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PCR-Based Assay for Rapid and Specific Detection of the New *Xanthomonas oryzae* pv. *oryzae* K3a Race Using an AFLP-Derived Marker

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We describe the development of a polymerase chain reaction method for the rapid, precise, and specific detection of the *Xanthomonas oryzae* pv. *oryzae* (Xoo) K3a race, the bacterial blight pathogen of rice. The specific primer set was designed to amplify a genomic locus derived from an amplified fragment length polymorphism specific for the K3a race. The 1,024 bp amplicon was generated from the DNA of 13 isolates of Xoo K3a races out of 119 isolates of other races, pathovars, and *Xanthomonas* species. The assay does not require isolated bacterial cells or DNA extraction. Moreover, the pathogen was quickly detected in rice leaf 2 days after inoculation with bacteria and at a distance of 8 cm from the rice leaf 5 days later. The results suggest that this PCR-based assay will be a useful and powerful tool for the detection and identification of the Xoo K3a race in rice plants as well as for early diagnosis of infection in paddy fields.

Keywords: Bacterial blight, detection, race, *Xanthomonas oryzae* pv. *oryzae*

Introduction

Xanthomonas oryzae pv. *oryzae* (Xoo) is a gram-negative bacterium that causes bacterial blight (BB) of rice (*Oryza sativa* L.). BB is one of the most serious diseases of rice in Asian countries. The bacteria enter the rice plant through the hydathodes or wounded leaves and multiply in the vascular system. The disease can cause yield losses as high as 50% in severely infected plants in the field [9].

Some bactericides have been developed and used to control BB disease, but none of them is highly effective and economical in the case of outbreaks. The most effective and economical way to control the disease is to use resistant cultivars. However, the large-scale and long-term cultivation of varieties carrying a single resistance gene results in the evolution of new pathogenic races. Indeed, the introduction of resistance genes into rice was correlated with a change in the pathogenic diversity of Xoo [10].

Studies conducted in Asian countries on the pathotypic and genetic diversity of Xoo are based on differential interactions with rice cultivars containing different resistance genes. These studies employed repetitive DNA elements as probes to detect restriction fragment length polymorphisms and PCR-based DNA fingerprinting [2–4, 6–8, 13]. Unfortunately, these methods are lengthy and labor intensive. As an alternative, PCR-based methods have been developed for the detection and identification of Xoo at the pathovar and species levels [1, 5, 14]. An available triplex PCR specifically detects the race of Xoo strains [15], but is unable to effectively distinguish Xoo K3a races from the other races of Korean Xoo. A race-specific primer, which can be used for identifying a particular Xoo race, is still lacking. Therefore, a PCR-based rapid assay that can detect and discriminate the Xoo K3a race is required.

In the present study, we describe the development of a race-specific marker derived from an amplified fragment length polymorphism (AFLP) for the detection and identification of the Xoo K3a race in rice plants. PCR

amplification using a race-specific primer validated the specificity of this primer set for detecting the Xoo K3a race and should be a reliable technique for the detection and diagnosis of this pathogen.

Materials and Methods

Bacterial Strains, Culture Conditions, and DNA Isolation

All bacterial strains used in this study are listed in Table 1. The Xoo and other xanthomonad strains were obtained from the Korean Agricultural Culture Collection (KACC, Korea), the Ministry of Agriculture, Forestry and Fisheries (MAFF, Japan), the Belgian Co-ordinated Collections of Micro-organisms (BCCM, Belgium), and from Dr. T. H. Noh at the National Institute of Crop Science (RDA, Korea). All *Xanthomonas* strains were cultured on YDC medium (2% D-glucose, 2% $CaCO_3$, 1% yeast extract, and 1.5% agar) at 28°C for 48 h. The *Escherichia coli* strain was grown at 37°C for 24 h in Luria-Bertani (LB) medium containing 200 μg/ml of ampicillin. Genomic DNA was isolated using a genomic DNA extraction kit (MagExtractor Genome, Japan) according to the manufacturer's instructions.

Development of a Race Marker using AFLP Analysis

AFLP was performed using a published method [16], with some modification. Genomic DNA (600 ng) was digested with 1 μl of FastDigest *Eco*RI and *Mse*I (Thermo Scientific) at 37°C for 30 min, and the restriction fragments were ligated to *Eco*RI and *Mse*I double-stranded adapters (Table 2) at 16°C for 5 h. The preselective PCR was performed using AccuPower PCR Premix (Bioneer, Korea) in a 25 μl reaction mixture containing 1 μl of DNA (50 ng/μl), 10 pmol of E0 primer, and 10 pmol of M0 primer (Table 2). The preselective PCR amplification was carried out in a T100 Thermal Cycler (Bio-Rad, USA) according to the program as follows: initial denaturation at 94°C for 5 min followed by 25 cycles at 94°C for 30 sec, 57°C for 1 min, and 72°C for 1 min; and a final extension for 5 min at 72°C. Pre-amplification PCR products were diluted 3-fold with sterile distilled water and used as templates. The AFLP reaction with primers having two selective bases (Table 2) was performed for 35 cycles under the conditions as follows: denaturation for 30 sec at 94°C, annealing for 20 sec at 67°C followed by lowering the temperature (0.7°C) in the next 12 cycles, and then at 57°C for the remaining 23 cycles; and a final extension for 1 min at 72°C. The amplified products were separated on a 1.2% agarose gel, stained with ethidium bromide, and visualized using a UV transilluminator.

The specific AFLP fragments were excised from the gel and DNA was eluted using a DNA fragment purification kit (MagExtractor PCR & Gel Clean up; Toyobo, Japan) according to the manufacturer's instructions. Eluted DNA was directly cloned into the pGEM-T Easy Vector (Promega, USA) and used to transform competent cells (DH5α, RBC) according to the supplier's information. The sequencing reaction and sequence data analysis were performed using an ABI Prism 3100 Automatic DNA Sequencer (Applied Biosystems).

Primer Design and PCR Specificity

The race-specific primers, K3aF (5′-TCTGATTCGCAACGCTTT TGAGGAC-3′) and K3aR (5′-CTTCCTAATCAATAGTCACCT TGAA-3′), were designed according to the sequences of the Xoo K3a race. The specificity of the race-specific primer set was tested against Xoo K3a races, other races, pathovars, and *Xanthomonas* species. The PCR was performed using a premixed type polymerase (Taq PreMix, TNT Research, Korea) in a 20 μl reaction mixture containing 50 ng of template DNA and 10 pmol of each race-specific primer. PCR amplification was carried out in a T100 Thermal Cycler (Bio-Rad, USA) according to the program as follows: initial denaturation at 96°C for 5 min followed by 25 cycles at 96°C for 15 sec, 62°C for 15 sec, and 72°C for 30 sec; and a final 5 min extension at 72°C. Subsequently, 5 μl of each reaction mixture was subjected to electrophoresis through a 1.2% agarose gel, stained with ethidium bromide, and visualized using a UV transilluminator.

PCR Assay for Detection of the Pathogen in Rice Plants

The leaves of 50-day-old susceptible rice cultivar IR24 were inoculated by clipping the leaf tips with sterile scissors and then dipping them into a saturated culture (10^9 cells/ml) of Xoo strain HB01001 [15]. The plants were grown in a greenhouse at 25–30°C, with a relative humidity of 60%. To evaluate the ability of the assay to directly detect the pathogen in infected rice plants, leaf samples were collected 1, 2, 3, 4, 5, 6, 7, and 8 cm from the lesion site 5 days after inoculation. Artificially infected rice leaves were harvested 1, 2, 3, 4, and 5 days after inoculation (1 cm from the lesion site). Each sample was soaked in 200 μl of sterile distilled water for 20 min. After soaking, 10 μl of the exudates was used as a template for the PCR assay as described above.

Results

Specificity of a Race Marker Using AFLP Analysis

To develop a marker for the specific detection of the Xoo K3a race, 64 AFLP primer combinations were tested for their ability to identify five K3a races and 17 other races of Korean Xoo (data not shown). From these primer combinations, a race-specific 1,062 bp DNA fragment for K3a was selected, cloned, and sequenced. Based on this sequence, the K3aF/K3aR primer set was designed to amplify a 1,024 bp fragment that specifically detects K3a races of Korean Xoo (Fig. 1).

The specificity of the putative race-specific primer was evaluated using genomic DNA extracted from the Xoo K3a race and the other *Xanthomonas* strains listed in Table 1. The PCR product was only produced from 13 isolates of

Table 1. List of bacterial strains used in this study.

No.	Bacterial strain	Source[a]	Geographical origin	Race
1	*Xanthomonas oryzae* pv. *oryzae*	KACC10331	Korea	K1
2	*Xanthomonas oryzae* pv. *oryzae*	KACC10861	Korea	K1
3	*Xanthomonas oryzae* pv. *oryzae*	KACC10878	Korea	K1
4	*Xanthomonas oryzae* pv. *oryzae*	KACC10880	Korea	K1
5	*Xanthomonas oryzae* pv. *oryzae*	HB 01013	Korea	K1
6	*Xanthomonas oryzae* pv. *oryzae*	HB 02010	Korea	K1
7	*Xanthomonas oryzae* pv. *oryzae*	HB 02022	Korea	K1
8	*Xanthomonas oryzae* pv. *oryzae*	HB 02027	Korea	K1
9	*Xanthomonas oryzae* pv. *oryzae*	HB 02033	Korea	K1
10	*Xanthomonas oryzae* pv. *oryzae*	HB 02045	Korea	K1
11	*Xanthomonas oryzae* pv. *oryzae*	HB 03011	Korea	K1
12	*Xanthomonas oryzae* pv. *oryzae*	HB 03087	Korea	K1
13	*Xanthomonas oryzae* pv. *oryzae*	HB 04027	Korea	K1
14	*Xanthomonas oryzae* pv. *oryzae*	HB 04068	Korea	K1
15	*Xanthomonas oryzae* pv. *oryzae*	KACC10873	Korea	K2
16	*Xanthomonas oryzae* pv. *oryzae*	KACC10877	Korea	K2
17	*Xanthomonas oryzae* pv. *oryzae*	HB 01014	Korea	K2
18	*Xanthomonas oryzae* pv. *oryzae*	HB 02030	Korea	K2
19	*Xanthomonas oryzae* pv. *oryzae*	HB 03026	Korea	K2
20	*Xanthomonas oryzae* pv. *oryzae*	HB 03028	Korea	K2
21	*Xanthomonas oryzae* pv. *oryzae*	HB 04046	Korea	K2
22	*Xanthomonas oryzae* pv. *oryzae*	HB 04048	Korea	K2
23	*Xanthomonas oryzae* pv. *oryzae*	HB 04059	Korea	K2
24	*Xanthomonas oryzae* pv. *oryzae*	HB 01015	Korea	K3
25	*Xanthomonas oryzae* pv. *oryzae*	HB 02003	Korea	K3
26	*Xanthomonas oryzae* pv. *oryzae*	HB 02019	Korea	K3
27	*Xanthomonas oryzae* pv. *oryzae*	HB 02024	Korea	K3
28	*Xanthomonas oryzae* pv. *oryzae*	HB 02038	Korea	K3
29	*Xanthomonas oryzae* pv. *oryzae*	HB 02041	Korea	K3
30	*Xanthomonas oryzae* pv. *oryzae*	HB 03002	Korea	K3
31	*Xanthomonas oryzae* pv. *oryzae*	HB 03009	Korea	K3
32	*Xanthomonas oryzae* pv. *oryzae*	HB 03010	Korea	K3
33	*Xanthomonas oryzae* pv. *oryzae*	HB 03013	Korea	K3
34	*Xanthomonas oryzae* pv. *oryzae*	HB 03014	Korea	K3
35	*Xanthomonas oryzae* pv. *oryzae*	HB 03029	Korea	K3
36	*Xanthomonas oryzae* pv. *oryzae*	HB 03032	Korea	K3
37	*Xanthomonas oryzae* pv. *oryzae*	HB 03034	Korea	K3
38	*Xanthomonas oryzae* pv. *oryzae*	Hb 03035	Korea	K3
39	*Xanthomonas oryzae* pv. *oryzae*	HB 03042	Korea	K3
40	*Xanthomonas oryzae* pv. *oryzae*	HB 03045	Korea	K3
41	*Xanthomonas oryzae* pv. *oryzae*	HB 03046	Korea	K3
42	*Xanthomonas oryzae* pv. *oryzae*	HB 03047	Korea	K3
43	*Xanthomonas oryzae* pv. *oryzae*	HB 03054	Korea	K3
44	*Xanthomonas oryzae* pv. *oryzae*	HB 03055	Korea	K3
45	*Xanthomonas oryzae* pv. *oryzae*	HB 03058	Korea	K3
46	*Xanthomonas oryzae* pv. *oryzae*	HB 03059	Korea	K3

Table 1. Continued.

No.	Bacterial strain	Source[a]	Geographical origin	Race
47	*Xanthomonas oryzae* pv. *oryzae*	HB 03060	Korea	K3
48	*Xanthomonas oryzae* pv. *oryzae*	HB 03061	Korea	K3
49	*Xanthomonas oryzae* pv. *oryzae*	HB 03063	Korea	K3
50	*Xanthomonas oryzae* pv. *oryzae*	HB 03065	Korea	K3
51	*Xanthomonas oryzae* pv. *oryzae*	HB 03067	Korea	K3
52	*Xanthomonas oryzae* pv. *oryzae*	HB 03071	Korea	K3
53	*Xanthomonas oryzae* pv. *oryzae*	HB 03072	Korea	K3
54	*Xanthomonas oryzae* pv. *oryzae*	HB 03074	Korea	K3
55	*Xanthomonas oryzae* pv. *oryzae*	HB 03076	Korea	K3
56	*Xanthomonas oryzae* pv. *oryzae*	HB 03079	Korea	K3
57	*Xanthomonas oryzae* pv. *oryzae*	HB 03084	Korea	K3
58	*Xanthomonas oryzae* pv. *oryzae*	HB 03086	Korea	K3
59	*Xanthomonas oryzae* pv. *oryzae*	HB 04000	Korea	K3
60	*Xanthomonas oryzae* pv. *oryzae*	HB 04002	Korea	K3
61	*Xanthomonas oryzae* pv. *oryzae*	HB 04006	Korea	K3
62	*Xanthomonas oryzae* pv. *oryzae*	HB 04007	Korea	K3
63	*Xanthomonas oryzae* pv. *oryzae*	HB 04009	Korea	K3
64	*Xanthomonas oryzae* pv. *oryzae*	HB 04012	Korea	K3
65	*Xanthomonas oryzae* pv. *oryzae*	HB 04015	Korea	K3
66	*Xanthomonas oryzae* pv. *oryzae*	HB 04021	Korea	K3
67	*Xanthomonas oryzae* pv. *oryzae*	HB 04022	Korea	K3
68	*Xanthomonas oryzae* pv. *oryzae*	HB 04023	Korea	K3
69	*Xanthomonas oryzae* pv. *oryzae*	HB 04024	Korea	K3
70	*Xanthomonas oryzae* pv. *oryzae*	HB 04031	Korea	K3
71	*Xanthomonas oryzae* pv. *oryzae*	HB 04050	Korea	K3
72	*Xanthomonas oryzae* pv. *oryzae*	HB 04052	Korea	K3
73	*Xanthomonas oryzae* pv. *oryzae*	HB 01001	Korea	K3a
74	*Xanthomonas oryzae* pv. *oryzae*	HB 01002	Korea	K3a
75	*Xanthomonas oryzae* pv. *oryzae*	HB 01003	Korea	K3a
76	*Xanthomonas oryzae* pv. *oryzae*	HB 01005	Korea	K3a
77	*Xanthomonas oryzae* pv. *oryzae*	HB 01009	Korea	K3a
78	*Xanthomonas oryzae* pv. *oryzae*	HB 03050	Korea	K3a
79	*Xanthomonas oryzae* pv. *oryzae*	HB 03077	Korea	K3a
80	*Xanthomonas oryzae* pv. *oryzae*	HB 03091	Korea	K3a
81	*Xanthomonas oryzae* pv. *oryzae*	HB 04039	Korea	K3a
82	*Xanthomonas oryzae* pv. *oryzae*	HB 11005	Korea	K3a
83	*Xanthomonas oryzae* pv. *oryzae*	HB 11168	Korea	K3a
84	*Xanthomonas oryzae* pv. *oryzae*	HB 11238	Korea	K3a
85	*Xanthomonas oryzae* pv. *oryzae*	HB 11239	Korea	K3a
86	*Xanthomonas oryzae* pv. *oryzae*	HB 04034	Korea	K5
87	*Xanthomonas oryzae* pv. *oryzae*	HB 04037	Korea	K5
88	*Xanthomonas oryzae* pv. *oryzae*	HB 04040	Korea	K5
89	*Xanthomonas oryzae* pv. *oryzae*	HB 04044	Korea	K5
90	*Xanthomonas oryzae* pv. *oryzae*	HB 04047	Korea	K5
91	*Xanthomonas oryzae* pv. *oryzae*	HB 04054	Korea	K5
92	*Xanthomonas oryzae* pv. *oryzae*	HB 04058	Korea	K5

Table 1. Continued.

No.	Bacterial strain	Source[a]	Geographical origin	Race
93	*Xanthomonas oryzae* pv. *oryzae*	HB 04071	Korea	K5
94	*Xanthomonas oryzae* pv. *oryzae*	MAFF 311018	Japan	–
95	*Xanthomonas oryzae* pv. *oryzae*	MAFF 311019	Japan	–
96	*Xanthomonas oryzae* pv. *oryzae*	MAFF 311020	Japan	–
97	*Xanthomonas oryzae* pv. *oryzae*	KACC10883	The Philippines	–
98	*Xanthomonas oryzae* pv. *oryzae*	KACC10885	The Philippines	–
99	*Xanthomonas arboricola* pv. *poinsettiicola*	LMG5403	New Zealand	–
100	*Xanthomonas axonopodis* pv. *axonopodis*	KACC10935	Colombia	–
101	*Xanthomonas axonopodis* pv. *begoniae*	LGM551	UK	–
102	*Xanthomonas axonopodis* pv. *citri*	KACC10443	Korea	–
103	*Xanthomonas axonopodis* pv. *citri*	KACC10444	Korea	–
104	*Xanthomonas axonopodis* pv. *dieffenbachiae*	LGM695	Brazil	–
105	*Xanthomonas axonopodis* pv. *glycines*	KACC10445	Zambia	–
106	*Xanthomonas axonopodis* pv. *glycines*	KACC10446	Zimbabwe	–
107	*Xanthomonas axonopodis* pv. *malvacearum*	LGM761	Sudan	–
108	*Xanthomonas axonopodis* pv. *phaseoli*	LMG7455	Bulgaria	–
109	*Xanthomonas axonopodis* pv. *vesicatoria*	LMG905	–	–
110	*Xanthomonas campestris* pv. *campestris*	LMG8052	The Netherlands	–
111	*Xanthomonas campestris* pv. *campestris*	LGM8091	USA	–
112	*Xanthomonas translucens* pv. *cerealis*	LMG679	USA	–
113	*Xanthomonas translucens* pv. *hordei*	LMG882	Canada	–
114	*Xanthomonas translucens* pv. *vauterin*	LMG843	Belgium	–
115	*Xanthomonas translucens* pv. *translucens*	LMG875	Canada	–
116	*Xanthomonas alfalfae* subsp. *alfalfae*	LGM8078	India	–
117	*Xanthomonas cassavae*	LMG673	Malawi	–
118	*Xanthomonas cucurbitae*	LMG8662	Belgium	–
119	*Xanthomonas theicola*	LMG8684	Japan	–

[a]KACC, Korean Agricultural Culture Collection, Korea (http://www.genebank.go.kr/); MAFF, Ministry of Agriculture, Forestry and Fisheries of Japan; LMG, The Belgian Co-ordinated Collections of Microorganisms (BCCM), Belgium; HB, Department of Rice and Winter Cereal Crop, National Institute of Crop Science, Korea. '–' unknown.

Table 2. Oligonucleotide adapters and primers used for AFLP analysis.

No.	Primer name		Sequence (5′-3′)	No.	Primer name		Sequence (5′-3′)
1	Adapter	*Eco*RI Up	CTCGTAGACTGCGTACC	1	Adapter	*Mse*I Up	TACTCAGGACTCAT
2		*Eco*RI Down	AATTGGTACGCAGTCTAC	2		*Mse*I Down	GACGATGAGTCCTGAG
3	E0		GACTGCGTACCAATTCA	3	M0		GATGAGTCCTGAGTAAC
4	E1		GACTGCGTACCAATTCAGG	4	M1		GATGAGTCCTGAGTAACAT
5	E2		GACTGCGTACCAATTCACG	5	M2		GATGAGTCCTGAGTAACTT
6	E3		GACTGCGTACCAATTCAAC	6	M3		GATGAGTCCTGAGTAACAC
7	E4		GACTGCGTACCAATTCACA	7	M4		GATGAGTCCTGAGTAACTA
8	E5		GACTGCGTACCAATTCACC	8	M5		GATGAGTCCTGAGTAACAG
9	E6		GACTGCGTACCAATTCAGC	9	M6		GATGAGTCCTGAGTAACTC
10	E7		GACTGCGTACCAATTCACT	10	M7		GATGAGTCCTGAGTAACAA
11	E8		GACTGCGTACCAATTCAAG	11	M8		GATGAGTCCTGAGTAACTG

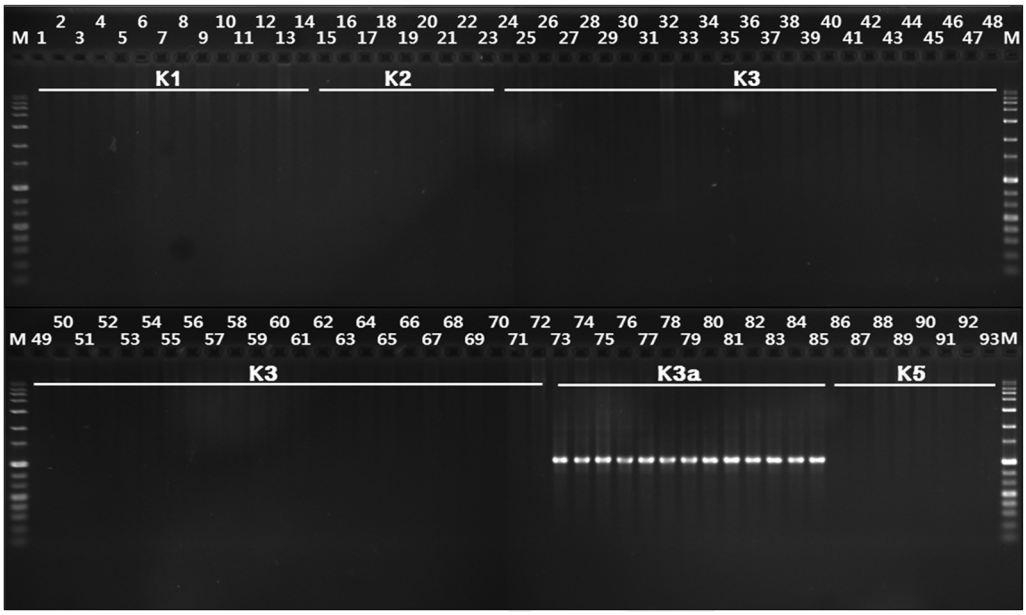

Fig. 1. Agarose gel electrophoresis of PCR products amplified from *X. oryzae* pv. *oryzae* strains using the race-specific K3aF/K3aR primer set.

Lane M: size marker (1 kb ladder; TNT Research, Korea); lanes 1–93: Xoo strains (numbers 1–93, respectively, in Table 1).

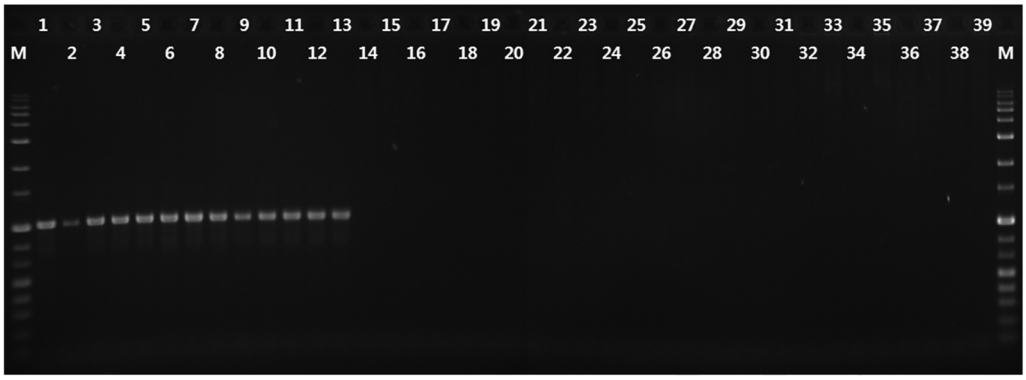

Fig. 2. Agarose gel electrophoresis of PCR products amplified from Xoo K3a races and other xanthomonads using the race-specific K3aF/K3aR primer set.

Lane M: size marker (1 kb ladder); lanes 1–13: Xoo K3a races (numbers 73–85, respectively, in Table 1); lanes 14–39, other xanthomonads (numbers 94–119, respectively, in Table 1).

Xoo K3a races among 39 isolates of other species and strains of *Xanthomonas* (Fig. 2). These results indicate that the primer set is highly specific for detecting the Xoo K3a race.

Direct PCR Assay for Detecting the Pathogen in Rice Plants

To evaluate the ability of the direct PCR assay to detect the pathogen in rice plants, artificially infected rice leaves (1 cm sections at the lesion site and at 2, 3, 4, 5, 6, 7, and 8 cm from the lesion) were soaked in sterile distilled water, and the exudates were used without further treatment as templates for the PCR assay. Amplicons were detected in all eight samples (Fig. 3).

To verify the ability of the PCR assay to predict disease outbreaks in paddy fields, artificially infected rice leaves were sampled at 0, 1, 2, 3, 4, and 5 days after inoculation, and the exudates were used as templates for the direct PCR assay. The expected 1,024 bp amplicon was detected in samples taken 2–5 days after inoculation (Fig. 4). These

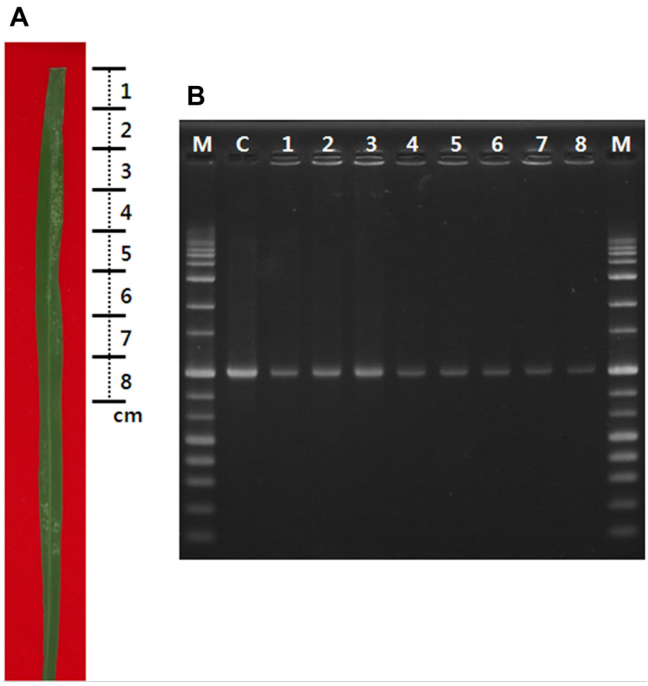

Fig. 3. Direct PCR detection of *X. oryzae* pv. *oryzae* K3a race in rice leaves.
(**A**) An artificially infected rice leaf 5 days after inoculation. (**B**) PCR assay for detection of the pathogen from different regions of the rice leaf sample shown in (**A**). Lane M: size marker (1 kb ladder); C, control Xoo HB01001 gDNA (50 ng/μl); lanes 1–8: samples (1–8 cm, respectively, from the lesion).

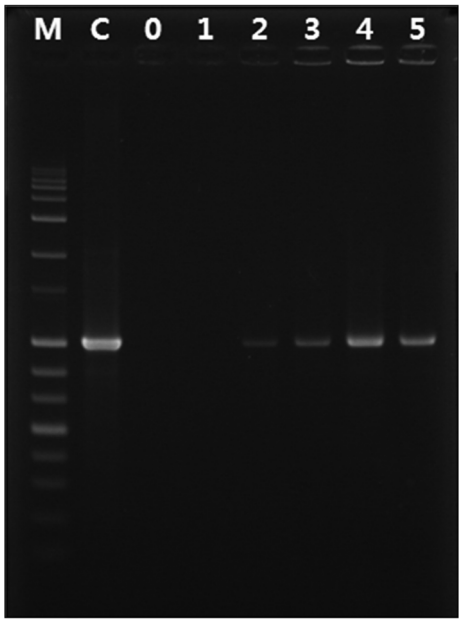

Fig. 4. PCR assay for pathogen detection in rice leaf samples up to 5 days after inoculation.
Lane M: size marker (1 kb ladder); C, control Xoo HB01001 gDNA (50 ng/μl); lanes 0–5: leaf samples, 0–5 days after infection.

results indicate that the PCR-based assay can be used directly to detect and identify Xoo K3a in infected rice leaf samples without isolating the bacteria and can be applied to predict disease outbreaks in paddy fields.

Discussion

The major objective of this study was to develop a rapid, precise, and specific PCR-based assay that can be used directly to identify the Xoo K3a race in infected rice plants, and to discriminate Xoo K3a from other races, pathovars, and *Xanthomonas* strains.

New races of Xoo are continuously being reported in rice-growing Asian countries [2, 8, 10, 11]. For example, K3a, which is pathogenic for rice cultivars containing Xa3 resistance genes, was isolated from the southwestern coastal areas of Korea [12]. Xoo strains are classified into several races according to their interactions with rice cultivars containing different resistance genes. Nine pathogenic races of Xoo were isolated in Nepal [3] and nine from China [8]. The Korean Xoo strains are classified into five races (K1 to K5) using five rice cultivars to distinguish among them [17]. However, such methods are too expensive, labor intensive, and lengthy to be useful for testing large numbers of samples and identifying new races of the pathogen. To address this problem, we applied the AFLP technique to identify DNA fragments specific for the new K3a race of Xoo. Polymorphic amplicons that were generated only from isolates of the K3a race were cloned and sequenced. The sequence data were used to design a race-specific primer that unambiguously distinguished isolates of the K3a race from other Korean Xoo races (Fig. 1).

Specific primers and DNA probes derived from the 16S–23S rDNA spacer region and repeated elements can detect and identify Xoo strains [1, 14]; however, none of them discriminated *X. oryzae* pv. *oryzae* from *X. oryzae* pv. *oryzcola*. Cho *et al*. [5] recently described the Bio-PCR method based on the amplification of a member of the *rhs* family gene for detecting Xoo at the pathovar and species levels. We also described a triplex PCR method for the detection and identification of Xoo races that discriminated between K1, including some K2 and K4 races, and the K3 and K5 races [15], but did not discriminate between K3a and K3 or K5 races. In contrast, the PCR-based assay described in the present study unambiguously discriminated

the Xoo K3a race from other races of Korean Xoo as well as from other *Xanthomonas* strains (Figs. 1 and 2). Interestingly, four strains that we previously classified as K3 and K5 races [15] were classified here as the K3a race. These results indicate that this race-specific primer set is highly specific for the Xoo K3a and can be used as a simple and rapid tool for identifying this pathogen.

Early diagnosis of the pathogen by rapid methods is important for assessing the health status of rice, because a latent infection can lead to a serious epidemic under favorable conditions. We show here that a PCR-based assay, which does not require isolating bacteria or extracting DNA, detected the pathogen in asymptomatic rice leaves 2 days after inoculation (Fig. 4). Therefore, this assay can be used as a reliable and useful method for the detection and identification of the Xoo K3a race in rice plants, as well as for the diagnosis of latent and nonsymptomatic infections in paddy fields to prevent disease outbreaks.

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