Electrophoretic karyotyping and gene mapping of seven formae speciales in *Fusarium solani*

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Abstract

Chromosomal DNAs of 22 strains in seven formae speciales of *Fusarium solani* were compared by pulsed field gel electrophoresis (PFGE) and gene mapping on the chromosomes. Using PFGE, complete separation of full components of the genome was not attained due to the limited resolution of large chromosomes, but 5 to 12 chromosomes with their sizes between 0.6 and 5.7 Mbp were resolvable for every strain. Although each strain had a unique banding profile, similarity in the banding profile was noticed among strains of the same forma specialis (f. sp.). In gene mapping, ribosomal RNA gene (rDNA) and putative pathogenesis-related genes encoding kievitone hydratase (*khs*), pisatin demethylase (*pda*), and pectate degrading enzyme (*pelA*), were located on the chromosomes separated by PFGE. rDNA was always detected on the stacked large bands of 5.2-5.7 Mbp. The *khs* was detected on a chromosome of 2.8-5.4 Mbp in all of the f. sp. *phaseoli* strains and one strain of f. sp. *pisi*. The *pda* was detected on a 1.4-5.6 Mbp chromosome in f. sp. *pisi* and *pelA* was localized on a 2.3-2.9 Mbp chromosome in f. spp. *pisi*, *xanthoxyli*, *batatas* and *mori*. Results of PFGE and Southern blot hybridization supported the idea that each f. sp. of *F. solani* (or mating population of teleomorph *Nectria haematococca*) has a distinctive genome organization, as previously inferred from molecular phylogenetic analyses.

Key words Electrophoretic karyotype・*Fusarium solani*・forma specialis・gene mapping

Introduction

Fusarium solani (Mart.) Appel and Wollenweber in section *Martiella* of the genus *Fusarium* is a ubiquitous soil-inhabiting fungus with worldwide. This species comprises phytopathogenic and saprophytic strains, of which the former can be further divided into groups, each with a distinctive host range. Traditionally, each of these groups based on the plant pathogenicity is treated as an infraspecific taxon called forma specialis (f. sp.). So far, eleven formae speciales (f. spp.) have been described in the species (Snyder and Hansen 1941; Sakurai and Matuo 1959; Matuo and Sakurai 1965; McClure 1951; Sakurai and Matuo 1961; Roy 1997). Of these formae speciales, seven are heterothallic with the common ascomycetous sexual stage of *Nectria haematococca* Berk. et Br. which belongs to the Hypocreales. Mating to produce perithecia occurs only within members of the same forma specialis, indicating that each forma specialis represents a single mating population (MP) (Matuo and Snyder 1973). On the other hand, four formae speciales have no known sexual stage.

Although *F. solani* was defined as a species based on the morphology of conidial state (Snyder and Hansen 1941), the presence of these MPs as well as the diversity of many other traits in the species suggests that this species is a biological species complex (VanEtten and Kistler 1988). This view was supported by the recent molecular phylogenetic analyses using nucleotide sequences of the specific genomic regions such as internal transcribed spacer region (ITS) of nuclear ribosomal RNA genes cluster (rDNA) and genes encoding translation elongation factor (O'Donnell and Gray 1995; Suga et al. 2000; O'Donnell 2000).

Given that *F. solani* is a biological species complex, it is intriguing to know how this status is reflected in the karyotype. In this paper, we

attempted to clarify the variation of karyotype in different forma specialis and MPs in *F. solani*, each of which is thought to represent a distinct biological species. Since comparison of the fungal chromosomes is difficult by conventional microscopic observation, we used pulsed-field gel electrophoresis (PFGE) for karyotyping. Chromosomes separated by PFGE were subjected to the subsequent gene mapping. For *F. solani*, karyotype analyses using PFGE have been limited to f. sp. *pisi* (*N. haematococca* MP VI) (Miao et al. 1991; Kim et al. 1995) and a few other strains with unknown pathogenicity (Nazareth and Bruschi 1994; Taga et al. 1998). To our knowledge, this is the first report of karyotype analyses of *F. solani* reflecting the extensive diversity within the species complex.

Materials and method

Fungal strains

Twenty-two strains of the seven formae speciales in *F. solani* were used in this study (Table 1). Strains except for f. sp. *piperis* MAFF236558 and MAFF236572 which were isolated from Pepper in Brazil, were isolated from each host plant in Japan. Strains of SUF (Culture Collection of *Fusarium* in Shinshu University, Japan) were listed in Bulletin of the Japan Federation for Culture Collections (1987). Each strain originated from a single conidium and was cultured on potato-dextrose agar (PDA). All strains were stored in the solution (1.5 % Sodium glutamate, 10 % Skim milk) at - 80 ℃.

Protoplasting and pulsed-field gel electrophoresis

Potato-dextrose broth (PDB) (Difco, Becton Dickinson and company,Sparks USA) inoculated with tiny PDA blocks of fungal culture

were reciprocally shaken for 2 days at 25 ℃ to produce budding spores. The spores were harvested by centrifugation after filtration through KimWipes (Kimberly-Clark Corp., Tokyo, Japan) and then were resuspended in PDB at a final concentration of 5.0×10^5/ml. The suspension was incubated at 25 ℃ with gentle shaking until the length of germ tubes reached to 2-3 times of the size of the budding spores. The germlings were harvested by filtration and washed with 1.2 M $MgSO_4$. For protoplasting, germlings were suspended in an enzyme solution [Novozym 234 (20 mg/ml) (Calbiochem-Novabiochem Co., Sandiego, USA), Cellulase Onozuka RS (10 mg/ml) (Yakult, Tokyo, Japan), Zymolyase 20T (10 mg/ml) (Seikagaku Kogyo, Tokyo, Japan), β-Glucuronidase Type H1 (10 mg/ml) (Sigma, St. Louis, USA), Driselase (10 mg/ml) (Kyowa Hakko Kogyo, Tokyo, Japan), a small amount of chitinase (Sigma) in 1.2 M $MgSO_4$] and agitated gently at 30 ℃ for 4-6 h. After filtration through four layers of KimWipes, protoplasts were harvested by centrifugation at 700×g for 10 min and then washed three times with 1.2 M NaCl. The final protoplast pellet was resuspended in SE (1 M sorbitol, 50 mM EDTA, pH 8.0) at a concentration of more than 2.0×10^8 cells per milliliter. Protoplast suspensions were mixed with equal volume of 1 % low-melting agarose in SE at 50 ℃ and molded into plugs. The plugs were soaked in a small volume of NDS buffer (0.5 M EDTA, 1 % *N*-lauroylsarcosine, 10 mM Tris-HCl, pH 8.0) and incubated for more than 14 h at 37 ℃. The plugs were rinsed briefly three times in 50 mM EDTA (pH 8.0) and stored in this solution at 4 ℃.

Pulsed-field gel electrophoresis

Chromosome separations were performed at 8 ℃ by contour-clamped homogeneous electric field (CHEF) electrophoresis with the CHEF-DRII

apparatus (Bio-Rad, Hercules, USA). The conditions to resolve large chromosomes were a 3600-1800 s swich time for 115 h at 50 V, a 1800-1300 s for 24 h at 50 V, a 1300-800 s for 30 h at 60 V and a 800-600 s for 27 h at 80 V with a 0.8 % Sea Kem Gold agarose gel (FMC BioProducts, Rockland, USA). The conditions for resolving small chromosomes were a switch time of 120 s for 13 h and then 180 s for 13 h at 180 V with a 0.8 % Sea Kem Gold agarose gel (Morales et al. 1993). The running buffer of 0.5× TBE (44.5 mM Tris, 44.5 mM boric acid, 1 mM EDTA) was replaced daily (Sambrook et al. 1989). Chromosomes of *Schizosaccharomyces pombe* strain 972 h (Bio-Rad) and *Saccharomyces cerevisiae strain* YNN295 (Bio-Rad) were used as size standards for 3.5-5.7 Mbp and 0.3-2.2 Mbp DNA, respectively. After the run, gels were stained for 1 h in 5 μg/ml ethidium bromide and then destained for 1 h in water. DNA bands were photographed by Imagemaster VDS (Amersham Pharmacia Biotech, Uppsala, Sweden). Molecular sizes of the chromosomal DNAs were estimated with reference to the size standards.

Polymerase chain reaction and Probe preparation

Genomic DNA used as template for polymerase chain reaction (PCR) was extracted as described before (Suga et al. 2000). PCR to amplify the internal transcribed spacer (ITS) region of rDNA was performed according to Suga et al. (2000) and PCR for other genes were performed with the following modifications: the primer set for kievitone hydratase gene *khs* (Li et al. 1995) were khs-1 (5'-AAGCATCCGAAACAGTACT-3') and khs-2 (5'-TCAAGGATGCTGCTAAGCTG-3') and the thermal cycle was 94 ℃ for 2 min, followed by 30 cycles of 94 ℃ for 1 min, 53 ℃ for 1 min, 72 ℃ for 1 min, and a final extension at 72 ℃ for 8 min; the primer set for the pisatin demethylase gene *pda* (Maloney and VanEtten 1994) were

pdaT9-5 (5'-AGCGAGCTCCAAGCCAAACTTGGCTGGGCT-3') and pdaT9-3 (5'-TTTGAGCTCAACACTTGAGGCAGACAGATT-3') and the thermal cycle was 94 ℃ for 2 min, followed by 30 cycles of 94 ℃ for 1 min, 55 ℃ for 1 min, 72 ℃ for 2 min, and a final extension at 72 ℃ for 9 min; the primer set for pectate degrading enzyme gene *pelA* (Gonzalez-Candelas and Kolattukudy 1992) were pelA-5 (5'-ATCGAATTCATGAAGTTCACTGCTGCTTTC-3') and pelA-3 (5'-ACGGAATTCGCAGCTCGTGGTGGAGCCAGT-3') and the thermal cycle was same as *pda*.

DNA fragments used as probes for gene mapping analysis were prepared by PCR amplification. A portion of *khs* and the ITS region of rDNA were obtained from strain S-3 of f. sp. *phaseoli* and a portion of *pda* and *pelA* were obtained from strain SS-1 of f. sp. *pisi* (MP VI). Nucleotide sequences of the amplified fragments were checked as described by Suga et al. (2000) using primer sets mentioned above.

Probes were labelled with a DIG DNA Labeling Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's recommendation.

Southern hybridization

Chromosomal DNAs separated by PFGE were depurinated in 0.25 M HCl for 15 min followed by denaturation in 0.5 M NaOH for 30 min. Then, they were vacuum-blotted onto a HybondN$^+$ membrane (Amersham Pharmacia Biotech) in 10× SSC (0.165 M Na_3 citrate, 1.65 M NaCl, pH 7.0) with the Model 785 Vacuum Blotter (Bio-Rad) for 90 min at 5 inches Hg. After prehybridization treatment, hybridizations were carried out in the hybridization buffer (50 ng/ml Dig-labeled probe, 0.25 M Na_2HPO_4 pH 7.2, 1 mM EDTA, 7 % SDS) at 65 ℃ overnight. After the hybridization, the membrane was washed three times at 65 ℃ for 20 min in the wash

solution (0.02 M Na_2HPO_4, 1 % SDS). Detection of hybridization was done with DIG Luminescent Detection Kit (Roche Diagnostics, Basel Switzerland) according to the manufacturer's instructions. Chemiluminescence signals were captured by Lumino-CCD (ATTO) using CDP-star (Roche Diagnostics).

Results

Chromosome-length polymorphism

Twenty-two strains of seven formae speciales (f. spp.) of *F. solani* were analyzed for their karyotypes by PFGE using two different running conditions. Repeated sample preparations showed that banding profile was reproducible. The result for f. spp. *phaseoli* and *pisi* (*N. haematococca* MP VI) with the running conditions to separate small chromosomes were shown in Fig. 1. The results for f. spp. *phaseoli*, *pisi* (MP VI), *xanthoxyli* (MP IV), *mori* (MP III), *batatas* (MP II), *robiniae* (MP VII) and *piperis* with the running condition to separate large chromosomes were shown in Fig. 2. Full resolution of large chromosomes exceeding 5.7 Mbp was difficult because such large chromosomes were compressed together in a high molecular weight band. With the running condition for small chromosomes, chromosomes ranging 0.6 to 2.0 Mbp were clearly resolved (Fig. 1). The combined results are summarized in Fig. 3 and Table 2. Among formae speciales, f. sp. *pisi* has the highest number of resolvable chromosome bands, from 9-12, f. sp. *xanthoxyli* has the lowest, either 5 or 6 and other f. spp. have intermediate numbers, from 6-8 (Fig. 3, Table 2). Of these chromosomal bands, some were thought to be co-migrating chromosomal DNAs based on their brighter fluorescence in ethidium bromide staining and these were shown as thick

bands in Fig. 3. Therefore, together with the upper limitation of resolution for large chromosomes, these numbers of bands represent a minimum estimate of chromosome number for each strain. With regard to the banding profile, each strain had different banding profile even in the same f. sp. (Fig. 3). Nevertheless, similarity in the banding profile was noticeable among strains in f. spp. *xanthoxyli* (MP IV), *piperis*, *mori* (MP III) and *batatas* (MP II) (Fig. 3).

Gene mapping on the chromosomes

rDNA and three putative pathogenesis-related (PPR) genes encoding kievitone hydratase (*khs*), pisatin demethylase (*pda*) and pectate lyase (*pelA*) were mapped on the chromosomes separated by PFGE. For PPR genes, strains to be subjected to Southern blot hybridization chosen based on the results of PCR amplifications (Table 2). Accordingly, as expected, *khs* and *pda* (or sequences homologous to these genes) were detected in all strains of f. sp. *phaseoli* and f. sp. *pisi* (MP VI), respectively. With the exception of one strain of f. sp. *pisi* (MP VI) in which *khs* was amplified, each of the two genes was not detectable in other formae speciales. In contast, existence of *pelA* was not specific to a certain forma specialis, but rather detected in f. spp. *pisi* (MP VI), *xanthoxyli* (MP IV), *mori* (MP III) and *batatas* (MP II).

The rDNA-ITS probe hybridized to a stacked large band (5.2-5.7Mbp) in Southerns for all 22 strains tested (Fig. 2, 3, Table 2). The khs probe hybridized to a single chromosome of 2.8-3.4 Mbp for six strains of f. sp. *phaseoli* and one strain of f. sp. *pisi* (MP VI). Although f. sp. *phaseoli* strain F00701-2 has chromosomes in this size range, the khs hybridizing signal for this strain was detected on a larger 5.4 Mbp sized-band (Fig. 3,

Table 2). The pda probe hybridized a single chromosome of 3.3-5.6 Mbp for three strains of f. sp. *pisi* (MP VI), while hybridizing to two smaller chromosomes (1.1 and 1.4 Mbp) in strain SS-1 (Fig. 2D, 3, Table 2). The pda probe was produced from strain SS-1 and direct sequencing suggested that the probe contained mainly the *pda*T9 gene and also small amount of another *pda* which has different nucleotide sequence from *pda*T9 (data not shown). Consequently, strain SS-1 seems to have two *pda* loci on different chromosomes. The pelA probe hybridized to a single chromosome of similar size (2.3-2.9 Mbp) in f. spp. *pisi* (MP VI) (Table 2), *xanthoxyli* (MP IV) (Fig. 2F, Table 2), *batatas* (MP II) (Table 2) and *mori* (MP III) (Fig. 2H, Table 2).

Discussion

In this study, 5-12 chromosomes with sizes between 0.6 - 5.7 Mbp were resolved by PFGE for strains from seven f. spp. of *F. solani* (Table 2). Although there is a high variation for the number of chromosome bands among f. spp., the variation within same f. spp. is smaller. The number of chromosomes of f. sp. *pisi* (*N. haematococca* MP VI) in this study is similar to previous reports, in which ten to sixteen chromosomes have been resolved on *N. haematococca* MP VI (Miao et al. 1991; Kim et al. 1995). However, we can not completely determine the accurate chromosome number from the results of PFGE. In f. sp. *xanthoxyli* (MP IV), the number of chromosomes separated by PFGE were quite different from the results obtained by microscopic observation, which showed more than ten chromosomes (Taga et al., unpublished data). PFGE can neither resolve large chromosomes nor co-migrating chromosomes of equal size (Miao et al. 1991; Taga et al. 1998; Zolan 1995). Direct observation by light microscopy (Taga et al. 1998), Southern blot hybridization using

probe of telomere sequence (Zolan 1995) or analysis of genetic linkage groups (Tzeng et al. 1992) would be useful for the accurate determination of chromosome number. Consequently in this study, we could only determine the minimum number of the chromosomes for each strain. Nevertheless, PFGE is useful for separation of chromosomes with sizes smaller than 5.7 Mbp.

Chromosome-length polymorphism (CLP) within forma specialis of *F. solani* was revealed in this study. CLP was present even within f. sp. *phaseoli*, in which a sexual stage has not been detected (Fig. 3). CLP has been reported within the asexual fungus *F. oxysporum* f. sp. *cubense* (Boehm et al. 1994). Chromosomal aberrations resulting in CLP, may be generated by mitotic processes (Kistler and Miao 1992; Zolan 1995). It was proposed that asexual fungi may have a high degree of CLP because chromosomal aberrations are more likely to persist in the absence of selection for homologous chromosomes during meiosis (Kistler and Miao 1992). In that regard, it is interesting that the asexual f. sp. *phaseoli* appears to have a higher degree of CLP than the sexual mating populations IV (f. sp. *xanthoxyli*), III (f. sp. *mori*), II (f. sp. *batatas*) and VII (f. sp. *robiniae*) (Fig. 3). Each sexual mating population of *Gibberella fujikuroi* also has a low degree of CLP (Xu et al. 1995). Although the frequency of the sexual stage of *F. solani* in nature is unknown, meiosis could explain the low degree of CLP within a mating population.

The forma specialis naming system is misleading for f. sp. *pisi* mainly because all strains of MP VI have been identified as f. sp. *pisi* in spite of the fact that MP VI includes strains with hosts other than pea (VanEtten and Kistler 1988). The host of strain SS-1 is pea but the host of the other three strains is mulberry or zelkova; nevertheless, the latter have been designated f. sp. *pisi* because they belong to MP VI as do authentic f. sp. *pisi* strains (Table 1). The gene *pda* (or a sequence homologous to *pda*)

was detected on 3.3-5.6 Mbp, single chromosome in these three strains, while it was detected on two smaller chromosomes (1.1 and 1.4 Mbp) in strain SS-1 (Fig. 2D, 3, Table 2). Recently a cluster of pea pathogenicity genes (PEP), including *pda,* that confers full pathogenicity to pea was found on small chromosome (1.6Mbp) of *Nectria haematococca* MPVI (Han et al. 2001). From the results of pathogenicity to pea and the size similarity of chromosomes carrying *pda*, it is suggested that strain SS-1 would have the PEP gene cluster on a 1.1 and/or a 1.4 Mbp chromosome.

This study showed that most strains of f. sp. *phaseoli* have *khs* on similar sized-chromosomes (2.8 to 3.4 Mbp), except for strain F00702-1 which has it on a larger chromosome (5.4 Mbp) (Fig. 2B, 3, Table 2). This might be the result of a chromosome translocation. In addition to the strains of f. sp. *phaseoli*, *khs* homology was detected on strain SS-1 of f. sp. *pisi* (MP VI) and it was located on a similar sized-chromosome (3.2 Mbp) to those of f. sp. *phaseoli* (Fig. 2B, 3, Table 2). There is a previous report that some strains of *N. haematococca* MP VI have *khs* homology (Li et al. 1995). It is strange why some strains of MP VI have *khs* homology, since f. sp. *phaseoli* and MP VI are somewhat distantly related based on molecular phylogeny studies (Suga et al. 2000; O'Donnell 2000). One possibility is that the chromosome or chromosomal segment containing *khs* homology has been lost in most strains of MP VI. Another possibility is that the presence of *khs* in *N. haematococca* MP VI may be attributable to lateral transfer. Han et al (2001) suggested that the PEP gene cluster of MP VI or the supernumerary chromosome containing the cluster might be laterally transferred. It would be interesting to determine whether *khs* homology, or a chromosome containing it, could be laterally transferred from f.sp. *phaseoli* to MP VI.

Using the same primers, PCR amplification of *pelA* was successful for *F. solani* f. spp. *pisi* (MP VI), *xanthoxyli* (MP IV), *batatas* (MP II), and *mori*

(MP III) which are relatively closely related based on molecular phylogeny (Table 2) (Suga et al. 2000; O'Donnell 2000) and the *pelA* homology was detected on similar sized chromosomes (2.3-2.9 Mbp) (Fig. 2F, 2H, 3, Table 2). This suggests that these f. spp. (or MPs) might share a common ancestor that is the origin of this gene.

In this study, we investigated genome organization of *F. solani* by comparison of chromosomes from strains of different formae speciales (or MPs). Results of this study support the contention that each forma specialis (or MP) has a distinct genome organization, that may be inferred by molecular phylogenetic analyses (Suga et al. 2000; O'Donnell 2000). Chromosomal analysis is a cumbersome task and would be impractical to apply to a large number of samples compared to molecular phylogenetic analysis. However, further investigation of how fungal genetic information is partitioned into chromosomes will advance our understanding of the genome organization and overall evolution of *F. solani*.

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Figure legend

Fig. 1

Chromosome separation of small chromosomes of *Fusarium solani* f. spp. *phaseoli* and *pisi*.

F. solani f. sp. *phaseoli* S-3 (lane 1) and f. sp. *pisi* SS-1 (lane 2) strains are shown. Pulsed field gel electrophoresis (PFGE) was carried out using the following conditions: a switch time of 120 s for 13 h and then 180 s for 13 h at 180 V using 0.8 % SeaKem Gold agarose (FMC BioProducts). The running buffer, 0.5× TBE (44.5 mM Tris, 44.5 mM borate and 1 mM EDTA) was held at 8 ℃. The gels were stained for 1 h in 5 μg/ml ethidium bromide and then destained for 1 h in water. Molecular sizes were shown on the left column.

Fig. 2

Chromosome separation of *Fusarium solani* and gene mapping by Southern blot hybridization

Pulsed field gel electrophoresis (PFGE) was carried out using the following conditions: a 3600-1800 s switch time for 115 h at 50 V, 1800-1300 s for 24 h at 50 V, 1300-800 s for 30 h at 60 V and 800-600 s for 27 h at 80 V using 0.8 % SeaKem Gold agarose (FMC BioProducts). The running buffer, 0.5× TBE (44.5 mM Tris, 44.5 mM borate and 1 mM EDTA) was held at 8 ℃ and replaced daily. The gels were stained for 1 h in 5 μg/ml ethidium bromide and then destained for 1 h in water. Molecular sizes were shown on the left column. DNA separated by PFGE was transferred onto a membrane and Southern blot hybridization was carried out using each probe. Arrows indicate the molecular size of hybridizing signals. *F. solani* f. sp. *phaseoli* S-3 (lane 1) and f. sp. *pisi* SS-1 (lane 2) strains are shown (A) and hybridizations were carried out using probes for *khs* from strain S-3 of f. sp. *phaseoli* (B). *F. solani* f. sp.

pisi strains SS-1 (lane 1), SUF305 (lane 2), and MAFF840047 (lane 3) are shown (C) and hybridizations were carried out using probes for *pda* from strain SS-1 of f. sp. *pisi* (D). *F. solani* f. sp. *xanthoxyli* strains SUF XV-1 (lane 1), and SUF XV-23 (lane 2) are shown (E) and hybridizations were carried out using probes for *pelA* from strain SS-1 of *F. solani* f. sp. *pisi* (F). *F. solani* f. sp. *mori* strain SUF235 (lane 1) (G) and hybridizations were carried out using probes for *pelA* from strain SS-1 of *F. solani* f. sp. *pisi* (H). *F. solani* f. sp. *batatas* strains SUF1327 (lane 1) and SUF1328 (lane 2), and f. sp. *robiniae* strain SUF577 (lane 3) (I) and f. sp. *piperis* strains MAFF236558 (lane 1) and MAFF236572 (lane 2) (K) are shown and results of hybridizations using probes for rDNA-ITS from strain S-3 of *F. solani* f. sp. *phaseoli* are shown in J and L respectively. PFGE conditions were the same as in Fig. 1 (A).

Fig. 3

Graphical representation of the electrophoretic karyotypes of twenty two strains belonging to eight formae speciales of *Fusarium solani*.

This graphic was generated from the results of two electrophoretic conditions (short and long runs); short run conditions are shown in Fig. 1 and were performed for separation of smaller size chromosomes; long run conditions for separating larger chromosomes are shown in Fig. 2. Formae speciales of each strain used are shown in Table 1. Hybridization to probes for *khs*, *pda*, *pelA* and rDNA-ITS are denoted by‘K’,‘P’,‘A’ and‘R’over the chromosome band, respectively. Molecular sizes are shown on both sides and the sizes of the chromosomal bands hybridizing to these probes are shown in Table 2.

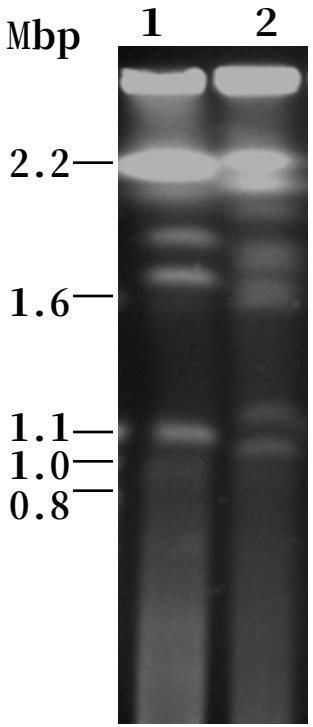

Fig.1

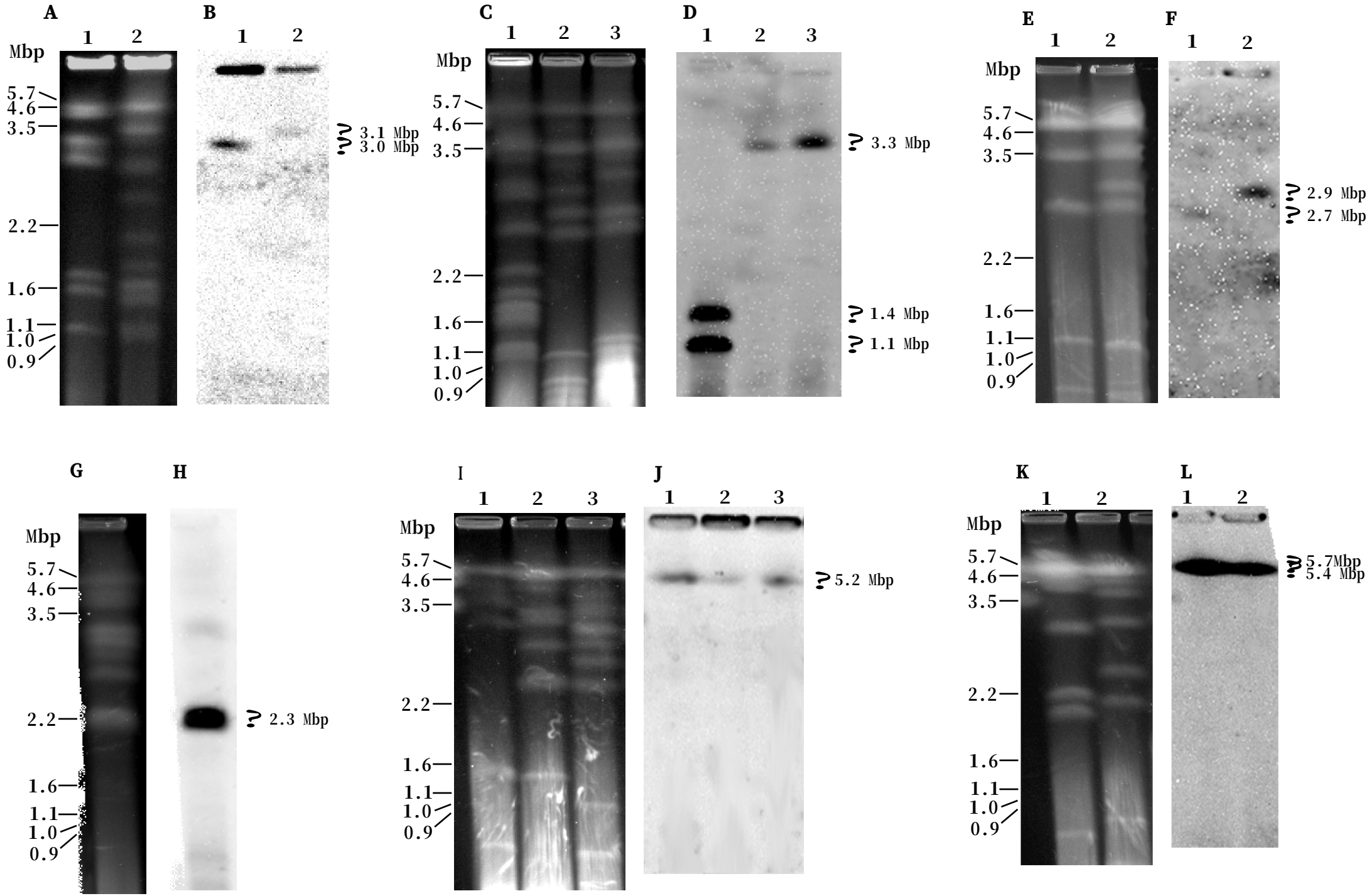
A
1 2
Mbp
5.7
4.6
3.5
2.2
1.6
1.1
1.0
0.9
B
1 2
3.1 Mbp
3.0 Mbp
C
1 2 3
Mbp
5.7
4.6
3.5
2.2
1.6
1.1
1.0
0.9
D
1 2 3
3.3 Mbp
1.4 Mbp
1.1 Mbp
E
1 2
Mbp
5.7
4.6
3.5
2.2
1.6
1.1
1.0
0.9
F
1 2
2.9 Mbp
2.7 Mbp
G
Mbp
5.7
4.6
3.5
2.2
1.6
1.1
1.0
0.9
H
2.3 Mbp
I
1 2 3
Mbp
5.7
4.6
3.5
2.2
1.6
1.1
1.0
0.9
J
1 2 3
5.2 Mbp
K
1 2
Mbp
5.7
4.6
3.5
2.2
1.6
1.1
1.0
0.9
L
1 2
5.7Mbp
5.4 Mbp

Fig. 2

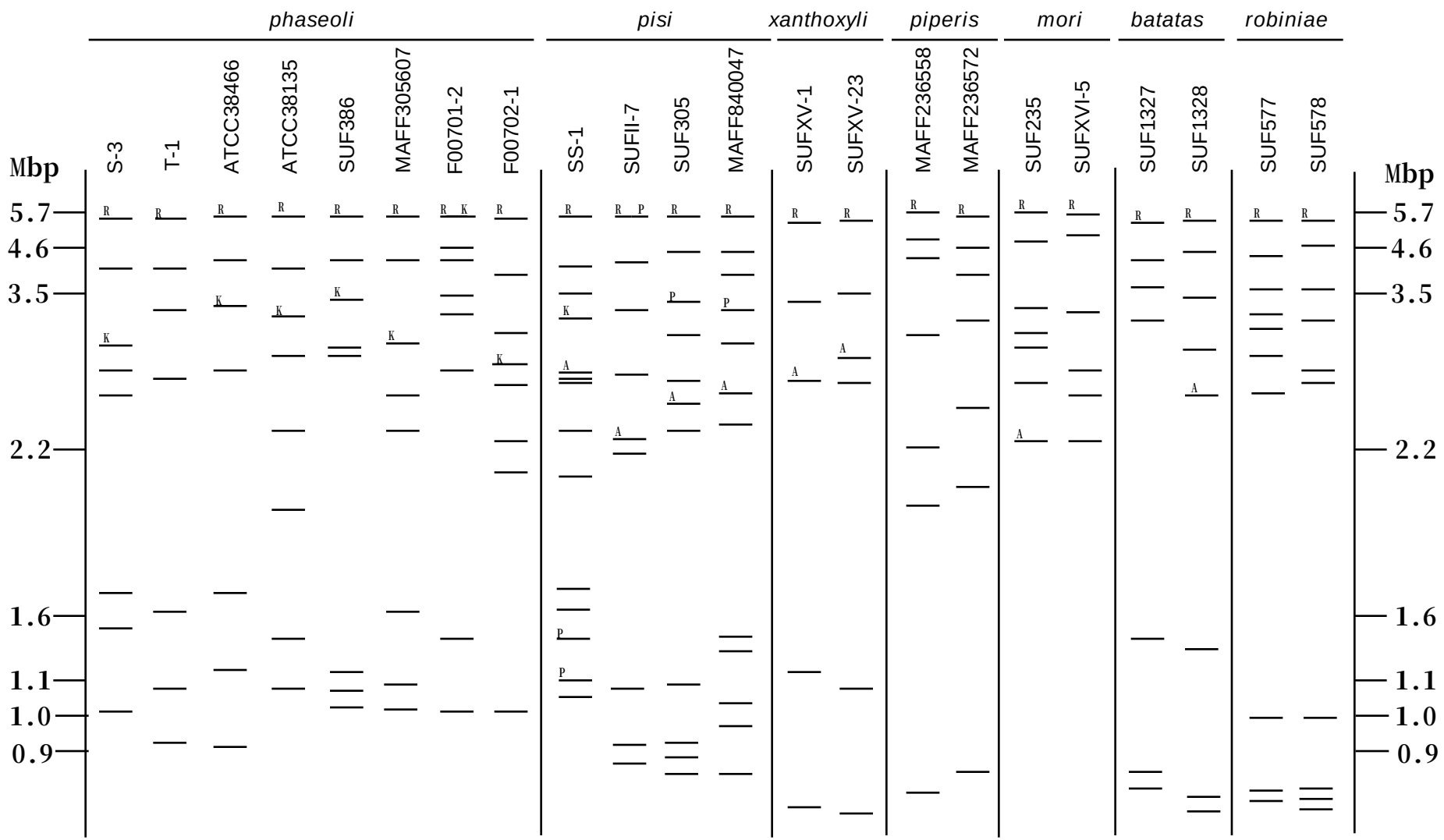
phaseoli
pisi
xanthoxyli
piperis
mori
batatas
robiniae
S-3
T-1
ATCC38466
ATCC38135
SUF386
MAFF305607
F00701-2
F00702-1
SS-1
SUFII-7
SUF305
MAFF840047
SUFXV-1
SUFXV-23
MAFF236558
MAFF236572
SUF235
SUFXVI-5
SUF1327
SUF1328
SUF577
SUF578
Mbp
5.7
4.6
3.5
2.2
1.6
1.1
1.0
0.9
R
K
A
P

Fig 3

Table 1. Strains of *Fusarium solani* used in this study

Strain [a]	Forma specialis	Teleomorph stage [b]	Host	Source
S-3	*phaseoli*	Unknown	Kidney bean	Hokkaido University
T-1	*phaseoli*	Unknown	Kidney bean	Hokkaido University
ATCC 38466	*phaseoli*	Unknown	Kidney bean	American Type Culture Collection
ATCC 38135	*phaseoli*	Unknown	Kidney bean	American Type Culture Collection
SUF 386	*phaseoli*	Unknown	Kidney bean	Shinshu University
MAFF 305607	*phaseoli*	Unknown	Kidney bean	Ministry of Agriculture, Forestry and Fisheries
F0070 1-2	*phaseoli*	Unknown	Kidney bean	Sakata Seed Corp.
F0070 2-1	*phaseoli*	Unknown	Kidney bean	Sakata Seed Corp.
SS-1	*pisi*	MP VI	Pea	Hokkaido University
SUF II-7 [c]	*pisi*	MP VI	Mulberry	Shinshu University
SUF 305 [c]	*pisi*	MP VI	Zelkova	Shinshu University
MAFF840047 [c]	*pisi*	MP VI	Mulberry	Ministry of Agriculture, Forestry and Fisheries
SUF XV-1	*xanthoxyli*	MP IV	Japanese pepper	Shinshu University
SUF XV-23	*xanthoxyli*	MP IV	Japanese pepper	Shinshu University
MAFF 236558	*piperis*	Unknown [d]	Pepper	Ministry of Agriculture, Forestry and Fisheries
MAFF 236572	*piperis*	Unknown [d]	Pepper	Ministry of Agriculture, Forestry and Fisheries
SUF 235	*mori*	MP III	Mulberry	Shinshu University
SUF XVI-5	*mori*	MP III	Mulberry	Shinshu University
SUF 1327	*batatas*	MP II	Sweet potato	Shinshu University
SUF 1328	*batatas*	MP II	Sweet potato	Shinshu University
SUF 577	*robiniae*	MP VII	Black locust	Shinshu University
SUF 578	*robiniae*	MP VII	Black locust	Shinshu University

[a] SUF: Culture Collection of *Fusarium* in Sinshu University, Japan; ATCC: American Type Culture Collection, Manassas, VA, U.S.A.; MAFF: Ministry of Agriculture, Forestry and Fisheries,Tsukuba,, Japan;

[b] Mating population (MP) of *Nectria haematococca*.

[c] Although this strain has been identified as *F. solani* f. sp. *pisi*, it actually should be considered *N. haematococca* MP VI (refer to discussion in text).

[d] Although number of mating population was not published, teleomorph stage was produced between strains isolated in Dominican Republic (Matsuda,, personal communication)

Table 2. Approximate size (Mbp) of chromosomes of *Fusarium solani* and distributions of *rDNA-ITS*, *khs*, *pda* and *pelA*

	Forma specialis and strain																					
	phaseoli								*pisi*				*xanthoxyli*		*piperis*		*mori*		*batatas*		*robiniae*	
Chr. No.	S-3	T-1[a]	ATCC 38466	ATCC 38135	SUF386	MAFF 305607	F0070 1-2	F0070 2-1	SS-1	SUFII-7	SUF305	MAFF 840047	SUF XV-1	SUF XV-23	MAFF 236558	MAFF 236572	SUF235	SUF XVI-5[b]	SUF 1327[b]	SUF 1328	SUF557	SUF578
I	5.4[c](R)[d]	5.4(R)	5.4(R)	5.2(R)	5.4(R)	5.4(R)	5.4(R,K)	5.2(R)	5.4(R)	5.6(R,P)	5.6(R)	5.6(R)	5.2(R)	5.3(R)	5.7(R)	5.4(R)	5.7(R)	5.6(R)	5.2(R)	5.2(R)	5.2(R)	5.2(R)
II	4.0	4.0	4.1	4.0	4.1	4.1	4.5	3.8	3.9	4.1	4.5	4.5	3.4	3.5	4.7	4.5	4.6	4.7	4.2	4.4	4.3	4.5
III	3.0(K)	3.3	3.3(K)	3.3(K)	3.4(K)	3.1(K)	4.1	3.1	3.5	3.3	3.4(P)	3.8	2.7(A)	2.9(A)	4.2	3.7	3.3	3.3	3.6	3.4	3.5	3.5
IV	2.8	2.7	2.8	2.9	3.0	2.6	3.5	2.8(K)	3.2(K)	2.8	3.1	3.3(P)	1.2	2.7	3.1	3.2	3.1	2.8	3.2	3.0	3.3	3.2
V	2.6	1.6	1.7	2.4	2.9	2.4	3.3	2.6	2.7(A)	2.3(A)	2.8	3.0	0.6	1.1	2.2	2.5	3.0	2.6	1.4	2.6(A)	3.1	2.8
VI	1.7	1.1	1.2	2.0	1.2	1.6	2.7	2.3	2.3	2.1	2.5(A)	2.6(A)		0.6	2.0	2.1	2.7	2.3	0.8	1.3	2.9	2.7
VII	1.5	0.9	0.9	1.4	1.1	1.1	1.4	2.1	2.0	1.1	2.3	2.4			0.7	0.8	2.3(A)		0.7	0.7	2.6	1.0
VIII	1.0		1.1	1.0	1.0	1.0	1.0	1.7	0.9	1.1	1.5								0.6	1.0	0.7	
IX								1.6	0.8	0.9	1.4									0.7	0.7	
X								1.4(P)		0.8	1.1									0.7	0.6	
?								1.1(P)		0.7	1.0											
?								1.0			0.7											

a) *khs* was amplified by PCR but this strain was not used for Southern blot hybridization with *khs* probe.

b) *pelA* was amplified by PCR but this strain was not used for Southern blot hybridization with *pelA* probe.

c) Underline means that band corresponding to this size is bolder than others on PFGE analysis.

d) Gene was amplified by PCR and signal was detected on the band by Southern blot hybridization. Hybridization to probes for *rDNA-ITS*, *khs*, *pda* and *pelA* are denoted by ‘ R ’, ‘ K ’, ‘ P ’ and ‘ A ’.