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Potential Applications of Recombinant DNA Probes for Relatedness Analysis of *Fusarium oxysporum*

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*Fusarium oxysporum*의 유연관계 분석을 위한 Recombinant DNA의 Probe로서의 이용 가능성

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ABSTRACT : Randomly chosen recombinant clones of *Fusarium oxysporum* were analysed to select useful probes for relatedness analysis of *Fusarium oxysporum*. Genomic DNA of *F. oxysporum* f. sp. *cubense*, digested with *Hin*dIII, was ligated to pUC118 and used to transform *Escherichia coli* strain DH5α. Three clones were identified that hybridized to multiple restriction fragments of some formae speciales of *F. oxysporum*. These probes detected repetitive sequences in *Hin*dIII or *Eco*RI digested DNAs. Repeated copy clone pFC46, pFC52 and pFC54 showed evident polymorphisms among ten formae speciales of this fungus. Since clone pFC 52 strongly hybridized to multiple *Eco*RI-digested restriction fragments of f. sp. *cubense*, it may be useful as a probe for analysis of other genetic characteristics of this forma specialis. The results suggest that our clones might be very useful as probes for relatedness analysis between or within formae speciales of *Fusarium oxysporum*.

***Key words* : Recombinant clone, *Fusarium oxysporum*, probe, relatedness analysis, repetitive sequence.**

Fusarium oxysporum is a common plant pathogenic fungus with a worldwide distribution. As a species, it probably causes more economic damage to agricultural crops than any other plant pathogen (4). Strains of *F. oxysporum* have been devided into formae speciales on the basis of virulence on a particular host or group of hosts. However, the exact relationship among the various strains of this fungus within a forma specialis has been questioned (11). Furthermore a change from one forma specialis to another forma specialis has been reported (2).

DNA and genetic analysis of plant pathogenic fungi and its utilization in plant pathology were developed only recently after successful applications in bacteria or yeast. Earlier studies on this subject were only the comparison of isozyme patterns or the total DNA amount from fungi. In such studies it was impossible to characterize the nature of DNAs responsible for the genetic diversity and for the rapid variation of pathogenicity, and to perform genetic analysis between inter- or intraspecies (1, 3). Restriction Fragment Length Polymorphism (RFLP) analysis method was developed to improve such weak point. Since this technique was successfully used in polymorphism analysis of *Bremia lactucae* by Hulbert and his colleagues (8), it has been widely used in advanced studies related to molecular genetics for human, plants, and the other plant pathogenic fungi (11, 19, 20).

Development of polymorphic DNA probes is the most important and essential factors that for such molecular genetic analysis. Because good probes hold promise for solution several problems in molecular genetics. Repetitive copy clone among probes may have very wide applications in molecular genetics including inheritance (6), classification of pathogens (5, 13, 17), estimation of genetic ralationship between strains (9~11) and genetic diversity and variation (12). In addition we can use such probes in the detection of unique gene loci on the

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genome concerning molecular genetics in plant pathogens (14).

Because of the lack of information and materials related to the gene or inheritance of plant pathogens, it was difficult to study the advanced molecular genetics in plant pathology in Korea. Therefore, the purpose of our research was to make and select useful probes, and to demonstrate the possibility of its use in the genetic relatedness analysis of *Fusarium oxysporum* which is one of the most important plant pathogenic fungi.

MATERIALS AND METHODS

Fungal strains. Ten formae speciales of *Fusarium oxysporum* were examined in this study. Their sources are listed in Table 1. All cultures were maintained on potato dextrose agar slant at 4℃.

DNA isolation. The fungi used in this study were grown in potato dextrose broth in petri plates without shaking at 27℃ for 5 days. Mycelium was filtered through several layers of cheese cloth, placed in microcentrifuge tubes, and lyophilized at −40℃ . Lyophilized mycelium was ground to a powder with a sterile wooden stick. DNAs were extracted as described by Kistler *et al.* (11). Resolved DNA was stored at −20℃.

Cloning *Fusarium oxysporum* f. sp. *cubense* DNA. Total genomic DNA from one strain of *Fusarium oxysporum* f. sp. *cubense* was completely digested with *Hin*dIII, ligated into *Hin*dIII-digested pUC118 and transformed into *Escherichia coli* strain DH5α as described by Vieira and Messing (18). Cells were plated on LB ampicillin plates containing X-gal and 60 white, Ap^r colonies were selected arbitrarily. Plasmid DNAs were extracted by the alkali lysis method (16), digested with *Pvu*II, and run on a 0.7% agarose gel.

Selection of repetitive copy clones. To identify potential clones carrying repetitive DNAs, a Southern blot of genomic clones was probed with labelled total DNA from *Fusarium oxysporum* f. sp. *cubense* as the case of *Magnaporthe grisea* (6). It was reasoned that clones hybridizing more strongly to this probe would more likely contain repeated DNA sequences.

Southern hybridization. Transfer of DNAs to nylon membranes was by capillary action essentially as described previously (16). Probes of plasmids and genomic DNAs were made by priming the Klenow fragment of DNA polymerase I with random hexanucleotides in the presence of deoxynucleotides plus digoxigenin-conjugated dUTP (dig-dUTP). Hybridization and immunological detection of the dig-dUTP were as described by the manufacturer(Boehringer-Mannheim).

RESULTS

To select repetitive copy clones that are the most useful materials for RFLP, fingerprint, relatedness and genetic analysis, a library of *Hin*dIII-digested

Table 1. Formae speciales of *Fusarium oxysporum* used for relatedness analysis in this study

Formae speciales of *F. oxysporum*	Host plants	Sources
asparagi	*Asparagus officinalis*	Yu and Ogoshi[a]
cepae	*Allium cepa*	Yu and Ogoshi
cubense	*Musa sapientum*	H. C. Kistler[b]
cucumerinum	*Cucumis sativus*	A.S.I.[c]
dianthi	*Dianthus elatier*	C.N.U.[d]
fragariae	*Fragaria ananassa*	A.S.I.
garlic	*Allium sativum*	Yu and Ogoshi
lilii	*Lilium longiflorum*	C.N.U.
lycopersici	*Lycopersicon esculentum*	C.N.U.
raphani	*Raphanus sativus*	H. C. Kistler

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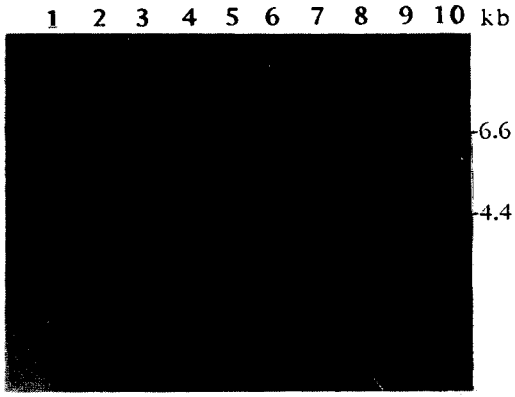

Fig. 1. Southern hybridization of clone pFC19 to genomic DNAs from 10 formae speciales of *Fusarium oxysporum*. DNA was digested with *Hin*dIII. Probe did not hybridized to f. sp. *raphani* and detected two bands to f. sp. *cubense*. The lanes contain DNA from *F. oxysporum* f. sp.: 1; *raphani*, 2; *lycopersici*, 3; *lilii*, 4; *garlic*, 5; *fragariae*, 6; *dianthi*, 7; *cucumerinum*, 8; *cubense*, 9; *cepae*, 10; *asparagi*.

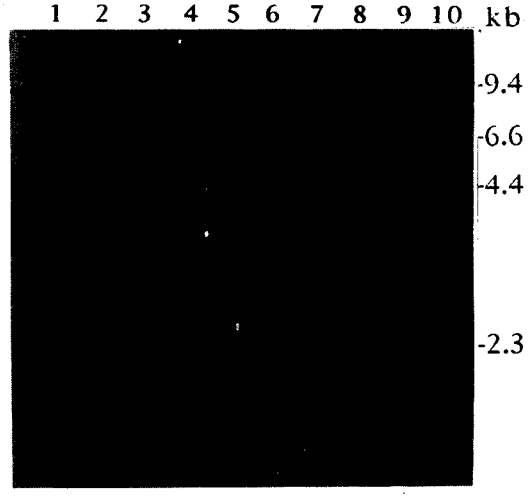

Fig. 2. Southern hybridization of clone pFC46 to genomic DNAs from 10 formae speciales of *Fusarium oxysporum*. DNA was digested with *Hin*dIII. Probe hybridized to multiple restriction fragments of ff. sp. *raphani*, *lycopersici* and *cepae* but no fragments in f. sp. *lilii*. The designations of strains are the same as Fig. 1.

DNA from *Fusarium oxysporum* f. sp. *cubense* was made in pUC118. Genomic DNA from this strain was labelled and used as a probe for Southern hybridization of 60 arbitrarily chosen genomic clones. In general, clones contain repetitive DNA sequences would hybridize more strongly to this probe. This method was use to select repetitive DNAs in *Magnaporthe grisea* (6). Seven clones which might contain a repetitive sequence were chosen among 60 random recombinant clones. Plasmid DNA of these seven clones was isolated, digested with *Pvu*II which cut the plasmids at position flanking the polylinker cloning site, and labelled. These seven plasmid DNAs were used as probes on *Hin*dIII-digested genomic DNAs of *Fusarium oxysporum*. Four of the seven clones examined in genomic Southerns hybridized primarily to one or two bands in all formae speciales. Clone pFC19 hybridized to only one or two size of restriction fragment digested with *Hin*dIII but failed to hybridize to DNA from f. sp. *raphani* (Fig. 1). Clone pFC 2, pFC 9 and pFC 23 that were single copy clone, also hybridized only with restriction fragments of one or two size (data not shown). Distinct genetic difference between formae speciales was not detected by these clones as probes.

Three clones, pFC46, pFC52 and pFC54 chosen by this method as strongly hybridizing clones, did detect repetitive sequences in several formae speciales. As shown in the Southern blots depicted in Fig. 2, probe pFC 46 hybridized 15 to 25 bands in DNA of *F. oxysporum* ff. sp. *cepae*, *cubense*, *lycopersici* and *raphani*. But the band pattern was very different between each formae speciales. Some other formae speciales including *fragariae* and *garlic*, also produced several bands, whereas no or a little hybridization was seen in the lane of ff. sp. *lilii* and *asparagi*.

Probe pFC 52 also hybridized to multiple *Hin*dIII restriction fragments in ff. sp. *raphani* and *garlic* as well as *cubense* which was hybridized most intensely (Fig. 3, A). In other formae speciales hybridization was weak and showed a few bands. Although this probe also hybridized several fragments digested by *Eco*RI, the band pattern was slightly different (Fig. 3, B). It produced also several bands with the DNAs from ff. sp. *lycopersici* and *cepae*, but this clone hybridized neither to DNA from *lilii* nor to DNA from the *dianthi*. However, there were about 40 strong bands on DNA from f. sp. *cubense* which is the source of an insert DNA in gene cloning.

When probe pFC 54 was used, further polymorphisms between *F. oxysporum* formae speciales were evident (Fig. 4). pFC54 hybridized very much to get possibility for genetic differentiation in DNA from

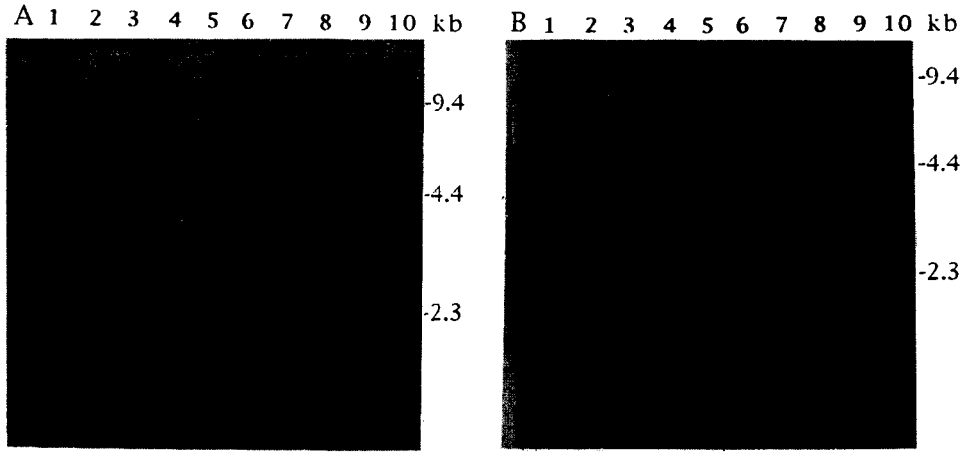

Fig. 3. Hybridization patterns of clone pFC52 to genomic DNAs from 10 formae speciales of *Fusarium oxysporum*. DNA was digested with *Hin*dIII (A) and *Eco*RI (B). Probe hybridized to several bands in DNA of ff. sp. *raphani, garlic, cubense* but a little bands in ff. sp. *lilii* and *dianthi*. The designations of strains are the same as Fig. 1.

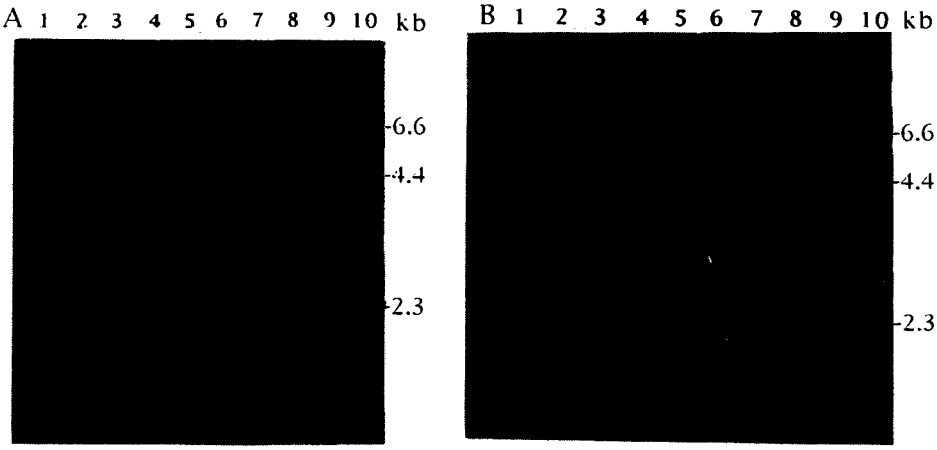

Fig. 4. Hybridization patterns of clone pFC54 to genomic DNAs from 10 formae speciales of *Fusarium oxysporum*. DNA was digested with *Hin*dIII (A) and *Eco*RI (B). Probe hybridized to multiple restriction fragments of ff. sp. *raphani, garlic, cubense* and *asparagi*. The designations of strains are the same as Fig. 1.

some, but not all. Weak hybridization was seen to *cucumerinum, dianthi* and *lilii*. In this case, probe pFC 54 distinguished each formae speciales, and multiple restriction fragment bands were observed at least in ff. sp. *lycopersici, garlic, cubense* and *cepae*. No hybridization was not observed in the lane of all formae speciales of *F. oxysporum* tested.

DISCUSSION

DNA probes are increasingly being used to identify plant pathogens (7, 15, 17) and analyse genetic similarity (9, 11) by hybridization assay and restriction fragment length polymorphisms (RFLPs) analysis of DNA. We have hybridized arbitrarily chosen genomic clones of *Fusarium oxysporum* f. sp. *cubense* to select useful probes for relatedness analysis of *F. oxysporum*. Three clones were identified to contain a repetitive sequence in DNA from ten formae speciales of *F. oxysporum* tested in this study. The repetitive DNAs selected have been used to infer feasibility as probes for relatedness analysis. Clone pFC 46, pFC 52 and pFC 54 had repetitive sequences in Southern blots of *Hin*dIII- and *Eco*RI-diges-

ted DNAs from several formae speciales of *F. oxysporum*. However, their band patterns distinctly differed to formae speciales. These hybridized to 15~40 fragments of ff. sp. *lycopersici, garlic, raphani* and *cepae* including *cubense* which used as an insert DNA for gene cloning, and no or very little hybridized to ff. sp. *lilii* and *asparagi*. These probes should be very effectively used to infer the phylogenetic relationship and used for molecular genetic analysis between, inter- and intra formae speciales of *Fusarium oxysporum*. Because the identification of repetitive DNA, which has restriction endonuclease fragments of particular lengths has provided a good tools for molecular genetics. When used repetitive sequences as probes in DNA hybridization analysis of restriction endonuclease-digested DNAs from various strains of *Fusarium oxysporum*, these probes identified distinctive banding patterns for each strain (11). Especially based on the result which pFC 52 hybridized to f. sp. *cubense* very strongly and numerously, it may be a probe to make fingerprint. This result indicates that DNA from strains which was the source of an insert DNA in gene cloning had a greater number bands and strong intensity in hybridization than DNA from strain of other formae speciales.

As *F. oxysporum* ff. sp. *garlic, lycopersici*, and *raphani* as well as *cubense* have 15~35 bands by our probes, it might be possible to analyse of RFLP and relatedness between strains of the same forma specialis. Furthermore, we are able to detect an ancestor strain of this fungus by using their results.

요 약

Fusarium oxysporum 분화형들의 유연관계 분석에 유용한 probe를 선발하고자 *F. oxysporum* f. sp. *cubense*의 genomic DNA를 *Hin*dIII로 자른 다음 pUC 118에 ligation시켰다. 이를 *Escherichia coli* strain DH 5α에 형질전환 시켜 얻은 임의 선발된 유전자 재조합 clone들의 특성을 분석하였다. 이들 clone중에 *F. oxysporum*의 몇몇 분화형에 다수 hybridization하는 세 개의 repeated copy clone이 선발되었다. 이들은 *Hin*dIII나 *Eco*RI으로 처리된 blots에서 repetitive sequence가 존재함을 밝혀냈다. Repeated copy clone pFC 46, pFC52와 pFC54는 10종의 분화형간에 분명한 polymorphism을 찾아냈으며 그들의 band pattern은 분화형에 따라 매우 달랐다. 특히 clone pFC52는 *F. oxysporum* f. sp. *cubense*에서 많은 수의 *Eco*RI restriction fragment와 강하게 hybridization하므로서 이 분화형의 다른 유전 특성 연구용 probe로서의 가능성을 보유하고 있었다. 이 결과는 이 repeated copy clone들이 probe로서 *Fusarium oxysporum*의 분화형간 또는 분화형내의 여러 가지 유연관계 분석시 매우 유용하리라는 점을 증명하고 있다.

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