 protein coding genes, respectively. Moreover, a total of 3,868, 3,318 and 4,754 protein coding genes were assigned a putative function (Table 2). These species showed genomic similarities with previously reported *Xanthomonas* species, *X. oryzae* pv. *oryzae*, *X. campestris* pv. *vesicatoria* and *X. fuscans* subsp. *fuscans* (Darrasse et al., 2013; Ochiai et al., 2005; Thieme et al., 2005). Similarly, high G+C contents were found in XAP, XH and XCZ, which is a common characteristic of most genera within the *Xanthomonadaceae* family (Darrasse et al., 2013). Furthermore, XAP, XH and XCZ genomic information facilitated the selection of species-specific primers based on variable regions in their genome. In addition, previous reports showed that, for the prevention of bacterial disease dissemination, early detection of *Xanthomonas* species is the most effective measure and conventional protocols carried out by the phytosanitary authorities are laborious, costly and excessively time consuming, which are major drawbacks for extensive surveillance of these pathogens (Lopez et al., 2003). However, over the last two decades, many DNA-based approaches, mostly PCR-based techniques, have been used for the detection of various pathogenic bacteria in general and *Xanthomonas* species in particular (Palacio-Bielsa, 2009). These PCR assays have been implemented successfully for the detection and identification of economically important *Xanthomonas* species such as *X. axonopodis* pv. *dieffenbachiae*, *X. axonopodis* pv. *citri*, *X. citri* subsp. *citri*, *X. campestris* pv. *citri* and *X. fragariae* (Adikini et al., 2011). Furthermore, previous studies also reported that *Xanthomonas* pathovars are difficult to differentiate as they are almost indistinguishable in bacteriological and biochemical traits (Bradbury, 1984).

Primer specificity for the detection of XAP, XH and XCZ. All 36 primer sets (12 for each strain) tested amplified against their respective strains (data not shown). The primer sets, XPN, XHG and XZRN, were then selected based on their best amplification results and specificity to their respective strains. The expected band of 966 bp, 1,049 bp and 328 bp for the three primer sets XHG, XPN and XZRN, respectively, were obtained (Fig. 1). These primer sets also allowed the amplification of 9 strains of XAP, XH and XCZ (data not shown). However, the evaluation results against 17 allied *Xanthomonas* species showed negative amplification with all tested strains. Consistent positive amplification was only achieved in their respective strains (XAP, XH and XCZ) (data not shown). Moreover, no amplification was observed when assessing these primer pairs against their respective host DNAs and microbial community of the host surface (Fig. 2).

Table 2. General features of three *Xanthomonas* species, *X. axonopodis* pv. *poinsettiicola* (XAP), *X. hyacinthi* (XH) and *X. campestris* pv. *zantedeschiae* (XCZ)

Attribute	XAP	XH	XCZ
Genome size (bp)	5,094,606	4,290,973	5,64,3059
DNA coding region (bp)	4,289,430	3,635,686	4,478,823
DNA G + C number of bases	64.77%	67.42%	64.52%
Total number of genes	5,287	4,459	8,090
Protein coding genes	5,221	4,395	7,986
RNA genes	66	64	104
rRNA genes	4	3	11
tRNA genes	53	51	73
Protein coding genes with predicted function	3,868	3,318	4,754
CDS assigned to COGs[a]	2,412	2,147	1,547
CDS assigned to KOGs[b]	652	593	333
CDS with signal peptides	585	452	502
Genes coding for transmembrane proteins	1,072	908	1,344

a) CDS; coding sequences, COG; clusters of orthologous group
b) KOG; eukaryotic orthologous group

Table 3. Number of genes associated with 25 general COG functional categories

Code	XAP		XH		XCZ		Description
	Value	% of total[a]	Value	% of total[a]	Value	% of total[a]	
E	178	6.6	176	7.3	99	5.72	Amino acid transport and metabolism
G	162	6.01	127	5.27	86	4.97	Carbohydrate transport and metabolism
D	24	0.89	23	0.95	19	1.1	Cell cycle control, cell division, and chromosome partitioning
N	92	3.41	91	3.77	60	3.47	Cell motility
M	161	5.97	131	5.43	97	5.61	Cell wall, membrane, and envelope biogenesis
B	1	0.04	0	0	1	0.06	Chromatin structure and dynamics
H	110	4.08	85	3.52	54	3.12	Coenzyme transport and metabolism
Z	1	0.04	2	0.08	1	0.06	Cytoskeleton
V	39	1.45	49	2.03	19	1.1	Defense mechanisms
C	147	5.45	150	6.22	105	6.07	Energy production and conversion
S	265	9.83	216	8.96	186	10.75	Function unknown
R	308	11.42	253	10.49	206	11.91	General function prediction only
P	135	5.01	139	5.76	86	4.97	Inorganic ion transport and metabolism
U	118	4.38	111	4.6	63	3.64	Intracellular trafficking, secretion, and vesicular transport
I	110	4.08	98	4.06	68	3.93	Lipid transport and metabolism
F	54	2	52	2.16	36	2.08	Nucleotide transport and metabolism
O	114	4.23	96	3.98	74	4.28	Posttranslational modification, protein turnover, chaperones
L	109	4.04	99	4.1	55	3.18	Replication, recombination, and repair
A	1	0.04	1	0.04	1	0.06	RNA processing and modification
Q	60	2.23	68	2.82	30	1.73	Secondary metabolites biosynthesis, transport, and catabolism
T	175	6.49	117	4.85	128	7.4	Signal transduction mechanisms
K	185	6.86	185	7.67	147	8.5	Transcription
J	147	5.45	143	5.93	109	6.3	Translation, ribosomal structure, and biogenesis
W	0	0	0	0	0	0	Extracellular structures
Y	0	0	0	0	0	0	Nuclear structure
	2875	54.38	2312	51.85	6543	80.88	Not in COGs

[a]The total is based on the total number of genes associated with COG categories.

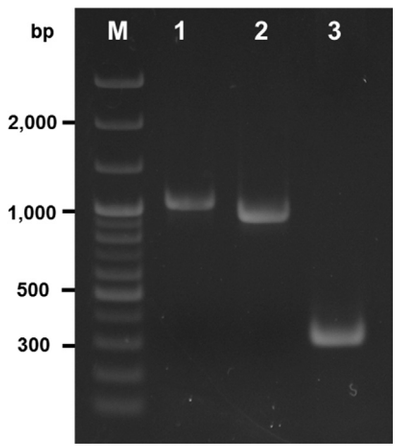

Fig. 1. Expected positive amplification for the three primer pairs XPN, XHG and XZRN against three *Xanthomonas* species, XAP, XH and XCZ, respectively. Lane M, 100 bp ladder; lane 1, XPN primer set against XAP, lane 2, XHG primer set against XH and lane 3, XZRN primer set against XCZ.

According to these results, these primers are specific to XAP, XH and XCZ. Thus, they can be used for the detection and routine diagnosis of these *Xanthomonas* strains in the field. To date, there is no report of species-specific primers for the detection of these species, although, many techniques, including DNA assays, are available for the detection of *Xanthomonas* species, which are mainly based on 16S ribosomal DNA amplification (Louws et al., 1994). However, these methods are not suitable for specific detection at the species and pathovar level. Therefore, in this study, we successfully developed species-specific primer pairs, which showed high specificity for the detection of these pathogens. The specificity of these primers relies upon the unique features of the template sequences that were used for primer designing. The XPN, XHG and XZRN primers were randomly designed from draft genome sequences. The portions of the sequence unique to XAP, XH and XCZ were identified by multiple sequence alignment and then used for these species-specific primer

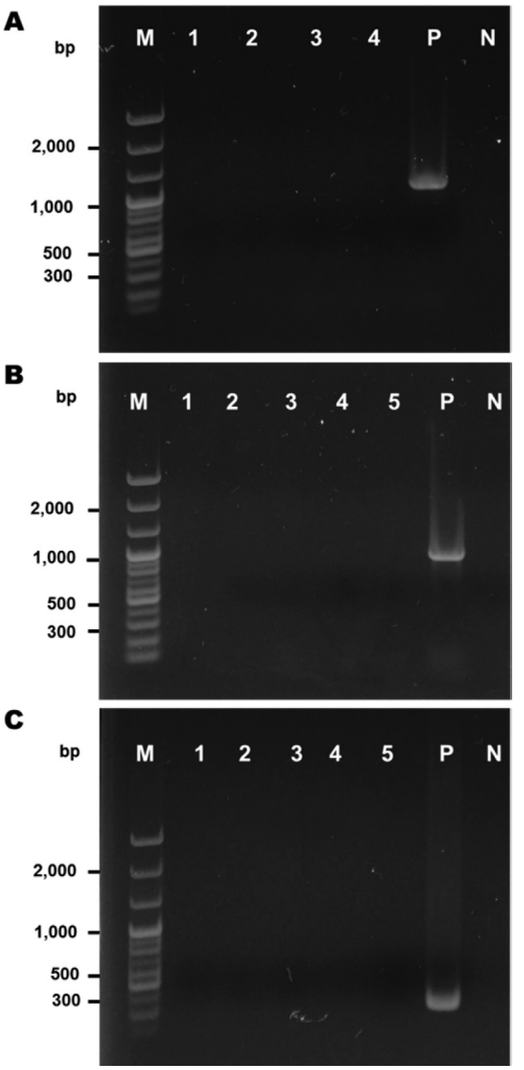

Fig. 2. Specificity of the selected primers (A) XPN against XAP host (*Euphorbia pulcherrima*), (B) XHG against XH host (*Hyacinthus orientalis*) and (C) XZRN against XCZ host (*Zantedeschia aethiopica*) DNA and microbial community of the respective host surface. (A) Lane M, 100 bp ladder; lane 1, host root; lane 2, host leaves; lane 3, root surface microbial community; lane 4, leaves surface microbial community; lane P, XAP and lane N, negative control. (B) and (C) Lane M, 100 bp ladder; lane 1, host root; lane 2, host leaves; lane 3, host bulb; lane 4, root surface microbial community; lane 5, bulb surface microbial community; lane P, XH and XCZ and lane N, negative control.

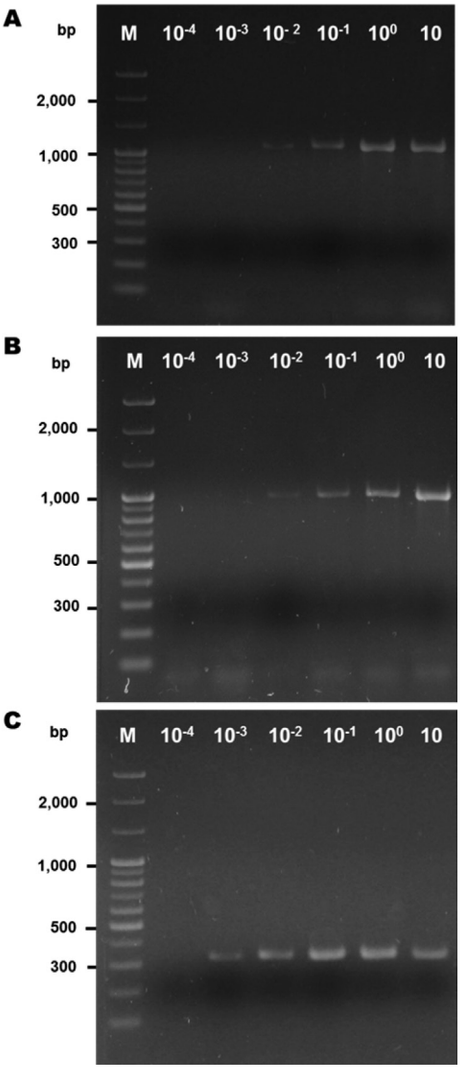

Fig. 3. Primer sensitivity test following genomic DNA 10-fold serial dilution of (A) XAP, (B) XH and (C) XCZ using XPN, XHG and XZRN primer sets, respectively. Lane 1, 100 bp ladder; lane 2–6, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 10^{0} and 10 dilutions, respectively.

designing. The respective genes are nucleoside hydrolase for primer XPN, glucosylphosphate transferase for XHG and resistance-Nodulation-Cell Division (RND) for XZRN. The lack of amplification in all allied *Xanthomonas* spp. demonstrates a high degree of specificity for these primers and their ability to consistently detect their respective species.

PCR sensitivity and efficiency. For pathogen quarantine and inspection, primer sets are often required to be not only specific to the templates, but also sensitive to small quantities of the pathogens. Therefore, these primers were found to be highly sensitive and could be used to detect small quantity of bacterial DNA. The detection limit for the primers, XPN and XHG, was 10^{-2} ng/µl, whereas XZRN was more sensitive and allowed the detection of 10^{-3} ng/µl of bacterial DNA per reaction (Fig. 3). Furthermore, the PCR efficiency in bacterial and host cell artificial mixture was determined. All three primer sets showed positive results and the specific PCR fragment for each primer set,

XPN, XHG and XZRN, was amplified from all four hosts and bacterial cell mixture (data not shown). The ability of these primer sets to detect XAP, XH and XCZ pathogen in small quantities suggests that this PCR assay can be used to detect these species in latently infected plants.

Ultimately, the PCR assay described here is a quick, sensitive and more suitable diagnostic tool for the detection of these pathogens. This selective and sensitive method circumvents many problems associated with the current detection techniques, enabling the reliable detection of XAP, XH and XCZ at early stages of infection and help monitor diseases.

References

Adikini, S., Tripathi, L., Beed, F., Tusiime, G., Magembe, E. M. and Kim, D. J. 2011. Development of a specific molecular tool for detecting *Xanthomonas campestris* pv. *musacearum*. *Plant Pathol*. 60:443–452.

Benschop, M., Kamenetsky, R., Le-Nard, M., Okubo, H. and De Hertogh, A. 2010. The global flower bulb industry: Production, utilization, research. *Hortic*. *Rev*. 36:1–115.

Bradbury, J. F. 1984. *Xanthomonas* Dowson 1939. In: Bergey's manual of systematic bacteriology, vol. 1, eds. by N. R. Krieg and J. G. Holt, pp. 199–210. The Williams & Wilkins Co., Baltimore, Md.

Darrasse, A., Carrere, S., Barbe, V., Boureau, T., Arrieta-Ortiz, M. L. et al. 2013. Genome sequence of *Xanthomonas fuscans* subsp. *fuscans* strain 4834-R reveals that flagellar motility is not a general feature of *xanthomonads*. *BMC genomics* 14:761.

Janse, J. D. and Miller, H. J. 1983. Yellow disease in *Scilla tubergeniana* and related bulbs caused by *Xanthomonas campestris* pv. *hyacinthi*. *Neth. J. Plant Pathol*. 89:203–206.

Joubert, J. J. and Truter, S. J. 1972. A variety of *Xanthomonas campestris* pathogenic to *Zantedeschia aethiopica*. *Neth*. *J*. *Plant Pathol*. 78:212–217.

Lee, B. M., Park, Y. J., Park, D. S. et al. 2005a. The genome sequence of *Xanthomonas oryzae* pathovar *oryzae* KACC10331, the bacterial blight pathogen of rice. *Nucleic Acids Res*. 33:577–586.

Lee, Y. A., Chen, K. P. and Chang, Y. C. 2005b. First report of bacterial leaf blight of white-flowered calla lily caused by *Xanthomonas campestris* pv. *zantedeschiae* in Taiwan. *Plant Pathol*. 54:239.

Lopez, M. M., Bertolini, E., Olmos, A., Caruso, P., Gorris, M. T., Llop, P., Penyalver, R. and Cambra, M. 2003. Innovative tools for detection of plant pathogenic viruses and bacteria. *Int*. *Microbiol*. 6:233–243.

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

Louws, F. J., Fulbright, D. W., Stephens, C. T. and de Bruijn, F. J. 1994. Specific genomic fingerprints of phytopathogenic *Xanthomonas* and *Pseudomonas* pathovars and strains generated with repetitive sequences and PCR. *Appl*. *Environ*. *Microbiol*. 60:2286–2295.

Mavromatis, K., Ivanova, N. N., Chen, I. M. A., Szeto, E., Markowitz, V. M. and Kyrpides, N. C. 2009. Standard operating procedure for the annotations of microbial genomes by the production genomic facility of the DOE JGI. *Stand Genomic Sci*. 1:63–67.

Ochiai, H., Inoue, Y., Takeya, M., Sasaki, A. and Kaku, H. 2005. Genome sequence of *Xanthomonas oryzae* pv. *oryzae* suggests contribution of large numbers of effector genes and insertion sequences to its race diversity. *Jpn*. *Agric*. *Res*. 39:275–287.

Palacio-Bielsa, A., Cambra, M. A. and Lopez, M. M. 2009. PCR detection and identification of plant-pathogenic bacteria: Updated review of protocols (1989–2007). *J. Plant. Pathol*. 91:249–297.

Rockey, W. 2012. Diversity within *Xanthomonas axonopodis poinsettiicola* and its role as causal agent of bacterial blight in commercial greenhouse ornamental crops. Master. thesis. University of Florida, Gainesville, USA.

Showmaker, K., Wang, X., Wang, H., Hsu, C. Y., Deng, P., Baird, S. M., Allen, T., Golden, B., Peterson, D., Nichols, R. and Lu, S. E. 2014. Draft genome sequence of *Xanthomonas citri* pv. *malvacearum* strain MSCT1 and development of pathovar specific PCR primers. *Am*. *Phytopathol*. *Soc*. 11p. (Abstact)

Smith, I. M., McNamara, D. G., Scott, P. R. and Holderness, M. 1997. Quarantine pests for Europe, 2nd ed. EPPO/CABI International, Wallingford, United Kingdom. 1425pp.

Thieme, F., Koebnik, R., Bekel, T., Berger, C., Boch, J., Büttner, D. et al. 2005. Insights into genome plasticity and pathogenicity of the plant pathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria* revealed by the complete genome sequence. *J. Bacteriol*. 187:7254–7266.

Van Doorn, J., Hollinger, T. C. and Oudega, B. 2001. Analysis of the type IV fimbrial-subunit gene *fimA* of *Xanthomonas hyacinthi*: Application in PCR-mediated detection of yellow disease in hyacinths. *Appl. Environ. Microbiol*. 67:598–607.