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Restriction Fragment Length Polymorphism of PCR Amplified Ribosomal DNA Among Korean Isolates of *Phytophthora*

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Genetic diversity of ninety-five Korean isolates of *Phytophthora* was investigated on the basis of PCR-RFLP of ribosomal DNA. The isolates were previously identified as following fifteen species by mycological and cultural characteristics; *P. boehmeriae, P. cactorum, P. cambivora, P. capsici, P. cinnamomi, P. citricola, P. citrophthora, P. cryptogea, P. drechsleri, P. erythroseptica, P. infestans, P. megasperma, P. nicotianae, P. palmivora* and *P. sojae.* The regions of small subunit (SSU) and internal transcribed spacer (ITS) of rDNA were amplified with primer pair, NS1 and ITS4, by polymerase chain reaction (PCR) and digested with nine restriction enzymes. *P. boehmeriae, P. cactorum, P. cambivora, P. capsici, P. cinnamomi, P. citricola, P. citrophthora, P. infestans, P. nicotianae* and *P. palmivora* showed specific band patterns for each species. However, *P. sojae* and *P. erythroseptica* presented identical band patterns and *P. cryptogea, P. drechsleri* and *P. megasperma* were divided into six groups, which were not compatible with delineation of the species. A group originated from cucurbits showed distinct band patterns from other groups, but the other five groups were closely related within 96.0% similarity, forming one complex group. Consequently, Korean isolates of *Phytophthora* were divided into thirteen genetic groups and each group was readily differentiated by comparing digestion patterns of *Ava*II, *Hae*III, *Mbo*I, *Hha*I and *Msp*I. Therefore, PCR-RFLP of rDNA using the five enzymes can be used to differentiate or identify the *Phytophthora* species reported in Korea so far.

***Keywords* :** genetic relationship, identification, PCR-RFLP, *Phytophthora*, ribosomal DNA.

The genus *Phytophthora* is one of the most important plant pathogens attacking almost all plant groups. Since de Bary established the genus in 1876, fifty-nine species and five varieties have been reported worldwide (Erwin and Ribeiro, 1996a) and twenty-one species are recorded in Korea (Jee, 1998). However, *P. carica* Hori and *P. fagopyri* Takimoto are not accepted as valid species internationally, and *P. colocasiae, P. macrospora* and *P. vignae* have not been found in the country recently (Jee, 1998). From a survey of *Phytophthora* diseases on plants in Korea from 1995 to 1997, 553 isolates collected from forty-six host plants were classified into fifteen species as *P. boehmeriae, P. cactorum, P. cambivora, P. capsici, P. cinnamomi, P. citricola, P. citrophthora, P. cryptogea, P. drechsleri, P. erythroseptica, P. infestans, P. megasperma, P. nicotianae, P. palmivora* and *P. sojae* (Jee, 1998).

Keys for species identification of the *Phytophthora* are mainly relied on morphological and cultural traits. However, morphological characteristics are highly variable among isolates within species and overlapping features exist between species, which often lead identification difficult (Erwin and Ribeiro, 1996b). Consequently, many researchers combined morphological traits with molecular characters in the taxonomy and phylogeny study of *Phytophthora* (Crawford et al, 1996; Mchau and Cofffey, 1995; Lee and Taylor, 1992).

Ribosomal DNA (rDNA) in fungi is organized in clusters of tandem repeats with several hundred copies per genome. The gene is conserved and contains sequence components possessing different evolutionary rates, which are phylogenetically and taxonomically informative for the study of genetic relatedness at generic or species level (Bruns et al. 1991). Ribosomal DNA was used to delineate species and clarify evolutionary relationship of *Phytophthora* spp. (Hong et al., 1998; Cooke and Duncan, 1997; Crawford et al., 1996; Forster et al., 1995; Lee and Taylor, 1992). Cooke and Duncan (1997) reported that PCR amplification of internal transcribed spacer (ITS) of rDNA followed by digestion with restriction enzymes is a quick and easy way to compare and identify isolates. Crawford et al. (1996) confirmed biological species of *P. medicaginis, P. trifolii* and *P. sojae* from *P. megasperma* species complex on the basis of rDNA sequence analysis. Hong et al. (1998) reported that twenty-one Korean isolates of *P. drechsleri* were divided into three groups by PCR-RFLP of small subunit (SSU) and ITS of rDNA.

In this study, SSU and ITS of ninety-five Korean isolates

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of *Phytophthora* were amplified and analysed by RFLP to examine genetic diversity of the isolates. In addition, identification of *Phytophthora* species using the technique was evaluated concomitantly.

Materials and Methods

Fungal isolates. The ninety-five isolates of *Phytophthora* spp. used in this study were isolated from forty-five host plants of vegetables, fruit trees and medicinal plants and cultural soils in Korea. One isolate of *P. melonis* obtained from Taiwan was included. Detailed information for the isolates is presented in Table 1. Cultures used in this study are now preserved in distilled water at 15°C and under liquid nitrogen as part of the Korean Agricultural Culture Collection. Information about these cultures could be searched on the internet, http://mgd.niast.go.kr.

Extraction of DNA. Extraction of genomic DNA basically followed the method of Lee and Taylor (1990). Actively growing mycelia on V8 juice agar were scrapped and inoculated on 1 ml V8 juice broth in a 1.5 ml microtube. After incubation with shaking for 2 days at 25°C, mycelial mats were harvested and washed with distilled water 2 times. Young mycelia were ground in the tube with glass rod and incubated in 400 μl lysis buffer [3% SDS, 50 mM EDTA, 50 mM Tris-HCl (pH 7.2), 1% 2-mercaptoethanol] at 65°C for 1 hour. Then, 400 μl of phenol/chloroform (1:1) was added and the tubes were vortexed, followed by centrifugation for 10 min at 12,000xg. The aqueous phase was transferred to a new tube, and DNA was precipitated by centrifugation after the addition of 0.1 volume of 3 M sodium acetate and 0.54 volume of isoprophanol. The pellet was rinsed with 70% ethanol, vacuum-dried and suspended in 50 μl of TE buffer.

Amplification of rDNA. Primer pairs, NS1 (5'-GTA-GTC-ATA-TGC-TTG-TCT-C-3')/ITS4 (5'-TCC-TCC-GCT-TAT-TGA-TAT-GC-3'), NS1/NS8 (5'-CCG-CGG-TTC-ACC-TAC-GGA-3') and ITS1 (5'-TCC-GTA-GGT-GAA-CCG-CGG-3')/ITS4, designed by White et al. (1990) were used for amplification of ITS and SSU, SSU, and ITS of rDNA, respectively (Fig. 1). PCRs were conducted in 100 μl reaction volumes. Each reaction tubes contained 1 μl (100 ng) of template DNA solution prepared above, 10 μl of 10 X buffer [50 mM KCl, 100 mM Tris-HCl (pH 9.0), 0.1% triton X-100, 15 mM $MgCl_2$], 4 μl of 2.5 mM (each) dNTP, 0.75 μl (each) of 100 uM primers, 0.5 μl of *Taq* Polymerase (5 unit/μl) and 83 μl of ddH_2O. Two drops of mineral oil was placed on the top of each reaction mixture. The thermal cycling parameters were denaturation, 1 min at 95°C, annealing, 1 min at 58°C, and polymerization, 2 min at 72°C. Thirty five cycles were conducted and the first denaturation and the last polymerization time were extended to 4 min and 8 min, respectively. The success of amplification was monitored by 1% agarose gel electrophoresis.

Digestion of rDNA by Restriction Enzymes. PCR-amplified SSU and ITS of rDNA of each isolate was digested with nine restriction enzymes (four cut: *Acc*II, *Alu*I, *Hae*III, *Hha*I, *Mbo*I, *Msp*I; five cut: *Ava*II, *Cfr*13I, *Hin*fI) according to the manufacture's instruction. The digested fragments were separated on 2% MetaPhore™ Agarose (FMC Bioproduct) with TBE buffer (45 mM Tris borate, 1 mM EDTA).

Analysis of RFLP patterns. Size of the bands separated on 2% MetaPhore agarose gel was determined by comparing them with a 100 bp size marker. If a band existed at a certain size, the isolate was recorded as 1, and if not, the isolate was recorded as 0 in a certain size. Thereby, binomial matrix was constructed, which included the data about all bands generated by nine restriction enzymes. NTSYS-pc, version 1.60 (Rohlf, 1990), were employed for numerical analysis of the data. Genetic similarity among isolates were calculated from dice formula and phenogram was constructed using the unweighted pair-group method with arithmetic averaging (UPGMA).

Results

The primers used in this study, NS1 and ITS4, successfully amplified ITS and SSU of rDNA on all isolates investigated. The sizes of amplified rDNA from all isolates were about 2,600 bp and could not be differentiated each other on 1% agarose gel electrophoresis. The primer pairs, ITS1/ITS4 and NS1/NS8, also amplified ITS region and SSU, respectively. The sizes of SSU were ca. 1,700 bp on all isolates. Sizes of ITS were differentiated among species on 2% MetaPhore agarose gel electrophoresis. Bands of the isolates of *P. boehmeriae, P. cinnamomi, P. erythroseptica, P. cambivora, P. sojae* and *Pml* group (cucurbits isolates previously identified as *P. drechsleri* and an isolate of *P. melonis* from Taiwan) were larger than 900 bp, but rests of the other species were smaller than 900 bp (data not shown).

There were no intraspecific differences among isolates except *P. cryptogea* and *P. drechsleri* on band patterns by nine restriction enzyme digestions of SSU and ITS (Table 1). Twenty-one isolates of *P. capsici* originated from various hosts, showing extensive variation in morphology and pathogenicity showed the same band patterns. Fifteen isolates of *P. nicotianae* from fifteen different hosts, six isolates of *P. cactorum*, five isolates of *P. citrophthora* and three isolates of *P. palmivora* also showed the same band patterns among isolates of each species. However, eight isolates of *P. cryptogea* showed four band patterns, *Pcr-dr*1 (one isolate from gerbera), *Pcr-dr*2 (one isolate from gerbera), P*cr-dr*3 (four isolates from gerbera) and *Pcr-dr*4 (two isolates from Chinese cabbage) (Table 1). Twenty-one isolates of *P. drechsleri* showed three band patterns, *Pcr-dr*1, *Pcr-dr*2 and *Pml* (Table 1). *P. cryptogea* and *P. drechsleri* shared two band patterns, *Pcr-dr*1 and *Pcr-dr*2. Two isolates of *P. erythroseptica* showed identical band patterns with two isolates of *P. sojae* on nine restriction enzyme digestion (Fig. 2A). *P. megasperma* isolates isolated from tomato at Koryeong showed identical band patterns with two isolates of *P. drechsleri* from tomato at the same area except for the two bands on *Msp*I (Fig. 2B). KACC F3022

Table 1. Isolates of *Phytophthora* spp. used and RFLP group determined from this study

Isolate no.	Species	Host	Geographic origin	Mating type	Alternative source and reference[d]	RFLP group from this study
KACC 40173[a]	*P. boehmeriae*	*Ailanthus altissima*	Hapcheon	Homo	P-96118 (Kim and Kim, 1993)	*Pbh*
KACC 40183	*P. cactorum*	*Fragaria Xananassa*	Koryeong	ND[c]	P-9815	*Pcc*
KACC 40174	〃	*Pyrus serotina*	Seosan	Homo	P-9776	〃
KACC 40175	〃	*Prunus persica* var. *vulgaris*	Chilgok	ND	P-9781 (Lim et al., 1998)	〃
KACC 40448	〃	*Malus pumila* var. *dulcissima*	Yeongdong	ND	P-9510	〃
KACC 40176	〃	*Malus pumila* var. *dulcissima*	Andong	Homo	Pb-09 (Jee et al., 1997c)	〃
KACC 40166	〃	*Malus pumila* var. *dulcissima*	Euiseong	Homo	Pb-36 (Jee et al., 1997d)	〃
KACC 40159	*P. cambivora*	*Malus pumila* var. *dulcissima*	Andong	A1	Pb-06 (Jee et al., 1997c)	*Pcm*
KACC 40160	〃	*Malus pumila* var. *dulcissima*	Euiseong	A1	P-9780	〃
KACC 40157	*P. capsici*	*Capsicum annuum*	Cheongsong	A1	Pa-11	*Pcp*
KACC 40473	〃	*Capsicum annuum*	Eumseong	A1	Pa-5	〃
KACC 40474	〃	*Capsicum annuum*	Yeongyang	A2	Pa-14	〃
KACC 40475	〃	*Capsicum annuum*	Jincheon	A1	Pa-23	〃
KACC 40476	〃	*Capsicum annuum*	Koisan	A2	Pa-61	〃
KACC 40477	〃	*Capsicum annuum*	Yeongam	A2	Pa-94	〃
KACC 40478	〃	*Capsicum annuum*	Muju	A1	Pa-107	〃
KACC 40479	〃	*Capsicum annuum*	Eumseong	A2	Pa-118	〃
KACC 40480	〃	*Capsicum annuum*	Haenam	A1	Pa-122	〃
KACC 40481	〃	*Capsicum annuum*	Cheongdo	A2	Pa-130	〃
KACC 40482	〃	*Capsicum annuum*	YangPyeong	A1	Pa-159	〃
KACC 40483	〃	*Capsicum annuum*	Milyang	ND	Pa-163	〃
KACC 40158	〃	*Capsicum annuum*	Tamyang	A2	Pa-109	〃
KACC F42	〃	*Lycopersicon esculentum*	Hanam	A1	P-9506	〃
KACC 40177	〃	*Lycopersicon esculentum*	Kimcheon	A1	P-9512 (Jee et al., 1998c)	〃
KACC 40470	〃	*Lycopersicon esculentum*	Puyeo	A1	P-9723	〃
KACC 40179	〃	*Citrullus lanatus*	Naju	A2	P-9650	〃
KACC 40471	〃	*Citrullus lanatus*	Yesan	ND	P-97131	〃
KACC 40178	〃	*Cucurbita sp.*	Suwon	A1	P-9540	〃
KACC 40472	〃	*Cucumis melo*	Kongju	A1	P-9632	〃
KACC 40181	〃	*Cucumis sativus*	Kongju	A1	P-9727	〃
KACC 40182	*P. cinnamomi*	*Larix leptolepis*	Suncheon	A2	P-9796	*Pcn*
KACC 40184	*P. citricola*	*Zizyphus jujuba* var. *inermis*	Kyeongsan	Homo	P-97101 (Jee et al., 1998b)	*Pctrc*
KACC 40188	*P. citrophthora*	*Malus pumila* var. *dulcissima*	Andong	A1	Pb-40	*Pctrp*
KACC 40186	〃	*Citrus sinensis*	Cheju	A1	P-9715 (Song et al., 1997)	〃
KACC 40185	〃	*Schizandra chinensis*	Chilgok	A1	P-9659	〃
KACC 40187	〃	*Citrus junos*[b]	Namhae	A1	SP-13	〃
KACC F3021	〃	*Prunus persica* var. *vulgaris*	Yeongcheon	ND	P-98155	〃
KACC F43	*P. cryptogea*	*Gerbera jamesonii*	Pusan	ND	P-9533 (Jee et al., 1996)	*Pcr-dr3*
KACC F44	〃	*Gerbera jamesonii*	Kwangju	ND	P-9535 (Jee et al., 1996)	〃
KACC F45	〃	*Gerbera jamesonii*	Jeju	ND	P-9638 (Jee et al., 1996)	〃
KACC 40162	〃	*Gerbera jamesonii*	Seoguipo	ND	P-9672	〃
KACC 40413	〃	*Brassica campestris* ssp *pekinensis*	Yangju	A1	P-9509 (Jee et al., 1999)	*Pcr-dr4*
KACC 40189	〃	*Brassica campestris* ssp *pekinensis*	Seosan	A2	P-9724	〃
KACC 40469	〃	*Gerbera jamesonii*	Pusan	ND	P-9620 (Jee et al., 1996)	*Pcr-dr1*
KACC 40161	〃	*Gerbera jamesonii*	Icheon	A2	P-9536 (Jee et al., 1996)	*Pcr-dr2*
KACC 40190	*P. drechsleri*	*Lycopersicon esculentum*	Koryeong	A1	P-9615 (Jee et al., 1998c)	*Pcr-dr1*
KACC F4	〃	*Lycopersicon esculentum*	Koryeong	A1	P-9614 (Jee et al., 1998c)	〃
KACC 40463	〃	*Lactuca sativa*	Seoul	ND	P-9801	〃
KACC 40464	〃	*Spinacia oleracea*	Yeocheon	ND	P-9818 (Jee et al., 1999)	〃

Table 1. Continued

Isolate no.	Species	Host	Geographic origin	Mating type	Alternative source and reference[d]	RFLP group from this study
KACC 40191	*P. drechsleri*	*Angelica gigas*	Seosan	A2	P-9519	*Pcr-dr2*
KACC 40484	〃	*Angelica gigas*[b]	Suwon	A1	SP-33	〃
KACC 40196	〃	*Ligularia fischeri*	Pyeongchang	ND	P-9705	〃
KACC 40465	〃	*Rehmannia glutinosa*[b]	Suwon	ND	SP-42	〃
KACC 40466	〃	*Schizandra chinensis*[b]	Suwon	ND	SP-51	〃
KACC 40467[a]	〃	*Lycium chinense*	Cheongyang	A2	P-97105	〃
KACC 40195	〃	*Atractylodes japonica*	Hamyang	A2	P-96116 (Kim et al. 1997)	〃
KACC 40199	〃	*Actinidia chinensis*	Koheung	A2	P-9797	〃
KACC 40198	〃	*Larix leptolepis*	Namwon	A1	P-9771	〃
KACC 40193	〃	*Cucumis sativus*	Puyeo	A1	P-9617	*Pml*
KACC 40485	〃	*Cucumis sativus*	Kongju	Homo	P-9636	〃
KACC 40486	〃	*Cucumis melo*	Iksan	Homo	P-9626	〃
KACC 40194	〃	*Cucumis melo*	Kongju	A1	P-9634	〃
KACC 40192	〃	*Cucumis melo*	Iri	Homo	P-9532	〃
KACC 40487	〃	*Cucumis melo*	Kongju	A1	P-9737	〃
KACC 40197	〃	*Citrullus lanatus*	Puyeo	ND[c]	P-9742	〃
KACC 40488	〃	*Citrullus lanatus*	Puyeo	A2	P-9750	〃
KACC 40444	*P. melonis*	*Cucumis melo*	Taiwan	A1	P. J. Ahn, Taiwan	〃
KACC 40401	*P. megasperma*	*Lycopersicon esculentum*	Koryeong	Homo	P-9608 (Jee et al., 1998c)	*Pcr-dr5*
KACC F3020	〃	*Lycopersicon esculentum*	Koryeong	Homo	P-9606 (Jee et al., 1998c)	〃
KACC 40200	*P. erythroseptica*	*Pueraria lobata*	Yeongcheon	ND	P-96117 (Kim and Kim, 1993)	*Per-sj*
KACC 40449	〃	*Astragalus membranaceus*	Suwon	Homo	P-9766	〃
KACC 40706	*P. infestans*	*Lycopersicon esculentum*	Kongju	ND	inf-1	*Pin*
KACC 40707	〃	*Solanum tuberosum*	Cheju	ND	inf-6	〃
KACC 40402	*P. nicotianae*	*Solanum melongena*	Pocheon	A1	P-9504	*Pnc*
KACC 40408	〃	*Citrus sinensis*[b]	Cheju	A1	SP-02	〃
KACC 40164	〃	*Solanum tuberosum*	Namjeju	ND	P-9676	〃
KACC 40403	〃	*Epiphyllum trucatum*	Hwaseong	A2	P-9516 (Jee et al., 1998a)	〃
KACC 40165	〃	*Cinnamomum cassia*	Keochang	ND	P-9544	〃
KACC 40405	〃	*Lycium chinense*	Cheongyang	A2	P-9646	〃
KACC 40458	〃	*Angelica gigas*	Suwon	ND	SP-55	〃
KACC 40459	〃	*Zizyphus jujuba* var. *inermis*	Taegu	ND	P-9814	〃
KACC 40407	〃	*Lilium longiflorum*	Seosan	ND	P-9695 (Jee et al., 1998a)	〃
KACC 40460	〃	*Gypsophila elegans*	Namwon	A2	P-9538 (Jee et al., 1998a)	〃
KACC 40404	〃	*Anthurium andreanum*	Koyang	A2	P-9642 (Jee et al., 1998a)	〃
KACC 40461	〃	*Citrus sinensis*	Cheju	ND	SP-54	〃
KACC 40163	〃	*Citrus junos*	Koheung	A2	P-96106 (Jee et al., 1997a)	〃
KACC 40462	〃	*Rehmannia glutinosa*	Suwon	ND	P-97060	〃
KACC 40406	〃	*Sesamum indicum*	Chilgok	ND	P-9660	〃
KACC 40410	*P. palmivora*	*Ficus carica*	Yeongam	A2	P-9790	*Ppl*
KACC 40409	〃	*Cymbidium sp.*	Suwon	A2	P-9741	〃
KACC 40167	〃	*Chrysalidocarpus lutescens*	Koyang	A1	P-9601 (Jee et al., 1997b)	〃
KACC 40412	*P. sojae*	*Glycine max*	Hongseong	Homo	P-9662 (Jee et al., 1998d)	*Per-sj*
KACC 40468	〃	*Glycine max*	Suwon	Homo	P-98145	〃
KACC F3022	*Phytophthora* sp.	*Ficus benjamina*	Yongin	ND	P-9810	*Per-sj*
KACC F3023	〃	*Ficus benjamina*[b]	Koyang	ND	SP-03	*Pctrp*

[a] Accession number of Korean Agricultural Culture Collection (KACC), Molecular Genetics Division, NIAST.
[b] Obtained from cultural soil.
[c] Not determined.
[d] The number used by Jee, Hyeong-Jin in the Plant Pathology Division at NIAST and references.

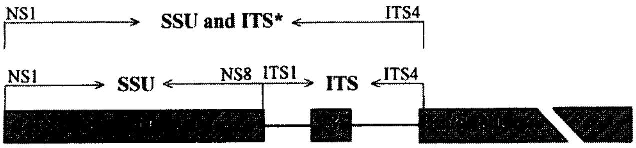

Fig. 1. The region of ribosomal DNA and the primers used in this study. *The region of small subunit (SSU) and internal transcribed spacer (ITS) was used for PCR-RFLP.

and KACC F3023 from benjamin's-tree which could not identified by morphological and cultural characteristics showed band patterns of *Per-sj* and *Pctrp*, respectively (Table 1).

Ninety-five isolates of *Phytophthora* were divided into seventeen groups as follows: *Pbh* (*P. boehmeriae*), *Pcc* (*P. cactorum*), *Pcm* (*P. cambivora*), *Pcp* (*P. capsici*), *Pcn* (*P. cinnamomi*), *Pctrc* (*P. citricola*), *Pctrp* (*P. citrophthora*), *Per-sj* (*P. erythroseptica* and *P. sojae*), *Pin* (*P. infestans*), *Pml* (*P. melonis*), *Pnc* (*P. nicotianae*), *Ppl* (*P. palmivora*) and *Pcr-dr* (*P. cryptogea-P. drechsleri*)1, 2, 3, 4 and 5 (Table 1). The band patterns of seventeen group except *Pcr-dr*5 are presented in Fig. 3. The band patterns of *Pcr-dr*5 were the same with those of *Pcr-dr*1 on eight restriction enzymes except *Msp*I, but were the same with those of *Pcr-dr*3 and *Pcr-dr*4 on *Msp*1 (Fig. 2). Especially, *Ava*II, *Hae*III, *Hha*I, *Mbo*I and *Msp*I out of nine restriction enzymes generated distinct band patterns among the 17 groups and differentiated them well (Fig. 3).

Analysis of the seventeen band patterns by NTSYS-pc presented that *Pcr-dr*1, *Pcr-dr*2, *Pcr-dr*3, and *Pcr-dr*4 of *P. cryptogea* and *P. drechsleri* and *Pcr-dr*5 of *P. megasperma* fell into a complex group within 96.0% similarity (Fig. 4). However, cucurbits isolates designated as *Pml* (previously identified as *P. drechsleri*) showed only 73% similarity with the complex group. The species producing apapillate sporangium as *Pcn, Pml, per-sj,* and *Pcm* were readily separated from papilate and semipapilate species.

Discussion

Results indicated that PCR-RFLP of rDNA is an useful tool to differentiate or identify most *Phytophthora* species since each isolate showed unique band patterns for the species. Among fifteen species tested, all species producing papillate or semi-papillate sporangium; *P. boehmeriae, P. cactorum, P. capsici, P. citricola, P. citrophthora, P. infestans, P. nicotianae, P. palmivora*, presented each distinct band pattern readily distinguishable from other species, but only two among seven species forming non-papillate sporangium, *P. cambivora* and *P. cinnamomi,* showed distinct band patterns for the species. Among the non-papillate species, *P. erythroseptica* and *P. sojae* were not distinguished from each other although they differed from other species. More complexly, *P. cryptogea, P. drechsleri* and *P. megasperma* were divided into six groups which were not clearly delineated for the species. One group originated from cucurbits was distinct enough from the other five groups with 73% similarity.

P. capsici has been known as a genetically variant species by Forster et al. (1995). Hwang et al. (1991) reported that seventeen isolates from pepper were divided into four groups by mitochondrial DNA (mt DNA) RFLP and seven Korean isolates were divided into two groups. However, twenty-one isolates of *P. capsici* originated from red pepper, tomato, watermelon, pumpkin, oriental melon and cucumber showed identical band patterns in this study (Table 1). Therefore, it is probable that Korean isolates of *P. capsici* are relatively recent evolutionary origin, although intraspe-

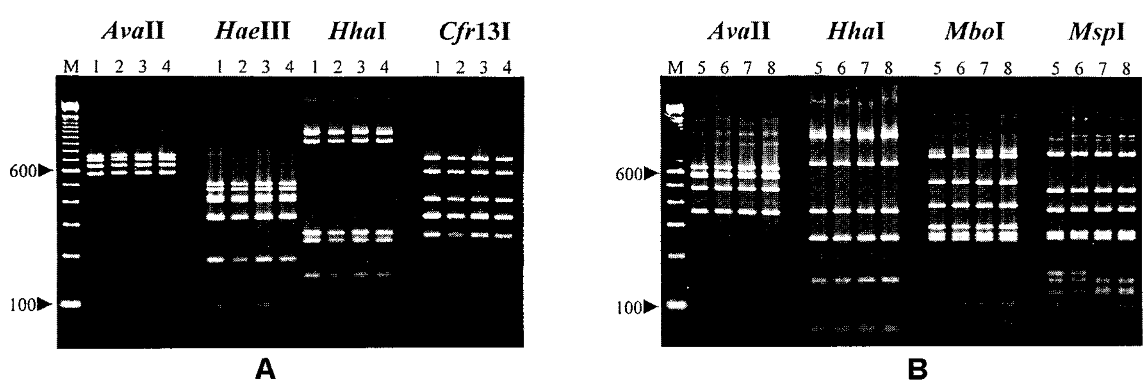

Fig. 2. A, Comparison of restriction fragments of *P. erythroseptica* isolates and *P. sojae* isolates. B, Comparison of restriction fragments of *P. drechsleri* isolates and *P. megasperma* isolates. The band patterns by the other 5 enzymes were the same between *P. erythrosepica* and *P. sojae* and between *P. drechsleri* and *P. megasperma.* SSU and ITS of each isolate was amplified by primer NS1 and ITS4 and digested by restriction enzymes, then the fragments were electrophoresed on 2% MetaPhor agarose. 1, KACC 40200 (*P. erythroseptica*); 2, KACC 40449 (*P. erythroseptica*); 3, KACC 40412 (*P. sojae*); 4, KACC 40468 (*P. sojae*); 5, KACC 40190 (*P. drechsleri*); 6, KACC F4 (*P. drechsleri*); 7, KACC 40401 (*P. megasperma*); 8, KACC F3020 (*P. megasperma*).

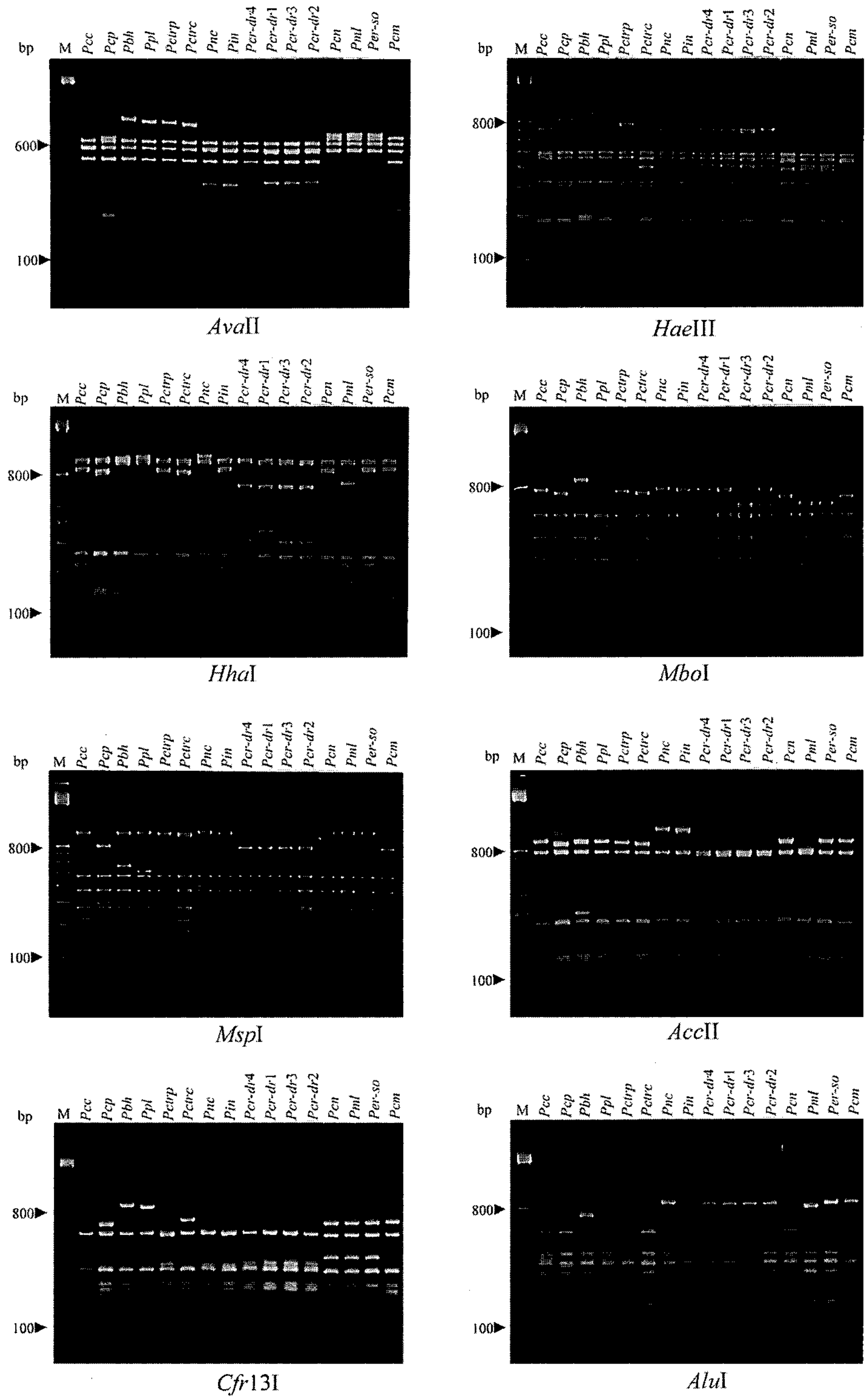

Fig. 3. Sexteen band patterns generated by digesting SSU and ITS of Korean isolates of *Phytophthora*. Band pattern of *Pcr-dr*5 group was not presented in this figure. The band pattern of the group was the same as that of *Pcr-dr*1 on eight enzymes except *Msp*I and was the same as that of *Pcr-dr*3 and *Pcr-dr*4 on *Msp*I. A part of the band pattern of *Pcr-dr*5 were presented in Fig. 2. RFLP group, *Pcc*, was originated from *P. cactorum*, *Pcp* from *P. capsici*, *Pbh* from *P. boehmeriae*, *Ppl* from *P. palmivora*, *Pctrp* from *P. citrophthora*, *Pctrc* from *P. citricola*, *Pnc* from *P. nicotianae*, *Pin* from *P. infestans*, *Pcr-dr*4, 1, 3 and 2 from *P. cryptogea-P. drechsleri*, *Pcn* from *P. cinnamomi*, *Pml* from *P. melonis*, *Per-sj* from *P. erythroseptica* and *P. sojae* and *Pcm* from *P. cambivora*.

cific homogeneity was due to the fact that sequence of rDNA is more conserved than that of mtDNA (Forster et al., 1995).

P. nicotianae has been known as a genetically uniform fungus although it has a wide host range (Forster et al., 1990). In accordance with the reports, Korean isolates of *P. nicotianae* collected from fifteen different host plants showed identical band patterns. Five isolates of *P. citrophthora,* six isolates of *P. cactorum* and three isolates of *P. palmivora* also showed the same band patterns although world collections of *P. citrophthora* were genetically variant (Forster et al., 1995). The other species such as *P. boehmeriae, P. cambivora, P. cinnamomi, P. citricola* and *P. infestans,* had too few isolates to discuss about their intraspecific variation.

P. erythroseptica and *P. sojae* are clearly differentiated in cultural and pathogenic characteristics although their morphological features are very similar (Erwin & Ribeiro, 1996a). However, four isolates of the two species showed completely same band patterns (Fig. 2A). In order to interpret the unexpected results, other genetic analyses including rDNA sequencing are required using a large number of isolates and type cultures.

KACC F3020 from tomato at Koryeong was identified as *P. megasperma* in spite of a little discrepancy with the original description of the species in that the isolate was homothalic and produced a few paragynous antheridia (Jee et al., 1998c). In this study, the isolate (*Pcr-dr*5) showed very similar band pattern with KACC F4 (*Pcr-dr*1) of *P. drechsleri* from tomato at the same area (Fig. 2B), and clustered in the *P. cryptogea- P. crechsleri* complex group, showinging 98.4% similarity (Fig. 4). Forster et al. (1995) reported that groups of *P. megasperma* were interspersed among those of *P. cryptogea/P. drechsleri* complex species on the analysis of ITSI of rDNA. Therefore, it is difficult to determine whether the isolate is *P. megasperma* or morphological variants of *P. drechsleri.*

A genetic heterogeneity of *P. drechsleri* of Korean isolates was studied previously by the authors (Hong *et al.* 1998). There were three intraspecific groups in *P. drechsleri,* designating as *Pd*G1 (*Pcr-dr*1 in this study), *Pd*G2 (*Pcr-dr*2 in this study) and *Pd*G3 (*Pml* in this study). Among the groups, *Pml* originated from cucurbits were distinct from *Pcr-dr*1 and *Pcr-dr*2 in the study. Eight isolates of *P. cryptogea* showed very similar band patterns with *Pcr-dr*1 and *Pcr-dr*2 of *P. drechsleri* in this study (Table 1). KACC 40469 and KACC 40161 showed band patterns identical to *Pcr-dr*1 and *Pcr-dr*2, respectively. Two isolates of *P. cryptogea* (*Pcr-dr*4) from chinese cabbage showed the difference of only two bands on *Msp*I from *Pcr-dr*2, and four isolates of *P. cryptogea* (*Pcr-dr*3) from gerbera showed the difference of only two bands on *Msp*I and three bands on

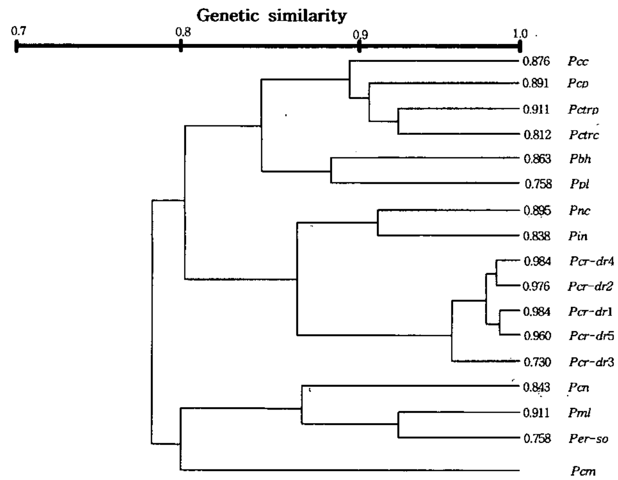

Fig. 4. Phenogram based on RFLP of PCR amplified rDNA showing the relationships among 17 groups of *Phytophthora* from Korea. The phenogram was constructed using the UPGMA from Dice similarity values. RFLP group, *Pcc,* was originated from *P. cactorum, Pcp* from *P. capsici, Pctrp* from *P. citrophthora, Pctrc* from *P. citricola, Pbh* from *P. boehmeriae, Ppl* from *P. palmivora, Pnc* from *P. nicotianae, Pin* from *P. infestans, Pcr-dr*4, 2, 1, 5 and 3 from *P. cryptogea-P. drechsleri* , *Pcn* from *P. cinnamomi, Pml* from *P. melonis, Per-sj* from *P. erythroseptica* and *P. sojae* and *Pcm* from *P. cambivora*

*Mbo*I from *Pcr-dr*2 (Fig. 3). The four groups except *Pml* in *P. cryptogea* and *P. drechsleri* clustered in a complex group, showing more than 96% homology on the basis of their band patterns (Fig. 4). However, the group, *Pml,* from cucurbits isolates of *P. drechsleri* was seperated from the complex group, showing 73% homology (Fig. 4). The isolate, KACC F3020, identified as *P. megasperma* also clustered in the *P. cryptogea-P. drechsleri* complex group on the phenogram. After all, types of *Pcr-dr*1, 2, 3, 4 and 5 formed *P. cryptogea-P. drechsleri* complex group. Therefore, ninety five Korean isolates of *Phytophthora* were divided into thirteen genetic groups as follows: *P. boehmeriae, P. cactorum, P. cambivora, P. capsici, P. cinnamomi, P. citricola, P. citrophthora, P. infestans, P. melonis, P. nicotianae, P. palmivora, P. erythroseptica-P. sojae and P. cryptogea-P. drechsleri* complex.

It seems to be difficult to elucidate detailed relationship among isolates of *Phytophthora* only with PCR-RFLP. In order to elucidate relationship more clearly, sequence analysis of rDNA is required. However, sequencing of many isolates is time consuming and costly. RFLP of PCR amplified rDNA is much simpler than sequence analysis, but the RFLP method is known to be informative for the analysis of relationships among isolates and useful for identification of fungal species (Chen, 1992; Ristaino et al., 1998). The results from this study can be also used for species identification of *Phytophthora* originated from Korea. Especially, the band patterns of *Ava*II, *Hae*III, *Hha*I, *Mbo*I, and *Msp*I are more valuable because the enzymes generated diverse and clear band patterns among species (Fig. 3).

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