J. Microbiol. Biotechnol. (2005), **15**(3), 502–509

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

A Genetic Marker Associated with the A1 Mating Type Locus in *Phytophthora infestans*

KIM, KWON-JONG[1], SEUNG-HEE EOM[2], SANG-PYO LEE[1], HEE-SUN JUNG[1], SOPHIEN KAMOUN[3], AND YOUN SU LEE[1]*

[1]*Division of Bio-resources Technology, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon 200-701, Korea*
[2]*Kangwon Agricultural Technology Administration, Chunchon 200-150, Korea*
[3]*Department of Plant Pathology, Ohio State University, OARDC, Wooster, OH 44691, U.S.A.*

Received: August 5, 2004
Accepted: October 29, 2004

Abstract Sexual reproduction plays an important role in the biology and epidemiology of oomycete plant pathogens such as the heterothallic species *Phytophthora infestans*. Recent worldwide dispersal of A2 mating type strains of *P. infestans* resulted in increased virulence, gene transfer, and genetic variation, creating new challenges for disease management. To develop a genetic assay for mating type identification in *P. infestans*, we used the Amplified Fragment Length Polymorphism (AFLP) technique. The primer combination E+AT/M+CTA detected a fragment specific to A1 mating type (Mat-A1) of *P. infestans*. This fragment was cloned and sequenced, and a pair of primers (INF-1, INF-2) were designed and used to differentiate *P. infestans* Mat-A1 from Mat-A2 strains. The Mat A1-specific fragment was detected using Southern blot analysis of PCR products amplified with primers INF-1 and INF-2 from genomic DNA of 14 *P. infestans* Mat-A1 strains, but not 13 *P. infestans* Mat-A2 strains or 8 other isolates representing several *Phytophthora* spp. Southern blot analysis of genomic DNAs of *P. infestans* isolates revealed a 1.6 kb restriction enzyme (*Eco*RI, *Bam*HI, *Ava*I)-fragment only in Mat-A1 strains. The A1 mating type-specific primers amplified a unique band under stringent annealing temperatures of 63°C–64°C, suggesting that this PCR assay could be developed into a useful method for mating type determination of *P. infestans* in field material.

Key words: *Phytophthora infestans*, molecular marker, mating type A-1, AFLP

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is one of the most destructive diseases of cultivated potato and tomato [1]. Heterothallic species of the oomycetous genus *Phytophthora* exhibit two mating types, which are called A1 and A2 [3]. These two mating types are physiologically differentiated by their ability to produce hormones that are required to induce gametangia under normal conditions [20]. When isolates of the A1 and A2 sexual compatibility types (mating types) of heterothallic species of *Phytophthora* are cultured together, antheridia and oogonia are induced, which associate and may fuse to form an oospore [3, 33, 36]. Sexual compatibility is thought to be determined by the interaction between diffusible mating hormones and their receptors. Until the late 1970s, only one mating type (A1) of *P. infestans* was distributed worldwide, and management of the asexually reproducing pathogen was somewhat successful. However, severe late blight epidemics occurred in recent years after aggressive A2 mating type strains migrated to Europe and North America [8, 13, 21, 31, 35]. The occurrence of a sexual cycle has resulted in increased virulence, gene transfer, and genetic variation in populations of *P. infestans*, creating new challenges for late blight management.

The genetic basis for mating type in *Phytophthora* has been most thoroughly addressed in *Phytophthora infestans*, and its mating type was shown to be determined by a single locus, which displayed a pattern of non-Mendelian inheritance [15, 16]. This pattern was similar to that observed for genes linked to balanced lethal loci [27], as only two genotypes of loci linked to the mating-type determinant were observed in progeny, compared to the four that would be expected for diploids.

A genetic survey of isolates representative of the entire species indicated that this unusual segregation pattern was a general feature of the mating type locus of *P. infestans*, as opposed to being a phenomenon displayed only by selected or aberrant isolates [9]. The conservation throughout the

*Corresponding author
Phone: 82-33-250-6417; Fax: 82-33-243-3314;
E-mail: younslee@kangwon.ac.kr

species of non-Mendelian segregation suggests that it may play an important role in the determination of mating type, possibly by eliminating potentially deleterious combinations of alleles or by suppressing mitotic crossing over.

Sexual reproduction plays an important role in the biology and epidemiology of *P. infestans*. Populations of *P. infestans* that contain A1 and A2 mating types carry the potential to complete their sexual life cycle. This results in the formation of oospores that enable the pathogen to survive for longer periods, as well as higher levels of virulence and high frequencies of resistance against the widely used fungicide metalaxyl, presumable because of increased gene flow [6, 22, 23, 37]. Therefore, a rapid genetic test to evaluate field populations of *P. infestans* for the frequency of A2 mating type has useful implications for disease management. Genetic studies of *P. infestans* and other oomycetes have been hampered by the lack of morphological and biochemical mutants but are benefiting from modern genetic tools [14, 17]. For example, the amplified fragment length polymorphism (AFLP) technique developed by Vos *et al*. [39] is a powerful, reliable, stable, and rapid assay with application in genome mapping, DNA fingerprinting, and marker-assisted breeding [19, 25, 28, 30, 32]. AFLP has been successfully used in genetic marker discovery and genome mapping in *Phytophthora* [38].

Table 1. Isolates of *P. infestans* Mat-A1 and Mat-A2 used in this study.

Isolate		Pathogen	Oospore a formation	Mating type	Host	Location
Na1-2	(1)*	*Phytophthora infestans*		A2	*Solanum tubersum* L.	Pyung Chang
Na1-5	(2)*	*P. infestans*	–	A2	"	"
Na2-1	(3)*	*P. infestans*	–	A2	"	"
KAW-40	(4)*	*P. infestans*	–	A2	"	Hoeng Gye
KAW-63	(5)*	*P. infestans*	–	A2	"	Pyung Chang
RDA-2	(6)*	*P. infestans*	–	A2	"	–
DGY1-2	(7)*	*P. infestans*	–	A2	"	Dae Gwan Yung
DGY2-4	(8)*	*P. infestans*	–	A2	"	"
DGY3-2	(9)*	*P. infestans*	–	A2	"	"
DGY3-4	(10)*	*P. infestans*	–	A2	"	"
DGY3-12	(11)*	*P. infestans*	–	A2	"	"
DGY3-17	(12)*	*P. infestans*	–	A2	"	"
20B02	(13)*	*P. infestans*	–	A2	"	Bu Yeo
YY-8	(14)*	*P. infestans* (1)**	+	A1	"	Yang Yang
YY-9	(15)*	*P. infestans* (2)**	+	A1	"	"
YY-11	(16)*	*P. infestans*	+	A1	"	"
YY-12	(17)*	*P. infestans*	+	A1	"	"
YY-19	(18)*	*P. infestans*	+	A1	"	"
YY-27	(19)*	*P. infestans*	+	A1	"	"
KC-5	(20)*	*P. infestans*	+	A1	"	Kim Chen
KJ-2	(21)*	*P. infestans*	+	A1	"	Kim Je
KR-3	(22)*	*P. infestans*	+	A1	"	–
WS2-1	(23)*	*P. infestans*	+	A1	"	Wang San
WS2-3	(24)*	*P. infestans*	+	A1	"	"
WS4-1	(25)*	*P. infestans*	+	A1	"	"
WS6-16	(26)*	*P. infestans*	+	A1	"	"
WS9-1	(27)*	*P. infestans*	+	A1	"	"
N/A	N/A	*P. megasperma* (3)**	N/A	N/A	*Lycopersicon esculentum* MILL.	–
N/A	N/A	*P. sojae* (4)**	N/A	N/A	*Glycine max* MERR.	–
N/A	N/A	*P. cactorum* (5)**	N/A	N/A	–	–
N/A	N/A	*P. cinnamomi* (6)**	N/A	N/A	–	–
N/A	N/A	*P. citricola* (7)**	N/A	N/A	*Zizyphus jujuba* MILL.	–
N/A	N/A	*P. cryptogea* (8) **	N/A	N/A	*Brassica campestris* subsp.	–
N/A	N/A	*P. capsici* (9, 10)**	N/A	N/A	*Capsicum annuum* L.	–

*Numbers in parenthesis indicate the lane numbers in Fig. 4.

**Numbers in parenthesis indicate the lane numbers in Fig. 5.

[a]The cross between A2 (Na1-2) and unknown isolate of *P. infestans*.

+ Oospores formation. –No oospores formation.

To improve management of the late blight disease, it is necessary to develop a diagnosis system that would evaluate the frequency of different mating types in latent infection of *P. infestans*. The aim of this study, therefore, was to use AFLP to identify and develop a genetic marker for mating type determination in *P. infestans*.

MATERIALS AND METHODS

Strains and Cultivating Condition

Thirteen isolates of *P. infestans* Mat-A2, fourteen isolates of *P. infestans* Mat-A1 (Table 1), and eight isolates of *Phytophthora* species (Table 1 and Lanes 3–10 in Fig. 5) were used in this study. DGY and WS series of *P. infestans* isolates were kindly provided by Dr. B. S. Kim (Kangnung National University), and other sixteen isolates of *P. infestans* were isolated from infected plants. The *P. infestans* and other *Phytophthora* species isolates (Table 1) were cultured in a V8 juice medium (200 ml of V8 juice, 1 g of $CaCO_3$) in 1 l of distilled water for 10 days at 18–20°C [12].

Mating-Type Assay

Mating type was determined for twenty-six isolates of *P. infestans* by placing a plug of an unknown isolate on one side of a petri dish containing 20% clarified V8 medium with a plug of a known A2 isolate (Na1-2). The plates were incubated at 20°C for 14 days, after which formation of oospores was observed under a binocular microscope at low magnification (×20).

Extraction of Genomic DNA and AFLP Analysis

The genomic DNA of *P. infestans* was extracted by a modification of the method described by Goodwin *et al.* [11] for PCR and Southern blot analyses. Long-term stocks were maintained as an agar plug in 10% glycerol under liquid nitrogen [32]. The concentration of the RNase-treated DNAs was determined spectrophotometrically or on 0.8% agarose gels stained with ethidium bromide. AFLP DNA fingerprinting was performed essentially as described by Vos *et al*. [39]. Genomic DNA (1 μg) was digested with the restriction endonucleases *Eco*RI and *Mse*I, and double-stranded adapters were then ligated to the ends of the restriction fragments, followed by ethanol precipitation and resuspension in 40 μl of distilled water.

The sequence of the adapter fitting the *Eco*RI site was as follows:

5'-CTCGTAGACTGCGTACC
CTGACGCATGGTTAA-5'.

The sequence of the adapter fitting the *Mse*I site was as follows:

5'-GACGATGAGTCCTGAG
TACTCAGGACTCAT-5'.

Pre-amplification PCR was performed using standard adapter primers containing no selective nucleotides, followed by selective amplification using similar primers with two or three selective bases. Pre-amplification PCR was started with a cycle of 30s at 94°C, 1 min at 60°C, and 1 min at 72°C, and followed by 20 cycles. After the pre-amplification, the reaction mixture was diluted to 200 μl with distilled water. For the selective amplification of a limited number of DNA restriction fragments, the secondary template DNA was amplified with primers containing two or three selective 3' nucleotides (*Eco*RI+2 and *Mse*I+3 primers; Bioneer, Korea). For the selective amplification, the following PCR profile was used: the first cycle with 30s at 94°C, 30s at 65°C, and 1 min at 72°C, followed by 9 cycles with a stepwise lowering of annealing temperature by 1°C in each cycle and 29 cycles with an annealing temperature of 56°C.

The purpose of this first selective PCR amplification was to reduce the complexity of the template fragments and to generate large quantities of template suitable for the second PCR amplification with radioactively labeled selective primers. The primers used in the amplification step are called E+2 and M+3 primers, corresponding to the *Eco*RI and *Mse*I ends of the primary template, respectively. "+2" indicates that the primer contains two selective nucleotides. The sequence of the E+2 primer was 5'-GACTGCGTTA-CCAATTCAT-3' (E+AT), in which AT is the selective nucleotide, whereas the M+3 primers had the sequence 5'-GATGAGTCCTGAGTAACTA-3' (M+CTA). Amplification products were separated using standard 6% denatured polyacylamide gel electrophoresis and detected using autoradiographic procedures.

Cloning and Sequencing of Specific DNA Fragment

In order to develop the Mat-A1-specific DNA fragment of *P. infestans* as a probe for Southern hybridization and primer for PCR, the DNA fragment of the YY-8 isolate was isolated using a Geneclean turbo kit (Q. BIOgene, U.S.A.), followed by cloning into pGEM-T Easy vector (Promega, U.S.A.). Plasmid DNA was extracted by the alkaline lysis method and harvested by centrifugation at 15,000 rpm for 20 min at 4°C after incubation on ice for 20 min with the PEG solution (20% of polyethyleneglycol, 2.5 M NaCl). In order to determine the sequences of the specific DNA fragment, the purified plasmid DNA was used as a template to amplify the insert DNA. T7 and SP6 in T-vector (used as the primer for the experiment), and 5'-AGGAAGAAAGTGTCGATGT-3' (used as an intermediate primer) were synthesized and used as a bridge primer. The nucleotide sequence of the cloned DNA was determined with a DNA sequencer (Applied Biosystems, U.S.A.).

Southern Blot Hybridizations

PCR products amplified by the INF-1 and INF-2 primers were eletrophoretically separated on a 1% agarose TBE gel

(0.089 M Tris, 0.089 M Boric acid, 0.02 M EDTA), and genomic DNA digested with *Bam*HI, *Ava*I, and *Eco*RI were eletrophoretically separated on a 0.8% agarose TBE gel.

After the gels were stained with ethidium bromide, they were transferred to a nylon membrane (Hybond^{TM}-N+, Amersham, England). This membrane was prehybridized and hybridized in a hybridization incubator. The cloned DNA fragment (pINF) was prepared as a probe for Southern blot analysis by random-primed labeling. A total of 100 µl of the PCR labeling reaction mixture containing 10 ng of template DNA, 0.5 µM of each primer (M13F, M13R), 1×PCR buffer, 5 units of *Taq* polymerase (Dynazyme, U.S.A.), 0.1 mM each dGTP, dCTP, and dATP, 0.09 mM dTTP, and 1 mM digoxigenin dUTP (Boehringer Mannhein, Germany) was prepared and reacted in a program consisting of 30 sec at 94°C, 30 sec at 62°C, 30 sec at 72°C for 30 cycles, and 8 min at 72°C. The color detection was processed using a DIG nucleic acid detection kit, and all procedures were carried out using the instruction manual supplied (Boehringer Mannheim, Germany).

Synthesis of Primers for Screening of *P. infestans* Mat-A1

Based on the determined sequences of a specific fragment (218 bp) of *P. infestans* Mat-A1, the forward and reverse 20-mer primer sets that amplify a fragment (170 bp) which can easily be used to screen *P. infestans* Mat-A1, were synthesized using Primer3 Output System (Primer3 cgi v. 0.6).

Determination of Detection Limit for Conidial Suspensions and DNA Levels by PCR

For detection of the Mat-A1 marker in infected potato leaf, five isolates of *P. infestans* Mat-A1 (Table 1, isolates 14–18) and Mat-A2 (Table 1, isolates 1–5) were inoculated in potato (Dejima; S). In order to assess the sensitivity of the PCR amplification with the primer set for molecular detection, the inoculation was performed under conidial suspensions ranging from 1.2×10^3 to 1.2×10^6 conidia/ml. Different amounts of conidial suspension ranging form 1.2×10^3 to 1.2×10^6 conidia/ml and DNA ranging from 10 ng to 10 pg were prepared by continual dilutions. The genomic DNAs were extracted from *P. infestans*-infected leaf tissues by a modification of the method described by Goodwin *et al.* [5, 11, 18].

RESULTS AND DISCUSSION

Mating-Type Test

Twenty-six South Korean isolates of *P. infestans* were paired with known A2 isolate, Na1-2, to determine their mating types. In pairings between A2-type Nal-2 and other isolates of *P. infestans*, oospores were formed only with those compatible isolates within the agar where the two isolates meet. An example of a compatible isolate combination (between Nal-2 and YY-8), which produced oospores, is shown in Fig. 1. In contrast, only mycelia and zoospores were observed on the edge of plates of incompatible combinations (Figs. 1A, 1C). Pairings of Na1-2 isolate with tester isolates of mating types A1 and A2 revealed that 46% (12/26) of the isolates were mating type A2 and 54% (14/26) were mating type A1 (Table 1; Fig. 1).

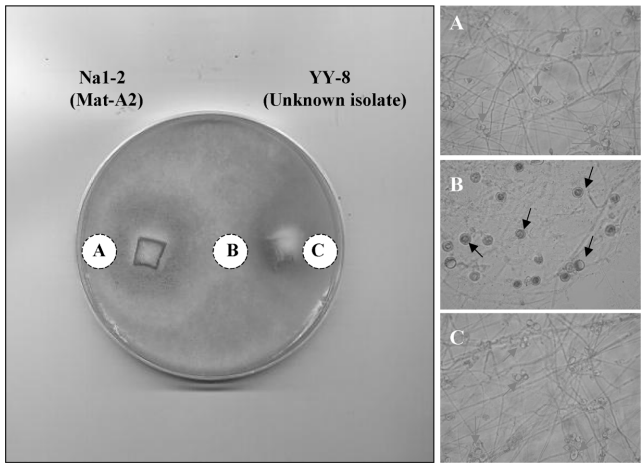

Fig. 1. Mating culture established by growing an A2 isolate (left; Na1-2) together with an unknown isolate (right; YY-8).
A and C: zoospores and mycelia produced; arrows/B: oospores formed.

A Mating-Type Specific AFLP Marker

We used AFLP to identify genetic markers associated with mating type. Genomic DNA was extracted from four Mat-A1 isolates of *P. infestans* (YY-8, YY-9, YY-11, and YY-12) and three Mat-A2 isolates (Na1-2, Na1-5, and Na2-1) and used in AFLP assays. Among sixty combinations of AFLP primers, the primer combination E+AT (5'-GACTG-CGTTACCAATTCAT-3')/M+CTA (5'-GATGAGTCCTGA-GTAACTA-3') repeatedly produced a specific DNA fragment, which was detected only from the isolates of *P. infestans* Mat-A1, including the isolate YY-8. This primer combination resulted in 30–35 fragments when the PCR products were electrophoresed on 6% polyacrylamide gel and stained with silver nitrate. Out of these fragments, a 218 bp fragment was specific to the *P. infestans* Mat-A1 strains (Fig. 2). This fragment was further investigated for the development of a specific marker for Mat-A1 strains of *P. infestans*.

Cloning and Sequencing of the *P. infestans* Mat-A1-Specific DNA Fragment

The specific DNA fragment of 218 bp was gel-purified, ligated into the pGEM-T-Easy vector (Promega, U.S.A.), and transformed in *E. coli* (JM 109). Plasmid DNA was extracted and the sequence of 218 bp was determined using T-vector primers (T7 and SP6; Fig. 3). The sequence was used in BLASTN and BLASTX searches of the

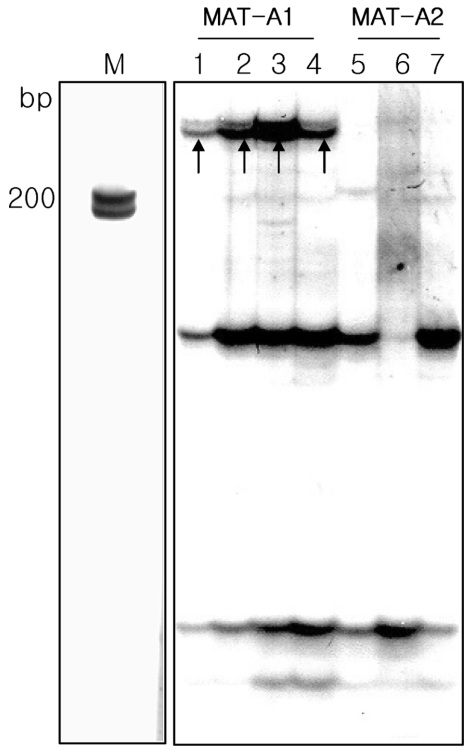

Fig. 2. AFLP profiles generated with primer combinations E+AT/M+CTA. Arrows indicate the specific AFLP product (218 bp) derived from *P. infestans* Mat-A1.
Lane M, 1 kb DNA ladder; lanes 1–4, *P. infestans* Mat-A1: YY-8 (1), YY-9 (2), YY-11 (3), and YY-12 (4); lanes 5–7, *P. infestans* Mat-A2: Na1-2 (5), Na1-5 (6), and Na2-1 (7).

nonredundant and dBEST databases of GenBank, as well as the *Phytophthora* sequences hosted in the *Phytophthora* Functional Genomics Database (PFGD, www.pfgd.org). No significant hits were detected, suggesting that the identified genetic marker is novel.

A PCR Assay of Mating-Type in *P. infestans*

Based on the sequence of the 218 bp fragment, we designed a pair of primers and used them to differentiate *P. infestans* Mat-A1 from Mat-A2 by PCR amplification. The

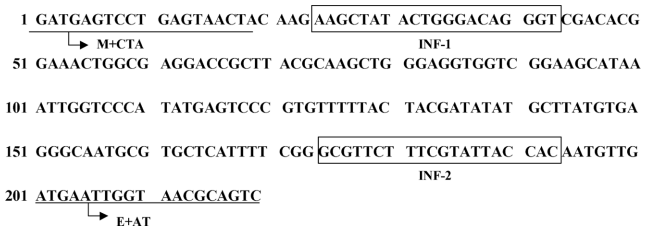

Fig. 3. Nucleotide sequences of *P. infestans* Mat-A1-specific DNA fragment. Underlined sequences were the E+AT/M+CTA primer combination used in PCR.
Sequences in boxes were forward (INF-1) and reverse (INF-2) primers for the detection of a specific DNA fragment in *P. infestans* Mat-A1.

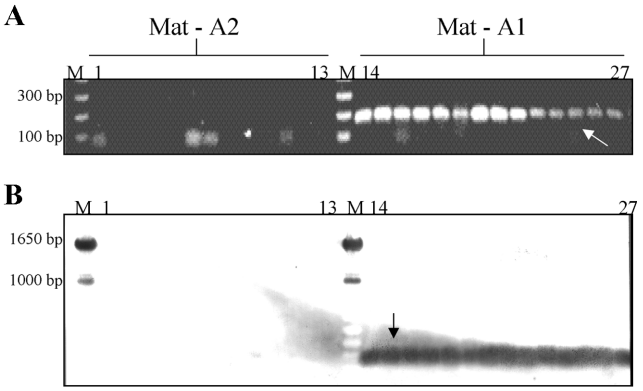

Fig. 4. PCR profiles (**A**) and PCR-Southern hybridization profiles (**B**) of isolates of *P. infestans* Mat-A1 and *P. infestans* Mat-A2.
The 170 bp fragments were generated only in the isolates of *P. infestans* Mat-A1 with specific primers (INF-1, INF-2; arrow). Lane M, 1 kb DNA ladder; lanes 1–13, isolates of *P. infestans* Mat-A2; lanes 14–27, isolates of *P. infestans* Mat-A1. Isolates of *P. infestans* are listed in Table 1.

sequences of the forward primer was 5'-AAGCTATACTG-GGACAGGGT-3' (INF-1) from 24 to 43 bp, and the G+C content of the primer was 50%. The reverse primer was 5'-GCGTTCTTTCGTATTACCAC-3' (INF-2) from 174 to 193 bp, and the G+C content was 45%. Using the forward and reverse primers, thirteen isolates of *P. infestans* Mat-A2 and fourteen isolates of *P. infestans* Mat-A1 were assayed by PCR with the primers INF-1 and INF-2. A single fragment of 170 bp was amplified from Mat-A1 isolates of *P. infestans*, but not from Mat-A2 isolates (Fig. 4A). These primers also failed to amplify genomic DNA of eight isolates of different *Phytophthora* spp. (Fig. 5A).

To ensure that the INF-1 and INF-2 primers failed to amplify genomic DNA from Mat-A2 strains, the amplified PCR products were separated in 0.8% agarose gel, blotted to nylon membrane, and hybridized with a probe (pINF) encoding the *P. infestans* Mat-A1-specific region amplified from the isolate YY-8. Hybridization signals were only detected in amplicons from the Mat-A1 isolates of *P. infestans* (Fig. 4B, lanes 14–27 and Fig. 5B, lanes 1 and 2), but not the Mat-A2 isolates (Fig. 4B, lanes 1–13) and other *Phytophthora* spp. (Fig. 5B, lanes 3–10).

Genomic Southern Blots Indicate that the Mat-A1-Specific DNA Fragment is a Single-Copy Sequence

To estimate the INF marker copy number, we analyzed a genomic Southern blot of *P. infestans* isolates (Mat-A1: YY-8, WS9-1, KC-C, and YY-19; Mat-A2: Na1-2, Na1-5, KAW-40, and DGY3-4). Extracted DNA was digested with three kinds of restriction enzymes, *Bam*HI, *Ava*I, and *Eco*RI, and hybridized with pINF as a probe. These restriction enzyme sites are not present in the INF marker clone (Fig. 6). This analysis identified an approximately 1.6 kb DNA fragment among the three restriction enzyme sites in the genomic DNA sequence of Mat-A1 (YY-8, KC-C, and

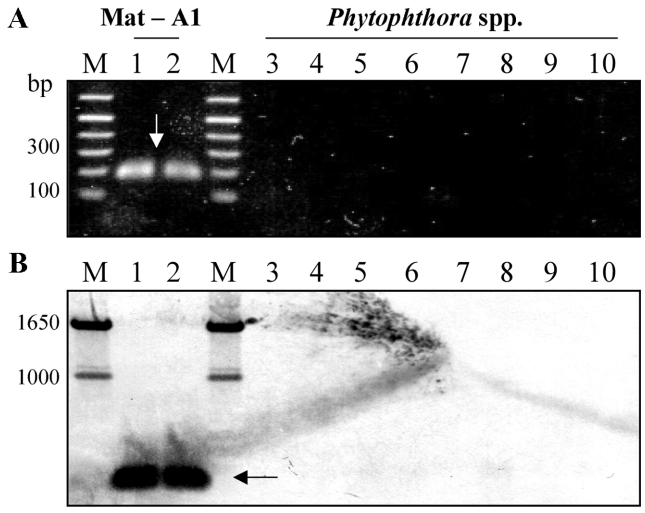

Fig. 5. PCR profiles (**A**) and PCR-Southern hybridization profiles (**B**) of isolates of *P. infestans* Mat-A1 and other *Phytophthora* spp.

The 170 bp fragment was generated only in the isolates of *P. infestans* Mat-A1 with specific primers (INF-1, INF-2; arrow). Lane M, 1 kb DNA ladder; lanes 1–2: positive control YY-8 (Mat-A1), YY-9 (Mat-A1); lane 3–10, isolates of *Phytophthora* species: *P. megasperma* (3), *P. sojae* (4), *P. cactorum* (5), *P. cinnamomi* (6), *P. citricola* (7), *P. cryptogea* (8), and *P. capsici* (9 and 10).

YY-19). It also identified a fragment of about 1.4 kb in *Bam*HI-digested WS9-1 isolate DNA. No signal was detected from isolates of *P. infestans* Mat-A2 (Fig. 6).

Optimization of PCR Conditions

To optimize PCR amplification conditions using primers INF-1 and INF-2, we investigated annealing temperature and template DNA quantity, since they could influence the

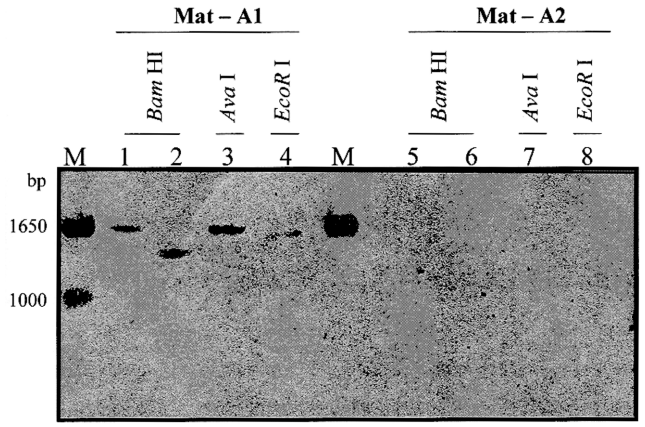

Fig. 6. Genomic Southern blot analysis of INF genes in *P. infestans*.

An approximately 1.6 kb single band was shown only in the isolates of *P. infestans* Mat-A1, when the cloned fragment was used as a probe (pINF). Lane M, 1 kb DNA ladder; lanes 1–4, *P. infestans* Mat-A1: YY-8 (1), WS9-1 (2), KC-5 (3), and YY-19 (4); lanes 5–8, *P. infestans* Mat-A2: Na1-2 (5), Na1-5 (6), KAW-40 (7), and DGY3-4 (8).

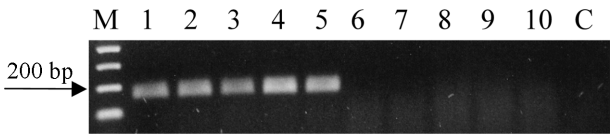

Fig. 7. Detection of the Mat-A1 marker in infected potato leaf (Dejima). Lane M, 1 kb DNA ladder;

Lanes 1–5, *P. infestans* Mat-A1-infected potato leaf; lanes 5–10, *P. infestans* Mat-A2-infected potato leaf; lane C, Non-infected control potato plant leaf.

rate and specificity of the amplification [12, 20, 24]. The theoretical annealing temperature calculated from the primers was 52°C. However, temperatures between 63–64°C were found to be suitable for the observation of the PCR products of the A1 mating type of *P. infestans* on stained agarose. Futhermore, 10 ng–200 pg of the A1 mating type of *P. infestans* genomic DNA was established to be sufficient for a detectable production by PCR (Fig. 8B).

In this study, DNA obtained from the target *P. infestans* (Mat-A1)-infected leaf tissues was amplified by PCR with the primers INF-1 and INF-2. Consequently, a 170 bp amplified fragment was detected only in *P. infestans* Mat-A1-infected leaf tissues. Furthermore, the pair primers were not amplified in *P. infestans* Mat-A2-infected leaf tissues and control leaf tissues (Fig. 7). In addition, these primer sets were amplified at a conidial suspension of 1.2×10^6 conidia/ml (Figs. 8A, 8c).

Therefore, complementary use of the primer combination E+AT/M+CTA and the forward and reverse primers will make it possible to quickly and precisely diagnose the mating type of *P. infestans* isolates in field material of the late blight disease.

Prospects for Late Blight Diagnostics

The primers and PCR assay described in this study should prove to be a useful tool to distinguish *P. infestans* Mat-A1

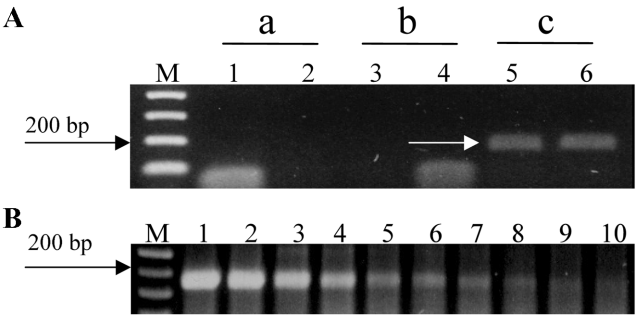

Fig. 8. Determination of detection limit for conidial suspensions (A) and DNA quantities (B) by PCR.

A. Lane M, 1 kb DNA ladder; lanes 1, 3, 5; *P. infestnas* Mat-A1 (KR-3)-infected plants; lanes 2, 4, 6; *P. infestnas* Mat-A1 (WS9-1)-infected plants. Lane a, 1.2×10^3 conidia/ml; lane b, 1.2×10^4 conidia/ml; lane c, 1.2×10^5 conidia/ml. **B.** Lanes M, 1 kb DNA ladder; 1, 10 ng; 2, 5 ng; 3, 1 ng; 4, 800 pg; 5, 600 pg; 6, 400 pg; 7, 200 pg; 8, 100 pg; 9, 50 pg; 10, 10 pg.

from *P. infestans* Mat-A2. This assay has significant practical applications, since Mat-A2 isolates are frequently associated with fungicide resistance and aggressiveness and should help implement disease management strategies [4, 7, 10, 24, 26, 29].

PCR technology can also be applied to detect pathogens *in vitro* and *in planta*, and can help in species identification [2, 9, 29, 34]. The described primers have useful potential to detect *P. infestans* among the microflora that are isolated from leaves, stems, or tubers of potato and tomato plants. Also, further studies to test the inheritance patterns of DNA marker, linked to Mat-A1 in F1 progeny through crossing of Na1-2 (*P. infestans* Mat-A2) and YY-8 (*P. infestans* Mat-A1), will further establish the extent to which the discovered marker is linked to Mat-A1.

Acknowledgment

This research was supported by a grant from Kangwon National University from 2003 to 2004. The authors are grateful for the financial support.

REFERENCES

1. Birch, P. R. J. and S. C. Whisson. 2001. *Phytophthora infestans* enters the genomics era. *Mol. Plant Pathol.* **2:** 257–263.
2. Bishop, H. 1940. A study of sexuality in *Sapromyces reinschii*. *Mycologia* **32:** 505–529.
3. Brasier, C. M. 1992. Evolutionary biology of *Phytophthora*. Part I: Genetic system, sexuality and the generation of variation. *Annu. Rev. Phytopathol.* **30:** 153–177.
4. Chang, T. T. and W. H. Ko. 1990. Resistance to fungicides and antibiotics in *Phytophthora parasitica*: Genetic nature and use in hybrid determination. *Phytopathology* **80:** 1414–1421.
5. Choi, J. Y., Y. H. Je, J. G. Kim, Y. C. Choi, W. T. Kim, and K. Y. Kim. 2004. Simultaneous detection and differentation of *Vairimorpha* spp. and *Nosema* spp. by multiplex polymerase chain reaction. *J. Microbiol. Biotechnol.* **14:** 737–744.
6. Deahl, K. L., S. P. Demuth, and A. Rivera-Pena. 1995. Identification of mating types and metalaxyl resistance in North American populations of *Phytophthora infestans*. *Am. Potato J.* **72:** 35–49.
7. Fisher, D. J. and M. D. Hayes. 1984. Studies of mechanisms of metalaxyl fungitoxicity and resistance to metalaxyl. *Crop Prot.* **3:** 117–185.
8. Fry, W. E. and S. B. Goodwin. 1997. Re-emergence of potato and tomato late blight in the United States and Canada. *Plant Dis.* **81:** 1349–1357.
9. Gallegly, M. E. 1968. Genetics of pathogenicity of *Phytophthora infestans*. *Annu. Rev. Phytopathol.* **6:** 375–396.
10. Gisi, U. and Y. Cohen. 1996. Resistance to phenylamide fungicides: A case study with *Phytophthora infestans* involving mating type and race structure. *Annu. Rev. Phytopathol.* **34:** 549–572.
11. Goodwin, S. B., J. L. Spielman, J. M. Matuszak, S. N. Bergeron, and W. E. Fry. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* population in Northern and Central Mexico. *Phytopathology* **82:** 955–961.
12. Griffith, G. W., R. Snell, and D. S. Shaw. 1995. Late blight (*Phytophthora infestans*) on tomato in the tropics. *Mycologist* **9:** 87–89.
13. Innis, M. A. and D. H. Gelfand. 1990. Optimization of PCRs; *In* Innis, M. A., D. H. Gelfand, and J. Sninsky (eds.), *PCR Protocols. A Guide to Methods and Applications*.
14. Judelson, H. S. 1996. Recent advances in the genetics of oomycete plant pathogens. *Mol. Plant-Microbe Interact.* **9:** 443–449.
15. Judelson, H. S. 1996. Physical and genetic variability at the mating type locus of the oomycete, *Phytophthora infestans*. *Genetics* **144:** 1005–1013.
16. Judelson, H. S., L. J. Spielman, and R. C. Shattock. 1995. Genetic mapping and non-Mendelian segregation of mating-type loci in the oomycete, *Phytophthora infestans*. *Genetics* **141:** 503–512.
17. Kamoun, S. 2003. Molecular genetics of pathogenic oomycetes. *Eukaryotic Cell* **2:** 191–199.
18. Kim, M. K., H. S. Kim, B. O. Kim, S. Y. Yoo, J. H. Seong, D. K. Kim, S. E. Lee, S. J. Choe, J. C. Park, B. M. Min, M. J. Jeong, D. K. Kim, Y. K. Shin, and J. K. Kook. 2004. Multiplex PCR using conserved and species-specific 16S rDNA primers for simultaneous detection of *Fusobacterium nucleatum* and *Actinobacillus actinomycetemcomitans*. *J. Microbiol. Biotechnol.* **14:** 110–115.
19. Kim, Y. T., B. K. Park, E. I. Hwang, N. H. Yim, S. H. Lee, and S. U. Kim. 2004. Detection of recombinant marker DNA in genetically modified glyphosate-tolerant soybean and use in environmental risk assessment. *J. Microbiol. Biotechnol.* **14:** 390–394.
20. Ko, W. H. 1988. Hormonal heterothallism and homothallism in *Phytophthora*. *Annu. Rev. Phytopathol.* **26:** 57–73.
21. Lee, Y. S. and K. J. Kim. 2002. Genetic DNA marker for A2 mating type in *Phytophthora infestans*. *J. Microbiol.* **40:** 254–259.
22. Lee, Y. S. and K. S. Kim. 2000. Rapid and accurate species-specific detection of *Phytophthora infestans* through analysis of ITS regions in its rDNA. *J. Microbiol. Biotechnol.* **10:** 651–655.
23. Lee, Y. S., S. H. Eom, K. J. Kim, H. S. Jung, and S. P. Lee. 2003. Identification of DNA markers linked to metalaxyl insensitivity loci in *Phytophthora infestans*. *Mycobiology* **31:** 229–234.
24. Lee, Y. S. and S. P. Lee. 2002. Development of rapid molecular detection marker for *Colletotrichum* spp. in leaf and fruit tissues of sweet persimmom. *J. Microbiol. Biotechnol.* **12:** 989–992.
25. Lui, S. Y., K. L. Ling, and B. Ho. 2003. rDNA gene in an unlikely marker for metronidazole resistance in the Asian *Helicobacter pylori* isolates. *J. Microbiol. Biotechnol.* **13:** 751–758.

26. McPherson, M. J., R. J. Oliver, and S. J. Gurr. 1992. The polymerase chain reaction, pp. 123–145. *In* Gurr, S. J., M. J. McPherson, and D. J. Bowles (eds.), *Molecular Plant Pathology. A Practical Approach*, vol. **1**. IRL Press, NY, U.S.A.
27. Muller, H. J. 1918. A case of balanced lethal factors. *Genetics* **3:** 422–499.
28. Old, R. W. and S. B. Primrose. 1994. Polymerase chain reaction, pp. 178–190. *In* S. B. Primrose, R. M. Twyman, and R. W. Old (eds.), *Principles of Gene Manipulation*. Blackwell Science. Cambridge, MA, U.S.A.
29. Paabo, S. 1990. Amplifying ancient DNA; PCR Protocols, pp. 159–166. *In* Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White (eds.), *A Guide to Methods and Applications*. Academic Press, Inc., Harcourt Brace Jovanovich, NY, U.S.A.
30. Reineke, A. and P. Karlovsky. 2000. Simplified AFLP protocol: Replacement of primer labeling by the incorporation of alpha-labeled nucleotides during PCR. *Biotechniques* **28:** 622–623.
31. Ristaino, J. B. 2002. Tracking historic migrations of the Irish potato famine pathogen, *Phytophthora infestans*. *Microbes Infect*. **4:** 1369–1377.
32. Rusell, J. R., J. D. Fuller, M. Macaulay, B. G. Hatz, A. Jahoor, W. Powell, and R. Waugh. 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. *Theor. Appl. Genet*. **95:** 714–722.
33. Shaw, D. S. 1988. The *Phytophthora* species, pp. 27–51. *In* Sidhu G. S. (ed.), *Advances in Plant Pathology*, vol. **6**. Academic Press, New York, U.S.A.
34. Shaw, D. S. 1991. *Genetics*, pp. 131–170. *In* Ingram, D. S. and D. H. Williams (eds.), *Phytophthora infestans*, vol. **7**. Academic Press, New York, U.S.A.
35. Smart, C. D. and W. E. Fry. 2001. Invasions by the late blight pathogen: Renewed sex and enhanced fitness. *Biol. Invas*. **3:** 235–243.
36. Tooley, P. W. 1988. Use of uncontrolled freezing for liquid nitrogen storage of *Phytophthora* species. *Plant Dis.* **72:** 680–682.
37. Tooley, P. W., W. E. Fry, and M. J. Villarreal Gonzalez. 1985. Isoenzyme characterization of sexual and asexual *Phytophthora infestans* population. *J. Hered*. **76:** 431–435.
38. Van der Lee, T., I. D. Witte, A. Drecth, C. Alfonso, and F. Govers. 1997. AFLP linkage map of the oomycete *Phytophthora infestans*. *Fungal Genet. Biol.* **21:** 278–291.
39. Vos, P., R. Hoger, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A. Fijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. *Nucl. Acid Res.* **23:** 4407–4414.