Mycoscience 40: 29–34, 1999

Cloning and sequence analysis of the plasmid DNA found in *Rhizoctonia solani* AG-2-2 LP isolate and its potential use for fungal detection*

Susumu Takamatsu[1],, Manami Nakano[1], Kaewalin Kunasakdakul[2], Hideyuki Yokota[1] and Hitoshi Kunoh[1]**

[1] Laboratory of Plant Pathology, Faculty of Bioresources, Mie University, 1515, Kamihama, Tsu 514–8507, Japan
[2] Department of Plant Pathology, Faculty of Agriculture, Chiangmai University, Chiangmai 50200, Thailand

Accepted for publication 24 November 1998

A plasmid DNA (PE-42 plasmid) obtained from *Rhizoctonia solani* AG-2-2 LP isolate PE-42, the causal agent of large patch disease of zoysiagrass (*Zoysia* spp.), was partially cloned. Sequence analyses of the 1.2-kb and 0.2-kb cloned fragments revealed that the nucleotide sequence of the 0.2-kb fragment was similar to that of the 5′ region of the 1.2-kb fragment (pSH4). Southern hybridization analysis of total DNA of a large patch isolate using the 1.2-kb fragment as a probe showed two bands differing slightly in size. These results indicated that the PE-42 plasmid consisted of at least two components having similar nucleotide sequences with different sizes. The nucleotide sequence of the pSH4 fragment showed no significant homology with known DNA sequences. The pSH4 fragment hybridized to all of the 22 large patch isolates tested, but not to other subgroup isolates in AG-2-2, other anastomosis groups of *R. solani*, or other pathogens of zoysiagrass. These results indicated that the pSH4 fragment can be used as a specific probe to detect the large patch fungus. The detection limit for the large patch fungus using the pSH4 fragment as a probe was 0.1 μg of the total DNA of the fungus, which was significantly higher than those for other fungi. However, with improvement of the detection sensitivity and simplification of the detection procedure, the pSH4 fragment has potential for use in molecular diagnosis of the large patch disease of zoysiagrass.

Key Words——large patch disease; molecular diagnosis; plasmid; *Rhizoctonia solani*; zoysiagrass.

Accurate diagnosis of turfgrass diseases for appropriate control is one of the capabilities required for green keepers of golf courses. Tani and Beard (1997) described more than 50 varieties of turfgrass diseases. Rapid and accurate diagnosis is essential for effective control of these diseases, leading to reduction of the amount and frequency of fungicide application in golf courses. However, since more than half of the diseases cause patch or spot death of turfgrasses, their diagnosis is complicated and time-consuming, especially in their early stages. Recently, various diagnostic procedures using molecular biological techniques have been established for a variety of plant diseases including turfgrass diseases (Goodwin et al., 1989, 1995; Henson, 1989; Judelson et al., 1996; Kageyama et al., 1997; Moukhamedov, 1994; Nicholson and Parry, 1996; O'Gorman et al., 1994; Parry and Nicholson, 1996; Tisserat et al., 1994). Compared with the traditional diagnostic procedures, the molecular ones are more accurate, less time-consuming, and more easily protocolled.

Rhizoctonia spp. cause several important diseases on turfgrasses: 'brown patch' caused by *Rhizoctonia solani* Kühn AG-1 and AG-2-2 IIIB, 'large patch' by *R. solani* AG-2-2 LP, 'yellow patch (spring dead spot)' by binucleate *Rhizoctonia* AG-D I, 'Rhizoctonia patch (elephant footprint)' by binucleate *Rhizoctonia* AG-D II, and 'pseudo-Rhizoctonia brown patch' by *Rhizoctonia circinata* var. *circinata* (Tani and Beard, 1997). Among them, large patch disease (abbreviated as 'large patch' below) is the most important *Rhizoctonia* disease of zoysiagrass (*Zoysia* spp.) on golf courses in Japan.

In the previous paper (Takamatsu et al., 1998), we found a 3.0-kb plasmid DNA fragment in the *R. solani* AG-2-2 LP isolate PE-42 and designated it as the PE-42 plasmid fragment. This plasmid DNA hybridized to the DNAs of all of the *R. solani* AG-2-2 LP isolates tested, but not to those of the other anastomosis groups (AG) of *R. solani* or other zoysiagrass pathogens. This result indicated that the PE-42 plasmid DNA was specific to *R. solani* AG-2-2 LP and applicable to molecular diagnosis of the large patch (Takamatsu et al., 1998). However, the plasmid DNA itself is not suitable as a probe for molecular diagnosis, because accumulation of the plasmid DNA required for this purpose is too time- and labor-consuming. Therefore, we cloned partial fragments of this plasmid DNA, sequenced the cloned fragments, and evaluated the specificity and sensitivity of the cloned fragments in this study.

* Contribution No. 140 from the Laboratory of Plant Pathology, Mie University.

** Corresponding author: E-mail takamatu@bio.mie-u.ac.jp; Fax +81–59–231–9637.

Materials and Methods

Fungal isolate The isolates of *R. solani* and other zoysiagrass pathogens used in this study are listed in Table 1. *Pythium graminicola* Subramanian was maintained on corn meal agar slants and other fungi on potato dextrose agar (PDA) slants.

DNA extraction and isolation of plasmid DNA A mycelial agar disk cut from plate cultures was incubated in 20 ml of potato dextrose broth at 25°C for 3 d. Cultures were then shaken (100 rpm) for 2 d. The mycelial mat was harvested on filter paper (Advantec Toyo, No. 1), rinsed several times with distilled water, and lyophilized. Total DNA was extracted as described in the previous report (Takamatsu et al., 1998). Total DNA extracted from the *R. solani* AG-2-2 LP isolate PE-42 was subjected to electrophoresis on 1.0% agarose gels. A plasmid DNA of 3.0 kb was recovered from the gel, using a DEAE-cellulose paper as described by Girvitz et al. (1980).

Cloning of plasmid DNA The PE-42 plasmid DNA was digested with three restriction enzyme combinations, *Sac*I/*Hin*dIII, *Acc*I/*Hin*dIII, and *Hin*cII/*Hin*dIII. DNA fragments were precipitated with ethanol, resuspended in TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA), and ligated to the plasmid vector pUC119 linearized with the same restriction enzyme combinations. The ligation mixtures were used to transform *Escherichia coli* strain JM109 according to the method of Hanahan (1981). White colonies which appeared on LB agar containing 100 mg/L ampicillin were transferred to LB liquid, cultured overnight, and used to extract plasmids by the boiling method (Holmes and Quingley, 1981). The presence of the insert DNA fragment was confirmed by electrophoresis and Southern hybridization using the PE-42 plasmid as the probe.

Southern transfer and hybridization Total DNAs extracted from fungal mycelia were fractionated by electrophoresis on a 1.0% agarose gel and transferred to nylon membranes (Hybond-N+, Amersham) by alkaline Southern blotting (Reed and Mann, 1985). Either the ECL direct nucleic acid labelling and detection system (Amersham) or the DIG DNA labelling and detection kit (DIG-ELISA, Boehringer Mannheim) was used for labelling of probe, hybridization, and detection of signals according to the manufacturers' instructions.

Results

Cloning and sequence analysis of the plasmid DNA Restriction enzyme analyses revealed the presence of one cleavage site each of *Hin*dIII and *Sac*I, and two or more cleavage sites of *Acc*I, *Hin*cII, *Msp*I, and *Pst*I in the PE-42 plasmid DNA (Takamatsu et al., 1998). Thus, the plasmid was cleaved with three combinations, *Sac*I/*Hin*dIII, *Acc*I/*Hin*dIII, and *Hin*cII/*Hin*dIII, and ligated to the plasmid vector pUC119 linearized with the same restriction enzyme combinations. Plasmid DNAs were extracted from 20 transformants each obtained from the three enzyme combinations and subjected to agarose gel electrophoresis in 1.0% agarose to confirm the insert DNA fragments. In the *Sac*I/*Hin*dIII treatment, 13 clones including 1.2-kb insert fragments were obtained, one of which was designated as pSH4. Four clones having a 0.3-kb insert were also obtained with the same enzyme combination. Eleven clones having a 0.2-kb fragment were obtained in the *Acc*I/*Hin*dIII treatment. Twelve clones having a 0.2-

Table 1. Isolates of *Rhizoctonia solani* and other fungi used in this study, and results of hybridization using the pSH4 fragment as the probe.

Species	AG	Isolate tested	3.0-kb signal[a]
R. solani	AG-1 (IA)	Cs-Ka, Cs-a, C-54	−
	AG-1 (IB)	B-19, shiba-2, 472, R1	−
	AG-1 (IC)	BV-7, F-2, 189, BW3	−
	AG-2-1	Ps-4, TG-1, SH-8, HV-1	−
	AG-2-2 (IIIB)	C-96, C-116, RGR59, GBB	−
	AG-2-2 (IV)	RI64, B-70, A-10, K-1	−
	AG-2-2 (LP)	PE-42, R-K-6, R-K-43, R-K-48, R-K-50, R-K-62, 38-R, 126-2, SI-6, S1, S2, S3, S4, A1, A2, A3, A4, A5, A6, A7, A8, A9	+
	AG-3	ST11-6, ST-9	−
	AG-4	AH-1, RH-74, Rh-165, HI521-21, RR5-2, 1272	−
	AG-5	GM-10, ST6-1, HG95-12, SH-1	−
	AG-6	OHT1-1, NAT3-1, NKN2-1, HN1-1, YK3-3, AO1-7	−
	AG-7	1535, 1529	−
	AG-8	A125	−
	AG-BI	TE2-4S, SN1-2	−
Rhizoctonia sp.	AG-D	R-K-57, S5, S6	−
Pythium graminicola		UOP380	−
Curvularia sp.		TGH-3c	−

a) Total DNAs extracted from the respective fungi were fractionated by agarose gel electrophoresis, blotted onto nylon membranes, then probed with the pSH4 fragment. +: detected; −: not detected.

kb fragment and one clone having a 2.0-kb fragment were obtained with *Hin*cII/*Hin*dIII. Southern hybridization analysis using PE-42 plasmid DNA as the probe showed distinctive hybridization signals in the clones having the 1.2-kb fragment but not in the clones with the other fragments. The nucleotide sequence of the pSH4 fragment is shown in Fig. 1. The nucleotide length of the insert fragment of pSH4 was 1232 bp. Nucleotide sequences of the 0.2-kb fragments were also determined for three clones, revealing that the sequences were completely identical among the three clones and highly similar (88.4%), but not identical, to the 5′ end of the pSH4 fragment.

The nucleotide sequence of the pSH4 fragment was compared with known sequences in DDBJ, GenBank, and EMBL databases by using the FASTA program. It showed no significant homology with the known sequences in the databases including those of plasmids found in other AGs of *R. solani*. To find a potential open reading frame (ORF), the nucleotide sequence of the pSH4 fragment was analyzed in all possible reading frames. When the ATG triplet was used as the initiation codon, no appropriate ORF longer than 200 bp was found. Two ORFs (ORF1 and ORF2 in Fig. 1) were found when the GTG triplet was used as the alternative initiation codon. The two putative ORFs were translated to amino acid sequences and subjected to homology search using the PIR and SWISS-PROT databases. However, no significant homology was found with the known sequences in the databases.

Specificity of the pSH4 fragment Total DNAs were extracted from 68 isolates of *R. solani*, including 14 AGs and six subgroups, and subjected to Southern hybridization analyses using the pSH4 fragment as the probe in the ECL or DIG-ELISA system. Distinctive hybridization signals were found at a 3.0-kb site in all 22 large patch isolates used in this experiment but not in isolates belonging to other AGs (Table 1). Indistinct hybridization signals were also observed at 6.0 kb and chromosomal DNA sites in the large patch isolates. A similar experi-

```
          ORF1 ➡
AAGCTTCTTC CTCCAGTGTG CTGTGCAATG CCCAATTCTT GTCCGCCTTA CAGTCGCCTA    60
                                                          ORF2 ⇨
AATCAGGCGC TGTAAGGGTC GTTCCACACT TAAACCTTGG TAGAAAGACC GTAGAGTGTG   120
TTTGCCACGC ACAGCGTGTA CCAACCACTC GACGATCTTT GGTCTTTCTT TTGTCCGATC   180
ATGGATCGGA CAAACAAGAC CGAGGTTTAA GGGTGTCGAC AATAGCCATT GCTCACGCAA   240
TGGTCTTAAG GACGTTTGGG CATTTCACCC GACGCGCCGA CCTTTTTCAA GGTCGGACGC   300
                                          ⬅
TAGCACACTG GGGCTTTCAA CTGGAGGAAG GCCGCGCGTA GTCAAATTAT CATTTTACGC   360
                                    ⇦
GCAGCGGCAT ACCCAGACCC GTCATGCTAG CCGCTAACAC CCCGGTTATT TTATTGGAAA   420
AATCCTAGAT TTTTCTAGGA ATAAATAACC TGAGGGACGT TAGCAAACGC TGTTGTGGCA   480
TCCGGTCTCT AGTGAGCGCG GTTGTAAGCC GCGCTGTTCT AGAAATTTCT TACCCCGCCG   540
CGGCGGATTG CCCTCACCCA CAGCGACCCC CAAGCTAGCG CGGGGGCGCC GGTCCTCCAT   600
TGAGGCCGGT GAACTGTGGG ATATGGGGTT ACCGTATGGG CGGGGTCAAG AAATGTCCCG   660
GCACTGGAAC ATTGGTGCTC TTCTTATCAT AGCTTATTGG CATTCTCGCC CTCTGTCAAA   720
GGACGAGACT TTTAATACCG TAGTCAAACT AGGTATACAA AAAACGTAGG TTTTTTGCTT   780
CTCTTTTATA CCAAAAAGAG AACCTAGGTT GACTTCGGTA ACTTAGGCCA ATAGGCTATG   840
TATAGGTGAG CAAATGTTCC AGCACAGGCG ACCGGATGCT CTGCAGGACG CCACAAGGGC   900
TCAGTGACGT CGCTTTCGAA TCCGACCCTA GCGGAACGAA AGCTCGGGCC GCCGAGTCGT   960
TCCGATCGGC TTGGCCAGAA GAAAACCTCT CAACTAGTTG AAAGGTTTTC TAACTAACCA  1020
TTACCCCTGG AGGGGTAATG CTTTGATTGA ATGAATCTTA CCTAGCTTTC CCCGTTGAAC  1080
GGAAGCCTCA GGCCATTCCA TTCATAAGGG GAAAGGTAGA CGTCTGAGAC TCCATTAGCC  1140
CTAGCAGCTT TAGCTGCGTA GGGGTAATGG CCGACCCATA TTTGGGTCGG ATACACGGCC  1200
AGCCCGGCAG TATCTGCCGG CGGCCCGAGC TC                                 1232
```

Fig. 1. Nucleotide sequences of the pSH4 fragments (1,232 bp).
Black arrows (➡, ⬅) and white arrows (⇨, ⇦) indicate the range of the putative open reading frames surveyed using a computer program 'GENETYX' (Software Development). The nucleotide sequences will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the accession numbers AB015667.

ment was done using total DNAs extracted from the zoysiagrass pathogens such as binucleate *Rhizoctonia* AG-D, *P. graminicola*, and *Curvularia* sp. A distictive hybridization signal was found only in the large patch isolate PE-42 used as a positive control, but not in the three other pathogens (Table 1).

Sensitivity of pSH4 fragment To determine the detection limit, aliquots of 3.0, 1.0, 0.3, 0.1, 0.03, and 0.01 μg of the total DNAs extracted from the large patch isolates R-K-6 and 38-R were subjected to agarose gel electrophoresis, blotted to nylon membrane, and hybridized to the digoxigenin-labeled pSH4 fragment. Both isolates showed similar results, of which that for R-K-6 is shown in Fig. 2. Distinctive hybridization signals were detected with aliquots of 3.0, 1.0, 0.3, and 0.1 μg, but not 0.03 and 0.01 μg. The same detection limit was obtained when the ECL system was used (data not shown). In addition to the distinctive 3.0-kb signal, a 6.0-kb signal was also found in all experiments. Two bands differing slightly in size were usually found in both 3.0-kb and 6.0-kb signals.

Discussion

We obtained 2.0-, 1.2-, 0.3- and 0.2-kb cloned fragments by the restriction enzyme treatments of the PE-42 plasmid DNA. In hybridization analysis using the PE-42 plasmid DNA as a probe, hybridization signals were obtained only with the 1.2-kb fragment. Thus, the 1.2-kb fragment was derived from the plasmid DNA, while the origin of other fragments was unclear. Nucleotide sequences were determined for a 1.2-kb fragment (pSH4) and three 0.2-kb fragments. The sequences of the three 0.2-kb fragments showed a high similarity, but not identity, to the 5′ end of the 1.2-kb fragment. In the previous report (Takamatsu et al., 1998), it was suggested that the PE-42 plasmid DNA fragments included more than one plasmid, because the total length of the fragments generated by restriction enzyme treatments usually exceeded that of the PE-42 plasmid. The present result suggests that the 0.2-kb fragments originate from a plasmid having similar sequence but not identical to the pSH4 fragment, although the PE-42 plasmid DNA did not hybridize to the 0.2-kb fragment. Hybridization analysis of large patch isolates using the pSH4 fragment as the probe showed two bands at the 3.0-kb portion. The hybridization analysis as well as the sequence analysis indicates that the PE-42 plasmid DNA fragments consist of at least two plasmids having similar nucleotide sequences and somewhat different sizes. Miyasaka et al. (1990) also reported the presence of three plasmid DNAs having the same size and different restriction sites in an *R. solani* AG-4 isolate.

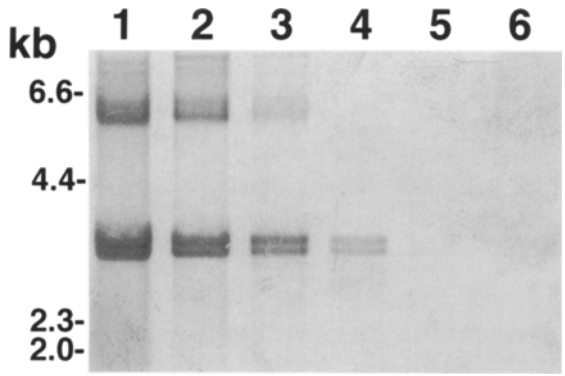

Fig. 2. Southern hybridization analysis of the digoxigenin-labeled probe pSH4 fragment to serial dilutions of the total DNA extracted from *Rhizoctonia solani* AG-2-2 LP isolate R-K-6.
Lanes 1, 3.0 μg; 2, 1.0 μg; 3, 0.3 μg; 4, 0.1 μg; 5, 0.03 μg; and 6, 0.001 μg.

Hongo et al. (1994) reported the presence of an ORF encoding a 7-kDa protein in the nucleotide sequence of a plasmid in *R. solani* AG-4 isolate. The ORF search of the pSH4 fragment revealed the presence of two potential ORFs of more than 200 bp in length. The putative amino acid sequences translated from the ORFs showed no significant homology to known proteins in the databases or to the 7-kDa protein reported by Hongo et al. (1994). Further experiments are necessary to examine whether the putative ORFs are functional.

In addition to the 3.0-kb plasmid signal, a 6.0-kb signal was usually detected in DNAs from the large patch isolates that hybridized to the pSH-4 fragment. We also reported that the 6.0-kb signal appeared when the PE-42 plasmid was used as the probe (Takamatsu et al., 1998). Miyashita et al. (1990) found a similar signal in plasmid pRS64 extracted from an *R. solani* AG-4 isolate and reported that the signal was derived from a dimeric form of the plasmid DNA. Thus, the possibility that the 6.0-kb band is the dimeric form of the PE-42 plasmid cannot be ruled out.

To use a certain DNA fragment as a probe for molecular diagnosis, that fragment should be consistently present only in the target fungus but not in any other organisms. Southern hybridization analysis using pSH4 fragment as the probe showed distinctive hybridization signals in all of the 22 large patch isolates collected from various sites in Japan. We also designed PCR primers to amplify the PE-42 plasmid based on the nucleotide sequences of the pSH4 fragment, and used them for survey of the nucleotide sequences identical to that of the PE-42 plasmid. Amplified DNA bands of the expected size were observed in 93 of 100 field isolates of large patch fungus isolated from the golf courses in Mie, Shiga, Gifu, and Toyama prefectures (unpublished data). These results indicate that the nucleotide sequence identical to the PE-42 plasmid is common to the plasmid of large patch fungus. On the other hand, no hybridization signals were observed in any other AGs of *R. solani* and in the other pathogens of zoysiagrass. Takamatsu et al. (1998) reported that the PE-42 plasmid DNA did not hybridize to the DNAs of two *R. solani* AG-2-2 IV isolates which were regarded as belonging to the same AG as the large patch fungus, nor to those of two AG-2-2 IIIB isolates which cause brown patch of zoysiagrass. In the present experiment using the pSH4 fragment as the

probe, we obtained the same, negative results for four isolates each of AG-2-2 IV and AG-2-2 IIIB. These results suggest that large patch fungus can be differentiated from AG-2-2 IV and AG-2-2 IIIB isolates by using the pSH4 fragment as a hybridization probe. A homology search using the nucleotide sequence of the pSH4 fragment showed no significant homology to deposited DNA sequences in the databases or to known nucleotide sequences of *Rhizoctonia* plasmids. From the above results, we concluded that the pSH4 fragment has sufficient consistency and specificity as a probe for specific detection of large patch fungus. A variety of serological techniques have been developed for specific detection of plant pathogens (Clark and Adams, 1977; Hibi and Saito, 1985). Those techniques were effective for virus detection but were less successful with bacteria and fungi due to the lack of specificity (Ball and Reeves, 1991). The present result showed that the specificity of the pSH4 fragment is much superior to that of serological techniques for detection of large patch fungus.

The sensitivity test of the pSH4 fragment showed that the detection limit for large patch fungus was 0.1 μg of total fungal DNA. Goodwin et al. (1989) examined the detection limit for *Phytophthora parasitica* Dastur using the nuclear repetitive sequence of the fungus as a probe, and reported that the detection limits were from 10 to 1 ng with a ^{32}P-labeled probe and about 1 ng with a sulfonated probe. Johansen et al. (1989) also used the repetitive sequence of *Clavibacter michiganense* subsp. *sepedonicum* (Spieck. & Kotth.) Davis et al. to detect the bacterium, and reported that the detection limits were 0.5 ng with a biotinylated probe and 1.5 ng with a sulfonated probe, which were similar to the limit of the ^{32}P-labeled probe. Our current detection limit was significantly higher than these. However, with improvement of the detection sensitivity and simplification of the detection procedure, the pSH4 fragment has potential for use in molecular diagnosis of the large patch disease of zoysiagrass.

Acknowledgements——This work was supported in part by a Grant-in-Aid for Scientific Research (A) (1) (No. 07556015) from the Ministry of Education, Science, Culture and Sports of Japan. The authors wish to thank Mr. N. Honda, Drs. M. Hyakumachi, T. Ichitani, K. Kobayashi, and H. Shigemitsu for providing *R. solani* isolates and zoysiagrass pathogens.

Literature cited

Ball, S. F. L. and Reeves, J. C. 1991. The application of new techniques in the rapid testing for seed-borne pathogens. Plant Varieties and Seeds **4**: 169–176.

Clark, M. F. and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. **34**: 475–483.

Girvitz, S. C., Bacchetti, S., Rainbow, A. J. and Graham, F. L. 1980. A rapid and efficient procedure for the purification of DNA from agarose gels. Anal. Biochem. **106**: 492–496.

Goodwin, P. H., Hsiang, T., Xue, B. G. and Liu, H. W. 1995. Differentiation of *Gaeumannomyces graminis* from other turf-grass fungi by amplification with primers from ribosomal internal transcribed spacers. Plant Pathol. **44**: 384–391.

Goodwin, P. H., Kirkpatrick, B. C. and Duniway, J. M. 1989. Cloned DNA probes for identification of *Phytophthora parasitica*. Phytopathology **79**: 716–721.

Hanahan, D. 1981. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166**: 557–580.

Henson, J. M. 1989. DNA probe for identification of the take-all fungus, *Gaeumannomyces graminis*. Appl. Environ. Microbiol. **55**: 284–288.

Hibi, T. and Saito, Y. 1985. A dot immunobinding assay for the detection of tobacco mosaic virus in infected tissues. J. Gen. Virol. **66**: 1191–1194.

Holmes, D. S. and Quingley, M. 1981. The rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114**: 193–197.

Hongo, M., Miyasaka, A., Suzuki, F. and Hashiba, T. 1994. Expression of the linear DNA plasmid pRS64 in the plant pathogenic fungus *Rhizoctonia solani*. Mol. Gen. Genet. **245**: 265–271.

Johansen, I. E., Rasmussen, O. F. and Heide, M. 1989. Specific identification of *Clavibacter michiganense* subsp. *sepedonicum* by DNA-hybridization probes. Phytopathology **79**: 1019–1023.

Judelson, H. S. and Messenger-Routh, B. 1996. Quantitation of *Phytophthora cinnamomi* in avocado roots using a species-specific DNA probe. Phytopathology **86**: 763–768.

Kageyama, K., Ohyama, A. and Hyakumachi, M. 1997. Detection of *Pythium ultimum* using polymerase chain reaction with species-specific primers. Plant Dis. **81**: 1155–1160.

Miyasaka, A., Chen, C. L. and Hashiba, T. 1990. Detection and properties of plasmid-like DNA in isolates from nine anastomosis and intraspecific groups of *Rhizoctonia solani*. J. Gen. Microbiol. **136**: 1791–1798.

Miyashita, S., Hirochika, H., Ikeda, J. and Hashiba, T. 1990. Linear plasmid DNAs of the plant pathogenic fungus *Rhizoctonia solani* with unique terminal structures. Mol. Gen. Genet. **220**: 165–171.

Moukhamedov, R., Hu, X., Nazar, R. N. and Robb, J. 1994. Use of polymerase chain reaction-amplified ribosomal intergenic sequences for the diagnosis of *Verticillium tricorpus*. Phytopathology **84**: 256–259.

Nicholson, P. and Parry, D. W. 1996. Development and use of a PCR assay to detect *Rhizoctonia cerealis*, the cause of sharp eyespot in wheat. Plant Pathol. **45**: 872–883.

O'Gorman, D., Xue, B., Hsiang, T. and Goodwin, P. H. 1994. Detection of *Leptosphaeria korrae* with the polymerase chain reaction and primers from the ribosomal internal transcribed spacers. Can. J. Bot. **72**: 342–346.

Parry, D. W. and Nicholson, P. 1996. Development of a PCR assay to detect *Fusarium poae* in wheat. Plant Pathol. **45**: 383–391.

Reed, K. C. and Mann, D. A. 1985. Rapid transfer of DNA from agarose gels to nylon membranes. Nucleic Acids Res. **13**: 7207–7210.

Takamatsu, S., Nakano, M., Yokota, H. and Kunoh, H. 1998. Detection of *Rhizoctonia solani* AG-2-2 IV, the causal agent of large patch of zoysiagrass, using plasmid DNA as a probe. Ann. Phytopathol. Soc. Japan **64**: 451–457.

Tani, T. and Beard, J. B. 1997. Color atlas of turfgrass diseases. Ann Arbor Press, Chelsea.

Tisserat, N. A., Hulbert, S. H. and Sauer, K. M. 1994. Selective amplification of rDNA internal transcribed spacer regions to detect *Ophiosphaerella korrae* and *O. herpotricha*. Phytopathology **84**: 478–482.