Mycobiology 31(4): 235-247 (2003)

DNA Fingerprinting Analysis of the Genus *Phytophthora* in Korea

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(Received September 15, 2003)

In order to investigate biodiversity and establish identification system for *Phytophthora* spp. in Korea, a variety of band pattern was produced by using the URP (universal rice primer). The fingerprint patterns of *Phytophthora* spp. showed many common and variable fragments according to their isolates in distinct genotypes. In particular, *P. drechsleri* was classified into four distinct types (I to IV). *P. drechsleri* (KACC 40498 and KACC 40499) and *P. cryptogea* (KACC 40413) appeared to have almost equal bands despite their being different species. Ninety isolates of *Phytophthora* spp. were clustered into 13 groups based on UPGMA (unweighted pair group method with arithmetic means) analysis. These DNA fingerprinting data would be helpful for inter- and intra-species identification of *Phytophthora* species.

KEYWORDS: Fingerprinting, *Phytophthora*, URP-PCR

The genus *Phytophthora* is one of the most important plant pathogens attacking almost all plant groups. Since de Bary established the genus with *P. infestans* as the type species in 1876, 95 species and 5 varieties have been reported worldwide (Erwin and Riberio 1996). Most are primary invaders of healthy plant tissue with limited saprotrophic ability. Many are responsible for serious diseases of economically important crops (Gregory 1983), while some cause extensive damage to natural plant communities (Brasier 1992a; Wills 1993; Zentmyer 1983). From a survey of *Phytophthora* diseases in plants in Korea from 1996 to 1999, 990 isolates were collected from 66 host plants and classified into 17 species, such as *P. boehmeriad*, *P. cactorum*, *P. cambivora*, *P. capsici*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, *P. drechsleri*, *P. erythroseptica*, *P. infestans*, *P. macrospora*, *P. megasperma*, *P. melonis*, *P. nicotianae*, *P. palmivora*, and *P. sojae* (Jee, 1999).

Oomycetes, such as *Phytophthora*, downy-mildews and *Pythium*, form a unique branch of eukaryotic plant pathogens with an independent evolutionary history (Kamoun *et al.*, 1999). Among the oomycetes, *Phytophthora* spp. causes some of the most destructive plant diseases in the world (Erwin and Ribeiro 1996; Kamoun 2000). For example, *P. infestans*, the cause of the Irish potato famine, remains a destructive pathogen responsible for multi-billion-dollar losses in potato and tomato production (Fry and Goodwin, 1997a, 1997b). Other economically important *Phytophthora* diseases include root and stem rot caused by *P. sojae*, which hampers soybean production in several continents.

The oomycetes represent a diverse group of organisms that include pathogens of plants and animals, as well as saprophytic species (i.e. water molds) (Margulis and Schwartz, 2000). The position of the oomycetes as a unique lineage of stramenopile eukaryotes, unrelated to true fungi but closely related to heterokont (i.e. brown) algae, has been well established using molecular phylogenies that are based on ribosomal RNA (rRNA) sequences (Kumar and Rzhetsky, 1996; Paquin *et al.*, 1997; van de Peer and de Wachter, 1997). Recently, these findings were supported by results of analyses of mitochondrial proteins (Lang *et al.*, 1999) and four protein-encoding chromosomal genes (Baldauf *et al.*, 2000). From these analyses, it became evident that oomycetes have the ability to infect plants independent of other eukaryotic plant pathogens and are likely to have unique mechanisms for doing so. Several recent studies have examined phylogenetic relationships within the oomycetes, which have contributed to a better understanding of their host specialization and pathogenicity (Hudspeth *et al.*, 2000; Leclerc *et al.*, 2000; Riethmuller *et al.*, 1999)

Korean isolates of *Phytophthora* were investigated by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) of internal transcribed spacer (ITS) region and small subunit (SSU) of rDNA. Ninety-five Korean isolates of 16 species were divided into 13 genetic groups. Two isolates of *P. erythroseptica* showed the same band pattern with that of *P. sojae*, while the isolates of *P. cryptogea*, *P. drechsleri*, and *P. megasperma* formed a complex group which was not compatible with the delineation of the species (Hong *et al.*, 1999, 1998).

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Since 1980, population-genetic studies based increasingly on molecular methods have improved our ability to both identify fungal species units and determine their natural relationships (Brasier, 1997). Despite this, species units in *Phytophthora* have remained poorly defined, because of its morphological and cultural characters (Brasier, 1992b).

The combined use of population and molecular criteria has also demonstrated that some traditional *Phytophthora* morphospecies, such as *P. megasperma*, comprise multiple species units (Foster *et al.*, 1989; Foster and Coffey, 1993; Hansen *et al.*, 1986), and that traditional taxonomic concepts in *Phytophthora* may bear little relationship to the evolutionary structure of the genus (Brasier, 1991; Brasier and Hansen, 1992; Hansen, 1991). In one case, morphologically distinct *Phytophthoras* have been shown to be part of a hybrid complex (Brasier *et al.*, 1999).

The classical taxonomy of *Phytophthora* is based on sporangial and sexual structures (Newhook *et al.*, 1978; Stamps *et al.*, 1990). Isozymes, as well as various kinds of nucleic acid analyses have recently been used in order to elucidate the phylogenetic relationships among different *Phytophthora* species (Cooke and Duncan, 1997; Crawford *et al.*, 1996; Mills *et al.*, 1991; Oudemans *et al.*, 1994).

Species identification has been based on morphological and physiological characters. These approaches are time consuming and require considerable knowledge of the genus (Tsao, 1983). The success of such an approach may depend on several factors, including interference from fast-growing secondary microflora (Tsao, 1990) and seasonal changes in pathogenic activity (Horner and Wilcox, 1996). Serological methods using enzyme-linked antibodies have been established for the detection of selected *Phytophthora* species (Miller *et al.*, 1997), but cross-reactions with other species and reduced sensitivity, especially in dark-rooted woody plants, still prevent their extensive application (Miller, 1996). Alternatively, species-specific oligonucleotide hybridization probes were developed in order to differentiate among *P. parasitica* Dastur, *P. capsici* Leonian, *P. cinnamomi* Rands, *P. megakarya* Brasier and Griffin, and *P. palmivora* var. *heterocystica* Babacauh (Goodwin *et al.*, 1989; Judelson and Messenger-Routh, 1996; Lee *et al.*, 1993).

Rapid, simple, and reliable identification of *Phytophthora* spp. has been successfully achieved using the PCR. DNA fingerprinting analysis has been used to assess genetic variability in a wide variety of organisms, including fungi (Deahl *et al.*, 1993; Gosselin *et al.*, 1995; Kelly *et al.*, 1994; Williams *et al.*, 1990).

URPs (universal rice primers), which can be used in PCR fingerprinting of various organisms including plants, animals and microorganisms, were developed from repetitive sequence of rice genome (Kang *et al.*, 2000, 1998). The URP-PCR technique is a useful tool for the characterization and grouping of fungal species at the inter- and intra-specific levels (Kang *et al.*, 2000).

Based on the above, this study conducted an analysis of DNA fingerprinting to address several issues concerning the *Phytophthora* taxonomy of Korean isolates. The objectives of this study were to detect identification of differentiate *Phytophthora* spp. isolates by using random amplified polymorphic DNA (RAPD) analysis and to contribute to a better understanding of somatic relationships among *Phytophthora* spp. isolates.

Materials and Methods

Fungal isolates and extraction of genomic DNA. Ninety isolates of *Phytophthora* spp. used in this study were selected from 95 isolates of a previous study (Hong *et al.*, 1999). Eighty-nine isolates of *Phytophthora* were obtained from the Korean Agricultural Culture Collection (KACC: http://kacc.rda.go.kr/) and an isolate was obtained from the Centraal Burean voor Schimmelcultures (CBS). Detailed information of the isolates is presented in Table 1. Extraction of genomic DNA from the isolates was conducted according to the procedure described by Hong *et al.* (1998), which basically followed that by Lee and Taylor (1990).

Oligonucleotide primer and PCR amplification. The PCR reactions were performed in a 50 u*l* PCR mixture containing 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 2.5 mM of each dNTP, 200 ng primer URP3 (Seoulin Biotech Co., Ltd., Korea), and 2.5 unit Taqpolymerase (Promega, USA). The total amount of genomic DNA from various organisms added to the PCR mixture was approximately 50 ng. PCR amplification was carried out in a PTC-200TM Gradient cycler (MJ Research, Inc., USA) using the following profile: 1 cycle of 4 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 55°C, 2 min at 72°C, and 1 cycle of a final extension for 7 min at 72°C. DNA fragments were detected by staining with ethidium bromide. To investigate the effect of annealing temperature on URP-PCR, gradient annealing temperatures ranging from 36° to 60°C were used in blocks of the PTC-200TM Gradient cycler. The URP-PCR products were electrophoresed on a 1.5% agarose gel in TAE buffer and visualized by staining with ethidium bromide and photographed under UV transilluminator.

Computer-assisted analysis of DNA patterns. All of the DNA patterns were analyzed with the Windows version of GelCompar II (version 4.0; Applied Math, Kortrijk, Belgium). The individual bands in each of the patterns produced by the different PCR methods were analyzed by applying the Dice coefficient to the peaks. The similarity

Table 1. Isolates of *Phytophthora* used in this study

Species	Isolate No.	Host	Alternative source and reference
P. palmivora	KACC 40409 AF228088*	*Cymbidiuum* sp.	P-9741 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. palmivora	KACC 40410	*Ficus carica*	P-9790 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. palmivora	KACC 40167 AF228087*	*Cocos nucifera*	P9601 Kor. J. Plant Pathol. 13(6):438-441, 1997
P. citrophythora	KACC 40186 AF228081*	*Citrus sinensis*	P-9715 Kor. J. Plant Pathol. 13(2):129-131, 1997
P. citrophythora	KACC 40185	*Schizandra chinensis*	P-9659 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. citrophythora	KACC 40187	*Citrus junos* soil	SP-13 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. cactorum	KACC 40166	*Malus pumila* var. *dulcissima*	Pb-36 Kor. J. Plant Pathol. 13:139-144, 1997
P. cactorum	KACC 40176 AF2280778*	*Malus pumila* var. *dulcissima*	Pb-09 Kor. J. Plant Pathol. 13(3):139-144; 145-151, 1997
P. cactorum	KACC 40448	*Malus pumila* var. *dulcissima*	P-9830 Kor. J. Plant pathol. 13(3):139-144; 145-151, 1997
P. cactorum	KACC 40175	*Prunus persica* var. *vulgaris* fruit	P9781 Kor. J. Plant Pathol. 14(1): 99-101, 1998
P. cactorum	KACC 40174 AF087480*	*Pyrus serotina*	P9776 KSPP. 1998. Recent technology of chemical control of plant diseases. P 228. (Abst.)
P. erythroseptica	KACC 40449 AF087474*	*Astragalus memvranaceus*	P-9832 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. capsici	KACC 40181	*Cucumis sarivus*	P-9727 NIAST. 1997. Compendium of vegetable diseases with color plates. P 185.
P. capsici	KACC 40473	*Capsicum annuum*	Pa-5 NIAST. 1997. Compendium of vegetable diseases with color plates. P 109.
P. capsici	KACC 40179	*Citrullus lanatus*	P-9650 NIAST. 1997. Compendium of vegetable diseases with color plates. P218
P. capsici	KACC 40180	*Cucumis meol*	P-9635 NIAST. 1997. Compendium of vegetable diseases with color plates. P202
P. capsici	KACC 40483	*Capsicum annuum*	Pa-163 NIAST. 1997. Compendium of vegetable diseases with color plates. P 109.
P. capsici	KACC 40475	*Capsicum annuum crown*	Pa-23 NIAST. 1997. Compendium of vegetable diseases with color plates. P 109
P. capsici	KACC 40481	*Capsicum annuum crown*	Pa-130 NIAST. 1997. Compendium of vegetable diseases with color plates. P 109.
P. capsici	KACC 40478	*Capsicum annuum crown*	Pa-107 NIAST. 1997. Compendium of vegetable diseases with color plates. P 109.
P. capsici	KACC 40470	*Lycopersicon esculentum*	P-9723 Kor. J. Plant Pathol. 14(1): 29-37
P. capsici	KACC 40476	*Capsicum annuum crown*	Pa-61 NIAST. 1997. Compendium of vegetable diseases with color plates. P 109.
P. capsici	KACC 40471	*Citrullus lanatus*	P-97131 NIAST. 1997. Compendium of vegetable diseases with color plates. P 219.

Table 1. Continued

Species	Isolate No.	Host	Alternative source and reference
P. capsici	KACC 40474	*Capsicum annuum*	Pa-14 NIAST. 1997. Compendium of vegetable diseases with color plates. P 109.
P. capsici	KACC 40479	*Capsicum annuum* crown	Pa-118 NIAST. 1997. Compendium of vegetable diseases with color plates. P 109
P. capsici	KACC 40477	*Capsicum annuum* crown	Pa-94 NIAST. 1997. Compendium of vegetable diseases with color plates. P 109
P. capsici	KACC 40482	*Capsicum annuum* crown	Pa-159 NIAST. 1997. Compendium of vegetable diseases with color plates. P 109.
P. capsici	KACC 40480	*Capsicum annuum* crown	Pa-122 NIAST. 1997. Compendium of vegetable diseases with color plates. P 109.
P. capsici	KACC 40178	*Cucurbita* sp.	P-9540 NIAST. 1997. Compendium of vegetable diseases with color plates. P 235.
P. capsici	KACC 40177 AF 228079*	*Lycopersicon esculeutum* crown	P-9512 RDA J. Crop Protection 40(1):29-37, 1998
P. capsici	KACC 40157 AF228078*	*Capsicum annuum* crown	Pa-11 Kor. J. Plant pathol. 14(4):299-302, 1998
P. capsici	KACC 40158	*Capsicum annuum* root, stem	Pa-109 NIAST. 1997. Compendium of vegetable disease with color plates, P109
P. drechsleri	KACC 40193 AF087473*	*Cucumis sativus*	P-9617 NIAST. 1997. Compendium of vegetable diseases with color plates. P185
P. drechsleri	KACC 40194	*Cucumis melo*	P-9634 NIAST. 1997. Compendium of vegetable diseases with color plates. P203
P. drechsleri	KACC 40192	*Cucumis melo*	P-9532 NIAST. 1997. Compendium of vegetable diseases with color plates. P201
P. drechsleri	KACC 40197	*Citrullus lanatus*	P-9742 NIAST. 1997. Compendium of vegetable diseases with color plates. P219
P. drechsleri	KACC 40488	*Citrullus lanatus*	P-9750 NIAST. 1997. Compendium of vegetable disease with color plates. P 219.
P. drechsleri	KACC 40486	*Cucumis melo* fruit	P-9626 NIAST. 1997. Compendium of vegetable disease with color plates. P 205.
P. drechsleri	KACC 40185	*Cucumis sativas*	P-9659 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. drechsleri	KACC 40487	*Cucumis melo* crown	P-9737 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. melonis	KACC 40444 AF228094*	*Cucumis melo* (Taiwan)	P.J. Ann Kor. J. Plant Pathol. 14(5): 519-525, 1998
P. nicotianaee	KACC 40460	*Gypsophila elegans* crown	P-9538 Kor. J. Plant Pathol. 14(5):452-457, 1998
P. nicotianaee	KACC 40462	*Rehmannia glutinose* crown	P-97060 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. nicotianaee	KACC 40461	*Citrus sinensis* soil	SP-54 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. nicotianaee	KACC 40407 AF228086*	*Lilium longiflorum*	P-9695 Kor. J. Plant Pathol. 14(5): 452-457, 1998

Table 1. Continued

Species	Isolate No.	Host	Alternative source and reference
P. nicotianaee	KACC 40164	*Solanum tuberosum*	P9676 Plant disease and agricultural (KSPP). 4(1): 79-89, 1998
P. nicotianaee	KACC 40165	*Cercidiphyllum japonicum*	P-9644 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. nicotianae	KACC 40163	*Citrus junos*	P-96106 1997, Kor. J. Plant pathol. 13:442-445, 1997
P. nicotianae	KACC 40404	*Anthurium andreanum*	P-9642 Kor. J. Plant Pathol. 14(5):452-457, 1998
P. nicotianae	KACC 40443	*nicotianae tabacum*	
P. nicotianae	KACC 40402	*Solanum melongena*	P-9504 NIAST. 1997. Compendium of vegetable diseases with color plates. P 129.
P. nicotianae	KACC 40458	*Angelica gigas* soil	SP-55 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. nicotianae	KACC 40406	*Sesamum indicum*	P-9660 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. nicotianae	KACC 40405	*Lycium chinense*	P-9646 Kor. J. Plant Pathol. 14(4): 294-298, 1998
P. nicotianae	KACC 40459	*Zizyphus jujuba* var. *inermis*	P-9814 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. nicotianae	KACC 40403 AF228085*	*Zygocactus truncactus*	P-9516 Kor. J. Plant Pathol. 14(5): 452-457, 1998
P. nicotianae	KACC 40408	*Citrus sinensis*	SP-02 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. drechsleri	KACC 40466	*Schizandra chinensis* soil	SP-51 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. drechsleri	KACC 40467 AF087472*	*Lycium chinense* crown	P-97105 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. drechsleri	KACC 40199	*Actinidia chinensis*	P-9797 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. drechsleri	KACC 40195	*Atractylodes japonica*	P-96116 Kor. J. Plant Pathol. 13(6): 433-437, 1997
P. drechsleri	KACC 40465	*Rehmannia glutinose* soil	SP-42 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. drechsleri	KACC 40484	*Angelica gigas* soil	SP-33 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. cryptogea	KACC 40161	*Gerbera jamesonii*	P-9536 Kor. J. Plant pathol. 12(3);374-376, 1996
P. drechsleri	KACC 40191	*Angelica gigas*	P-9519 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. erythroseptica	CBS 956.87 AF228082*	*Solanum tuberosum* (U.S.A)	P1699 Pl. Dis. Reptr. 61: 807-810, 1977
P. erythroseptica	KACC 40712	*Solanum tuberosum*	
P. cryptogea	KACC 40189 AF087475*	*Brassica campestris* ssp. *pekinensis*	P-9724 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. cryptogea	KACC 40162 AF087477*	*Gerbera jamesonii*	P-9672 Kor. J. Plant pathol. 12(3);374-376, 1996
P. cryptogea	KACC 40702 (CBS 468.81)	rot and stem of Begonia eliator; hybrid in greenhouse (Germany)	
P. drechsleri	KACC 40499 (CBS 359.52)	*Solanum tuberosum* (Argentina)	
P. drechsleri	KACC 40498 (CBS 292.35)	*Beta vulgaris* var. *altissima* (U.S.A)	
P. cryptogea	KACC 40413	*Brassica campestris* ssp. *pekinensis*	P-9509 NIAST. 1997. Compendium of vegetable diseases with color plates. p58

Table 1. Continued

Species	Isolate No.	Host	Alternative source and reference
P. drechsleri	KACC 40463	*Lactuca sativa* fine root	P-9801 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. drechsleri	KACC 40190	*Lycopersicon esculentum*	P-9615 RDA J. Crop Protection 40(1): 29-37, 1998
P. drechsleri	KACC 40464	*Spinacia oleracea*	P-9818 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. cryptogea	KACC 40500 (CBS 290.35)	*Aster* sp. (U.S.A)	
P. cryptogea	KACC 40469 AF087476*	*Gerbera jamesonii* crown	P-9620 Kor. J. Plant Pathol. 12(3):374-376, 1996
P. cryptogea	KACC 40701 (CBS 113.19)	root and stem of *Lycopersicon esculentum* or *Petunia* sp. (Irish)	
P. sojae	KACC 40412 AF228089*	*Glycine max*	P-9662 RDA J. Crop Protec. 40(1):16-22, 1998
P. sojae	KACC 40468	*Glycine max*	P-98145 RDA J. Crop Protec. 40(1):16-22, 1998
P. cinnamomi	KACC 40182 AF087478*	*Larix leptolepis*	P-9796 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. megasperma	KACC 40401	*Lycopersicon esculentum*	P-9608 RDA J. Crop Protection 40(1): 29-37, 1998
P. boehmeriae	KACC 40173 AF228076*	*Ailanthus altissima*	P-96118 Kor. J. Plant Pathol. 9:327(Abst.), 1993
P. citricola	KACC 40184 AF228080*	*Zizyphus jujuba* var. *inermis*	P-97101 Kor. J. Plant Pathol. 14(5):402-407, 1998
P. cambivora	KACC 40159 AF087479*	*Malus pumila* var. *dulcissima*	Pb-06 Kor. J. Plant pathol. 13(3):145-151, 1997
P. cambivora	KACC 40160	*Malus pumila* var. *dulcissima*	P-9780 Kor. J. Plant pathol. 13(3):145-151, 1997
P. infestans	KACC 40707 AF228083*	*Solanum tuberosum*	inf-6
P. cinnamomi	KACC 40183	*Fragaria ananassa*	P-9815 Plant disease and agriculture (KSPP). 4(1): 79-89, 1998
P. erythroseptica	KACC 40200	*Pueraria lobata*	P-96117 Kor. J. Plant Pathol. 9:319(Abst.), 1993

between pairs of linearly combined fingerprints was calculated using the product-moment correlation coefficient (r value) (Pearson, 1926), applied to the whole densitometric curves of the gel tracks (Hane *et al.*, 1993). Cluster analysis of the pairwise similarity values was performed using the unweighted pair group method with arithmetic means (UPGMA) clustering technique (Sneath and Sokal, 1973) and a band position tolerance of 1.5% was used for comparison of the DNA patterns. The analysis of the patterns was undertaken in accordance with the manufacturers instructions. Fingerprint patterns were constructed as described by Rademaker and De Bruijn (1997).

Results

DNA fingerprinting of *Phytophthora* spp. In this study, DNA fingerprinting analysis was developed for the identification and sub-typing of *Phytophthora* species. URP3 primer well characterized field isolates of *Phytophthora* spp. DNA fingerprinting patterns were evaluated using the criteria established by Gillespie *et al.* (1997). DNA fingerprint patterns were analyzed for: (i) number of DNA fragments, (ii) optical density, and (iii) size of fragments (bp). Primer URP3 provided consistently a set of unique and well-defined polymorphic DNA fragments. The number of bands produced by amplification of the genomic DNAs ranged from 6 to 17 of the total 1020 with sizes ranging from 233.85 (*P. sojae*) to 6130 bp (*P. erythroseptica*).

As shown in Fig. 1 and TABLE II, DNA fingerprinting analysis by using URP3 showed that the fingerprint patterns of the 90 isolates of *Phytophthora* spp. consisted of several variable fragments and distinct genotypes. In particular, the DNA patterns of *P. drechsleri* were divided into four distinct types (I to IV) (Table 2).

In the case of *P. drechsleri* (lanes 19 and 20) and *P.*

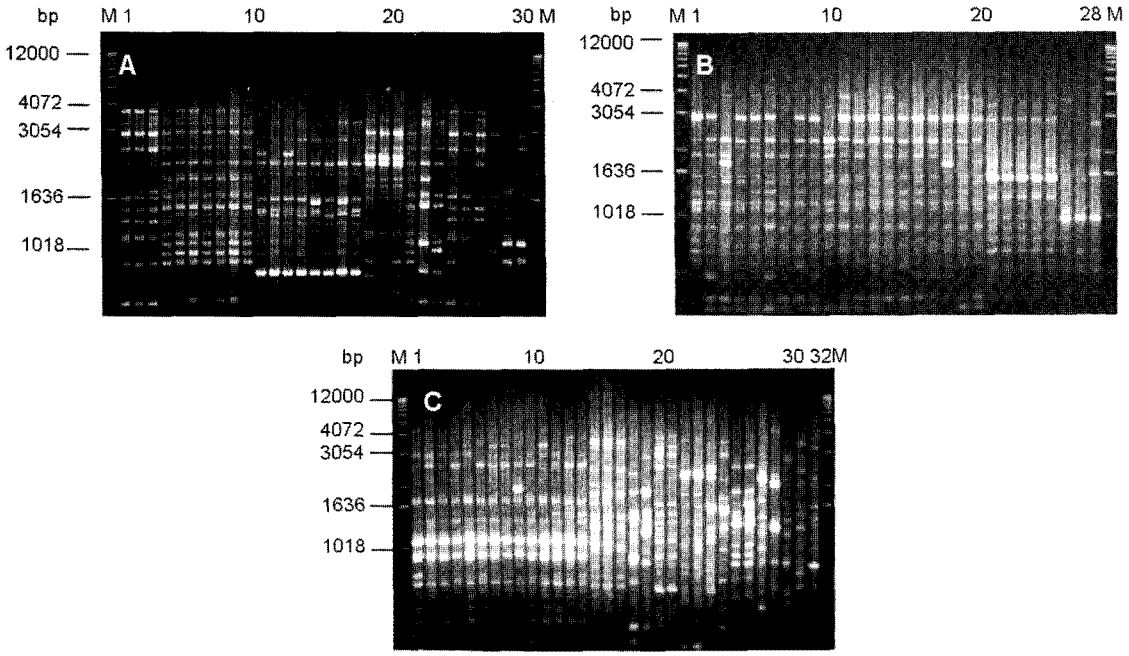

Fig. 1. PCR (polymerase chain reaction) profiles of the 90 *Phytophthora* isolates generated by URP-PCR (universal rice primer-PCR). M: 1 kb ladder (Promega). Plate A: Lanes 1-20, isolates of *P. drechsleri* (KACC 40190, KACC 40463, KACC 40464,KACC 40191, KACC 40195, KACC 40199, KACC 40465, KACC 40466, KACC 40467, KACC 40484, KACC 40192, KACC 40193, KACC 40194, KACC 40197, KACC 40185, KACC 40486, KACC 40487, KACC 40488, KACC 40498, and KACC 40499). Lanes 21-28, isolates of *P. cryptogea* (KACC 40413, KACC 40161, KACC 40162, KACC 40189, KACC 40469, KACC 40500, KACC 40701, and KACC 40702). Lanes 29 and 30, isolates of *P. nicotianae* (KACC 40407 and KACC 40408). Plate B: Lanes1-20, isolates of *P. capsici* (KACC 40157, KACC 40158, KACC 40177, KACC 40178, KACC 40179, KACC 40180, KACC 40181, KACC 40470, KACC 40471, KACC 40473, KACC 40474, KACC 40475, KACC 40476, KACC 40477, KACC 40478, KACC 40479, KACC 40480, KACC 40481, KACC 40482, and KACC 40483). Lanes 21-25, isolates of *P. cactorum* (KACC 40166, KACC 40174, KACC 40175, KACC 40176, and KACC 40448). Lanes 26-28, isolates of *P. palmivora* (KACC 40167, KACC 40409, and KACC 40410). Plate C: Lanes 1-14, isolates of *P. nicotianae* (KACC 40163, KACC 40164, KACC 40165, KACC 40402, KACC 40403, KACC 40404, KACC 40405, KACC 40406, KACC 40443, KACC 40458, KACC 40489, KACC 40460, KACC 40461, and KACC 40462). Lanes 15-17, isolates of *P. citrophthora* (KACC 40185,KACC 40186, and KACC 40187). Lanes 18-21, isolates of *P. erythroseptica* (KACC 40200, KACC 40449, CBS956.87, and KACC 40712). Lanes 22 and 23, isolates of *P. cambivora* (KACC 40159 and KACC 40160). Lanes 24 and 25, isolates of *P. cinnamomi* (KACC 40182 and KACC 40183). Lanes 26 and 27, isolates of *P. sojae* (KACC 40412 and KACC 40468). Lane 28, isolate of *P. boehmeriae* (KACC 40173). Lane 29, isolate of P. *citricola* (KACC 40184). Lane 30, isolate of *P. megasperma* (KACC 40401). Lane 31, isolate of P. *infestans* (KACC 40707). Lane 32, isolate of *P. melonis* (KACC 40444).

cryptogea (lane 21), most of their band patterns appeared equal though they are of different species (Fig. 1). This result suggests that these two different species may have the same genetic background.

P. erythroseptica showed that two isolates (KACC 40200 and KACC 40449) amplified only two common fragments (1915 and 1523 bp), but the others (CBS956.87 and KACC 40712) coincided completely with each other. Accordingly, this explains why CBS956.87 and KACC 40712 have a high homology. Meanwhile, because the same band did not show among *P. boehmeriae, P. citricola, P. megasperma, P. infestans*, and *P. melonis*, homology among these different species did not exist (Fig. 1C).

Distinguished observed band pattern in each major band was revealed by using the fingerprinting method in DNA fingerprinting analysis of four *Phytophthora* species (Table 3). The major bands suggested that the size of the DNA fragments amplified in several isolates of identical species is the same. Of these major bands, however, several bands (lanes 1 to 3 in *P. drechsleri*; lanes 3, 4, 7, 10, and 13 in *P. capsici*; lanes 1, 3, 5, 11, 12, and 13 in *P. nicotianae*; and lanes 29 and 30 in *P. cryptogea*) did not appear in the PCR amplification.

In this fingerprinting analysis by DNA fingerprinting method in various isolates of *Phytophthora* spp., although band patterns among some species and genus appeared to be the same, both homology and non-homology among diversified band patterns according to isolates were observed. However, in the identification of *Phytophthora* spp., fingerprinting could not present a more accurate

Table 2. DNA fingerprint profiles of *Phytophthora* species by primer URP3

Organism	Amplified DNA fragments			
	Primary (bp)	Variable (bp)	Genotype	Frequency (%)
P. drechsleri	I 3714, 2925, 2532, 1646, 1477, 1388, 1154, 912, 641	–	dre1	3 (15.0)
	II 2979, 2586, 2243, 1954, 1687, 1525, 1200, 1103, 1010, 931	–	dre2	2 (10.0)
		3752	dre3	5 (25.0)
	III 2243, 843	2440, 1631	dre4	2 (10.0)
		1631	dre5	2 (10.0)
		3294, 1580	dre6	2 (10.0)
	IV 3370, 2567, 2366, 2243, 1950, 1840	–	dre7	2 (10.0)
P. cryptogea	2557	3687, 2943, 2243, 1950, 1680, 1520, 1360, 1100, 936, 657	cry1	1 (12.5)
		3687, 2943, 2243, 1950, 1680, 1520, 1100, 850, 710, 657	cry2	1 (12.5)
		3687, 2943, 2243, 1680, 1520, 1100, 1031, 850, 710, 657, 595	cry3	1 (12.5)
		3687, 2943, 2243, 1680, 1520, 1360, 936, 657	cry4	3 (37.5)
		2943, 2243, 1950, 936, 710	cry5	1 (12.5)
		2943, 2243, 1680, 1520, 1360, 657, 591	cry6	1 (12.5)
P. capsici	2243, 1154, 881	2818, 1906, 1534	cap1	11 (55.0)
		2818, 1906, 1765, 1534	cap2	1 (5.0)
		3672, 2818, 1906, 1534	cap3	3 (15.0)
		1906, 1765, 1534	cap4	1 (5.0)
		2818	cap5	1 (5.0)
		2818, 1534	cap6	1 (5.0)
		1906, 1534	cap7	2 (10.0)
P. cactorum	2818, 1717, 1481, 1225, 974, 865, 769, 664	–	cac1	4 (80.0)
		1818	cac2	1 (20.0)
P. palmivora	966	–	pal1	1 (33.3)
		3529, 1049	pal2	1 (33.3)
		2659, 1550	pal3	1 (33.3)
P. nicotianae	1747, 1100, 933, 673	–	nic1	1 (7.1)
		1393	nic2	1 (7.1)
		1393, 725	nic3	1 (7.1)
		2625, 1393	nic4	6 (42.9)
		2625, 1994, 1393	nic5	1 (7.1)
		2879, 2565, 1588, 1482	nic6	1 (7.1)
		2565, 1482, 1373, 1184	nic7	1 (7.1)
		2625, 1510, 1393	nic8	1 (7.1)
		3422, 1393	nic9	1 (7.1)
		1510, 1393	nic10	1 (7.1)
		2625	nic11	1 (7.1)
P. citrophthora	3567, 3245, 2471, 2089, 1871, 1459, 1168, 724, 649	–	cit1	1 (33.3)
		1701	cit2	2 (66.6)
P. erythroseptica	1915, 1523	2363	ery1	1 (25.0)
		2883, 858	ery2	1 (25.0)
	3865, 3277, 2972, 2572, 2262, 1965, 1673, 1497, 1364, 1197, 1108, 919, 840, 623	–	ery3	2 (50.0)

Table 2. Continued

Organism	Amplified DNA fragments			
	Primary (bp)	Variable (bp)	Genotype	Frequency (%)
P. cambivora	1871, 1637	–	cam1	1 (50.0)
		1994, 1478	cam2	1 (50.0)
P. cinnamomi	1274	2270, 1179, 1094	cin1	1 (50.0)
		2965, 2204, 1788, 998	cin2	1 (50.0)
P. sojae	2555, 1925, 1053, 932, 843	1446, 1328	soj1	1 (50.0)
		233	soj2	1 (50.0)
P. boehmeriae	1737, 1441, 1205, 1000, 849, 487	–	–	–
P. citricola	4230, 3866, 3036, 1669, 1531, 862	–	–	–
P. megasperma	2767, 1390, 1183, 840, 695	–	–	–
P. infestans	3427, 2412, 2079, 1274, 749	–	–	–
P. melonis	3284, 2215, 1631, 1419, 816	–	–	–

Table 3. Distinguished observed band type of major bands revealed in several *Phytophthora* species

Species	Band size (bp)	Lane no.[a]																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
P. drechsleri	2243	×[b]	×	×	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
P. capsici	2818	○[c]	○	×	○	○	○	×	○	○	×	○	○	○	○	○	○	○	○	○	○
	1906	○	○	○	×	○	○	○	○	○	○	○	○	×	○	○	○	○	○	○	○
	1534	○	○	○	×	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
P. nicotianae	2625	×	○	×	○	×	○	○	○	○	○	×	×	○	○	–	–	–	–	–	–
	1393	○	○	×	○	○	○	○	○	○	○	○	○	×	○	–	–	–	–	–	–

[a]Contains lane No. all of A, B, C on FIG. 1. *P. drechsleri* (FIG. 1A) 1: KACC 40190; 2: KACC 40463; 3: KACC 40464. *P. capsici* (FIG. 1B) 3: KACC 40177; 4: KACC 40178; 7: KACC 40181; 10: KACC 40473; 12: KACC 40476. *P. nicotianae* (FIG. 1C) 1: KACC 40163; 3: KACC 40165; 5; KACC 40403; 11: KACC 40459; 12: KACC 40460; 13: KACC 40461.
[b]nonexistent band.
[c]existent band.

reciprocal relation among each *Phytophthora* spp. Accordingly, we have observed the same results by dendrogram using phylogenetic tree to investigate genetic relationship among species.

Genetic relationship analysis among *Phytophthora* genus. In this study, 85 of the total 90 isolates in 16 species of *Phytophthora* were classified into 13 groups (Fig. 2). Genetic relationship analyzed with the Windows version of GelCompar II (version 4.0; Applied Math, Kortrijk, Belgium) clustered preferentially a representative group of identical species and another group of similar band pattern. However, the same species group contains many isolates scattered in several other groups, of which the population includes numerous isolates classified into a representative group. For example, KACC 40161, an isolate of *P. cryptogea*, is visible in the *P. drechsleri* group (group 7), but many isolates of *P. cryptogea* are in group 8 and not included in group 7.

P. palmivora was classified into group 1 and *P. citrophthora* into group 2. *P. cactorum*, on the other hand, belonged to group 3 and *P. capsici* to group 4. In particular, *P. drechsleri* was observed in a variety of groups: three single groups (groups 5, 7, and 9) and one complex group each containing three isolates of *P. drechsleri* and an isolate of *P. cryptogea* (group 10). Group 5 contained 8 isolates, group 7 contained 7 isolates, and group 9 contained 2 isolates of *P. drechsleri*. *P. nicotianae* was classified into group 6. Group 8 included two isolates of *P. erythroseptica* and three isolates of *P. cryptogea*. *P. sojae* belonged to group 11 while *P. cambivora* belonged to group 12. Group 13 was composed of a complex group including two isolates of *P. cinnamomi* and each an isolate of *P. megasperma*, *P. boehmeriae*, *P. citricola*, and *P. infestans*.

The PCR-profile data were subjected to cluster analysis, and the dendrogram is shown in Fig. 2. From this, it can be seen that all strains were distinguishable from each other. The total cophenetic value of 90 isolates of *Phytophthora* species showed common homology of 85%. Cophenetic values (%) of isolates within each group are as follows: Three isolates of *P. palmivora* (group 1) showed 94% cophenetic value while that of three isolates of *P. citrophthora* (group 2) was 92%. Five isolates of *P. cactorum* (group 3) showed 82% cophenetic value. All of the 20 isolates of *P. capsici* (group 4) showed 84% cophe-

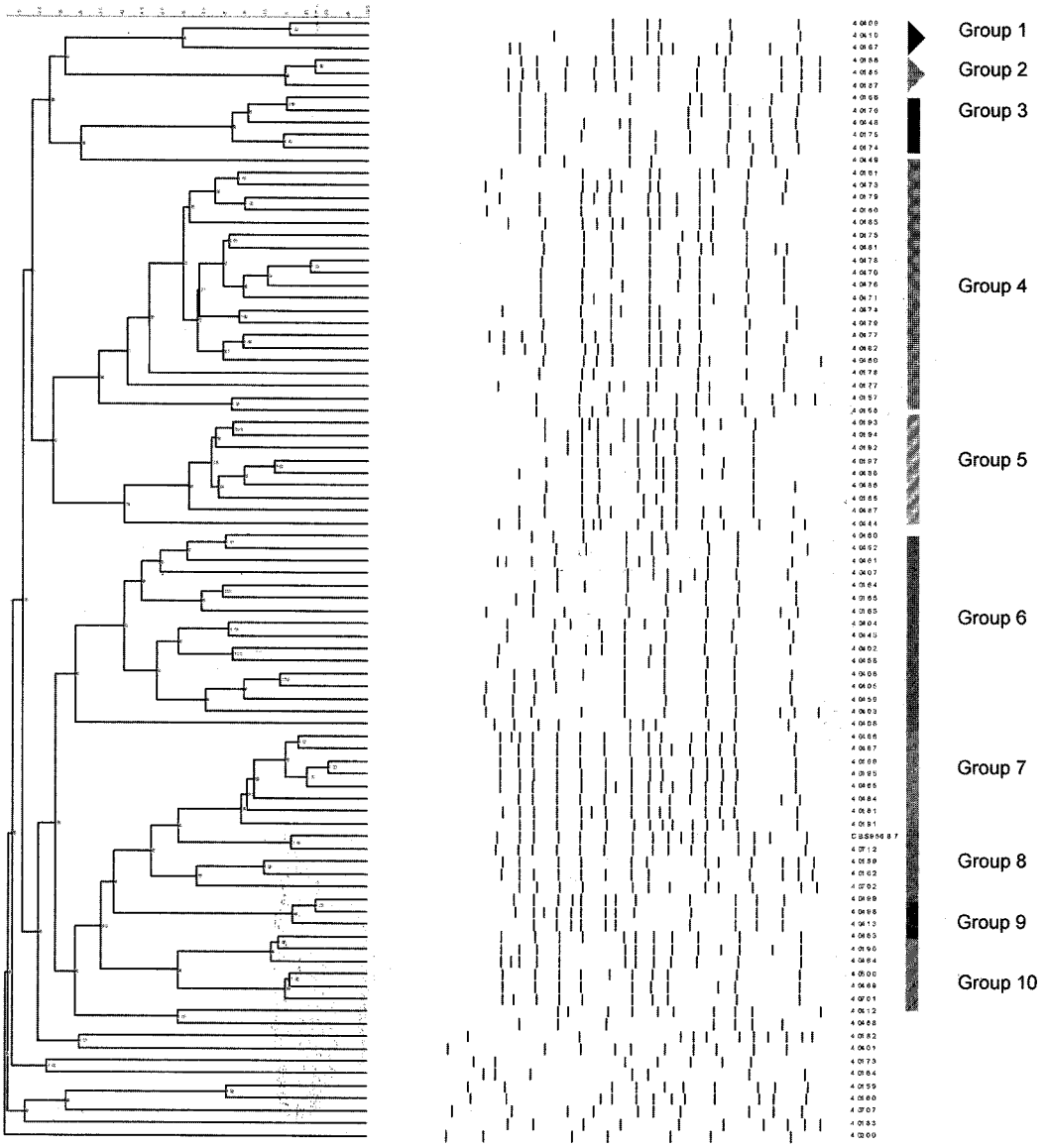

Fig. 2. UPGMA (unweighted pair group method with arithmetic means) dendrogram derived from URP-PCR DNA profiles of 90 *Phytophthora* isolates. Numbers on each clade represent cophenetic correlation values.

netic value. In three groups (groups 5, 7, and 9) composed of *P. drechsleri*, extremely different results occurred with group 5 showing the lowest at 61% cophenetic value in the entire groups as well as in the three groups. On the other hand, group 9 showed a high cophenetic value of 98% while that of group 7 was 84%. All of the 16 isolates of *P. nicotianae* (group 6), without exclusion, showed 76% cophenetic value. In the complex group 8, two isolates (CBS956.87 and KACC 40712) of *P. erythroseptica* showed a high homology of 100% cophenetic value while three isolates of *P. cryptogea* had 88% cophenetic value. The other *P. cryptogea* in the complex group 10 with *P. drechsleri* showed 90% cophenetic value. Both *P. sojae* (group 11) and *P. cambivora* (group 12) showed the highest homology of 100% cophenetic value among all the groups of *Phytophthora* spp. Cophenetic value between *P. cambivora* and *P. infestans* was high at 99%, while that with *P. cinnamomi* (KACC 40183) was 99%. For the cophenetic value (%) among each group, results between *P. palmivora* and *P. citrophthora* showed a cophenetic value of 99%, while that with *P. cactorum* was 96%. Between *P. capsici* and *P. drechsleri* of group 5, the cophenetic value was 92%.

In this study, two isolates (KACC 40409 and KACC 40167) of *P. palmivora*, two isolates (KACC 40174 and KACC 40176) of *P. cactorum*, two isolates (KACC 40159 and KACC 40160) of *P. cambivora*, two isolates (KACC 40157 and KACC 40177) of *P. capsici*, and two isolates (KACC 40468 and KACC 40412) of *P. sojae* were classified into the same group, respectively. Also, an isolate (KACC 40469) of *P. cryptogea* and an isolate (KACC 40463) of *P. drechsleri* (group 10 in this study) were clas-

sified into the same group. KACC 40449 and KACC 40200 of *P. erythroseptica* were classified into different groups, whereas, CBS956.87 and KACC 40712 of *P. erythroseptica* were classified into the same group.

Discussion

Primer URP3 used for identification and sub-typing of the 90 isolates of *Phytophthora* spp. provided a consistently unique and suitably defined polymorphic DNA fragments. Cluster analysis of *Phytophthora* spp. performed using the unweighted pair group method with arithmetic means (UPGMA) clustering technique (Sneath and Sokal, 1973) was useful in grouping the species.

In particular, *P. drechsleri* appeared to be more taxonomically confused and nonspecific than any other species of *Phytophthora*. In the case of *P. drechsleri* (lanes 19 and 20) and *P. cryptogea* (lane 21), most of their band patterns appeared to be equal (Fig. 1). This result suggests that even though the species are different, they seem to have the same genetic background. *P. drechsleri* is readily distinguished from other species in group 6 (Stamps *et al.*, 1990) but is considered in some reports to be co-specific with *P. cryptogea* (the other name) but different in other reports.

Also, *P. drechsleri* formed a variety of groups: three single groups (groups 5, 7 and 9) and one complex group each containing three isolates of *P. drechsleri* and an isolate of *P. cryptogea* (group 10). Differently from these results, however, Jee *et al.* (2000) classified them into two groups. Furthermore, Mills *et al.* (1991) concluded that the *P. cryptogea-P. drechsleri* complex was too diverse to warrant a merger of the two species. Therefore, molecular and taxonomical studies should be continued to determine whether *P. drechsleri* should be retained as a distinct species of *Phytphthora*. Furthermore, result of the genetic relationship analysis was consistent with the assertion of Hong *et al.* (2000) that *P. drechsleri* formed a complex group with *P. cryptogea* (Fig. 2). However, in this fingerprinting analysis, *P. drechsleri* was classified into several distinct clusters (Fig. 1A). Therefore, it is believed that it would be better to classify *P. drechsleri* among the four species.

In this study, two isolates (KACC 40409 and KACC 40167) of *P. palmivora*, two isolates (KACC 40174 and KACC 40176) of *P. cactorum*, two isolates (KACC 40159 and KACC 40160) of *P. cambivora*, two isolates (KACC 40157 and KACC 40177) of *P. capsici*, and two isolates (KACC 40468 and KACC 40412) of *P. sojae* were classified into the same group, respectively, consistent with the results of Hong *et al.* (2000).

Also, an isolate (KACC 40469) of *P. cryptogea* and an isolate (KACC 40463) of *P. drechsleri* (group 10 in this study) were classified into the same group supporting the results of Hong *et al.* (2000). However, two isolates (KACC 40449 and KACC 40200) of *P. erythroseptica* each classified into different groups differed from the findings of Hong *et al.* (2000) that KACC 40449 and KACC 40200 had a very close relationship. Also, two isolates (CBS956.87 and KACC 40712) of *P. erythroseptica* were classified into the same group similar with the results of Jee *et al.* (2000).

This study demonstrated the applicability of URP-PCR in differentiating taxonomically confused isolates within the genus *Phytophthora*. This method is fast, requiring only 2 days after the fungal cultures have produced sufficient mycelia for genomic DNA extraction. Because both PCR kits and URP primers are commercially available, the method is also convenient and can be performed in any laboratory with access to a thermal cycler. Most importantly, the accuracy of the results has been confirmed by band sizes of the reference positions on the sample gel. Thus, this report has described a molecular taxonomic approach for the identification of genus *Phytophthora* isolates.

From the results of this study, the classification of the *Phytophthora* isolates indicated adequately the genetic and pathogenic distinctions among this group. Therefore, this method proved to be a sensitive and highly reliable method for quickly identifying fungal pathotypes causing pathogenesis of *Phytophthora*.

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