

プラスミドDNAをプローブとして用いた日本芝ラージパッチ菌Rhizoctonia solani AG-2-2 IVの特異的検出

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Detection of *Rhizoctonia solani* AG-2-2 IV, the Causal Agent of Large Patch of Zoysiagrass, Using Plasmid DNA as a Probe*

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Abstract

Electrophoretic analysis of total DNA extracted from *Rhizoctonia solani* Kühn AG-2-2 IV isolate PE-42, the causal fungus of large patch of zoysiagrass, revealed a 3.0-kb band in addition to the band of chromosomal DNA. Because the band was regarded as a double-stranded plasmid DNA based on restriction enzyme analyses, it was designated as a PE-42 plasmid fragment. The PE-42 plasmid DNA recovered from agarose gel hybridized to DNA of all 22 large patch isolates used, but not to DNA of other zoysiagrass pathogens. Southern hybridization analysis using the PE-42 plasmid DNA as a probe showed that the DNA could be used as a marker to distinguish the large patch fungus from other intraspecific groups of *Rhizoctonia solani*. DNAs extracted from zoysiagrasses having various stages of large patch symptom and yellow patch symptom or having no symptoms (healthy) were subjected to Southern hybridization. The PE-42 plasmid DNA hybridized to the DNAs from zoysiagrass with large patch symptom, but not to DNAs from yellow patch and healthy plants. Thus, the PE-42 plasmid DNA was considered to be useful as a probe for molecular diagnosis of large patch of zoysiagrass.

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Key words : large patch, molecular diagnosis, plasmid, *Rhizoctonia solani*, zoysiagrass.

INTRODUCTION

Increasing of golf courses in Japan has resulted in a large demand of turfgrasses, and the extensive use of agricultural chemicals in golf courses has caused various social problems such as contamination the chemicals in soil and drinking water. Although a few golf courses are making efforts to maintain turfgrasses without chemicals, it is very difficult to prevent diseases and insects completely without any chemical application. Exact diagnosis of initial stages of diseases makes it possible to decide the timing of spot application of chemicals and thus to reduce the amount and frequency of chemical application.

Hybridization procedures^{6,7,15,30} or the polymerase chain reaction (PCR)^{5,8,12,18,20,24,29,31} are excellent tools for developing accurate, specific diagnosis of a variety of plant diseases. For example, Henson⁷) used a 4.3-kb mitochondrial DNA fragment as a hybridization probe to identify the fungus of take-all disease of wheat, *Gaeumannomyces graminis* var. *tritici*. Specific PCR primers were also designed based on the DNA sequence of the 4.3-kb fragment and successfully used to detect this fungus in infected plants^{8,29}). Similarly, Goodwin *et al.*⁶) designed PCR primers to detect this fungus based on

the DNA sequence of the internal transcribed spacers of the rDNA region.

Rhizoctonia blight, one of the most important diseases of turfgrasses, includes three different diseases based on the isolates of the causal organisms and host symptoms : 'brown patch' caused by *Rhizoctonia solani* AG-1 IA and AG-2-2 IIIB, 'large patch' by *R. solani* AG-2-2 IV, and 'yellow patch (spring dead spot)' by binucleate *Rhizoctonia* AG-D^{3,10}). Large patch is an important disease of zoysiagrass in Japan.

In this study, we surveyed DNA sequences specific to the large patch pathogen, *R. solani* AG-2-2 IV, and then developed a diagnostic procedure to identify the disease by using the DNA sequence as a hybridization probe.

MATERIALS AND METHODS

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

DNA extraction from fungi A mycelial agar disk precultured on PDA was incubated in 20 ml of potato dextrose broth at 25°C for 3 days, then grown in shake culture (100 rpm) at 25°C for 2 days. The mycelial

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mat was filtered through filter paper (ADOVANTEC TOYO No. 1), rinsed several times with distilled water, and lyophilized.

Total DNA was extracted according to the procedure described by Miyasaka *et al.*¹⁶⁾. One gram of lyophilized mycelium was ground to powder in liquid nitrogen and suspended in 16 ml 0.1 M citrate/phosphate buffer (pH 6.0) containing 0.1 M EDTA and then lysed by adding 20 ml 10% (w/v) sodium dodecyl sulfate (SDS) solution. After adding 5 M NaCl to make a final concentration of 1 M, this mixture was kept at 4°C overnight, then centrifuged at $15,000 \times g$ for 40 min. The supernatant was precipitated with 0.6 volumes of iso-propanol, and the precipitate was collected by centrifugation. The total DNA precipitate was resuspended in 5 ml TE-sarkosyl solution (20 mM Tris-HCl, pH 8.0; 0.25 mM EDTA; 0.4% N-lauroyl sarcosine) and purified by CsCl density-gradient centrifugation [5 g CsCl and $0.286 \mu\text{l}$ 10 mg/ml ethidium bromide (EtBr)]. The sample was centrifuged at $95,000 \times g$ for 24 hr. The bands removed from the gradients were extracted with *n*-butanol, precipitated with ethanol and dissolved in TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA).

Isolation of plasmid DNA Total DNA extracted from *R. solani* AG-2-2 IV isolate PE-42 was subjected to agarose gel electrophoresis in 1.0% agarose. The 3.0-kb plasmid DNA was recovered from agarose gel by using DEAE-cellulose paper according to the procedure described by Sambrook *et al.*²⁸⁾.

DNA extraction from plants Total DNA was extracted by CTAB (cetyltrimethylammonium bromide) method²⁷⁾. Five hundred mg of lyophilized zoysiagrass was ground to powder in liquid nitrogen with a mortar and pestle, suspended in 1.5 ml of 1×CTAB solution (1% CTAB; 50 mM Tris-HCl, pH 8.0; 10 mM EDTA; 0.7 M NaCl; 0.5% polyvinylpyrrolidone) and incubated at 65°C for 3 min. An equal volume of chloroform/isoamyl alcohol was added, then mixed and centrifuged at $11,000 \times g$ for 2 min. The aqueous phase was removed to a fresh tube. After adding 1/10 volume 10% CTAB solution (10% CTAB; 0.7 M NaCl), the chloroform/isoamyl alcohol extraction was repeated once. An equal volume of CTAB precipitation buffer (1% CTAB; 50 mM Tris-HCl, pH 8.0; 10 mM EDTA) was then added, and the solution was incubated at 65°C for 1 min and centrifuged at $10,000 \times g$ for 2 min. The total DNA precipitate was dissolved in 500 μl TE buffer containing 1 M NaCl and precipitated again by adding ethanol. The pellet was dissolved in 50 μl DNA buffer (10 mM Tris-HCl, pH 7.5; 0.1 mM EDTA; 10 mM KCl) and stored at -20°C until use.

Southern transfer and hybridization Total DNAs extracted from fungal mycelia or plant tissues were fractionated by electrophoresis in a 1.0% agarose gel, and transferred to a nylon membrane (Hybond-N⁺, Amersham) by alkaline Southern blotting²⁵⁾. Plasmid DNA derived from *R. solani* AG-2-2 IV isolate PE-42 was used as a probe of hybridization. An ECL direct

nucleic acid labelling and detection system (Amersham) was used for the labelling of probe, the hybridization and the detection of signals according to the manufacturer's instructions.

RESULTS

Plasmid DNA in large patch isolate

When the total DNA extracted from *R. solani* AG-2-2 IV isolate PE-42 was electrophoresed in an agarose gel, a distinctive 3.0-kb band was found in addition to the chromosomal DNA band (Fig. 1). To examine whether this band was DNA or RNA, the band was recovered from the agarose gel and treated with the following eight restriction enzymes: *AccI*, *Bam*HI, *Eco*RI, *Hinc*II, *Hind*III, *Msp*I, *Pst*I, and *Sac*I. Although the band was not digested by *Bam*HI and *Eco*RI, the other six enzymes generated more than two bands: 2.2, 1.25 and 0.54 kb in *Acc*I; 1.8, 1.65, 1.3 and 1.1 kb in *Hinc*II; 3.0, 2.1 and 1.05 kb in *Hind*III; 1.45, 0.86, 0.55 and 0.35 kb in *Msp*I; 3.0, 2.1, 1.15 and 1.05 kb in *Pst*I; 3.0, 2.3 and 0.72 kb in *Sac*I (Fig. 2). The original 3.0 kb band was found by *Hind*III, *Pst*I and *Sac*I treatments and did not disappear even after a prolonged enzyme treatment. Because the 3.0-kb band is mainly composed of a double-stranded DNA, it was designated as a PE-42 plasmid fragment.

Specificity of the plasmid DNA sequence in several zoysiagrass pathogens

Total DNAs extracted from nine large patch isolates including PE-42 were run in agarose gel electrophoresis. Although the plasmid band stained with EtBr in seven of nine isolates (Fig. 3A), it was not clear in the other two isolates (isolates R-K-50 and 38-R). These DNAs were then blotted to a nylon membrane for hybridization

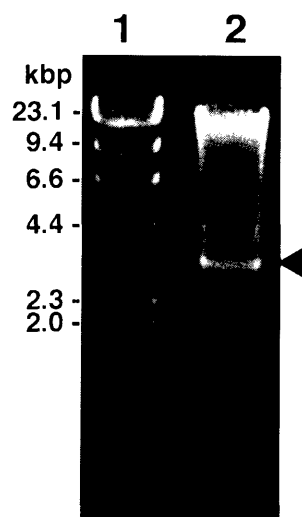


Fig. 1. Electrophoretic analysis of total DNA extracted from *Rhizoctonia solani* AG-2-2 IV isolate PE-42 from large patch of zoysiagrass (lane 2). A 3.0-kb band (arrowhead) was observed in addition to large-sized, chromosomal DNA. Lane 1 is λ DNA digested with *Hind*III.

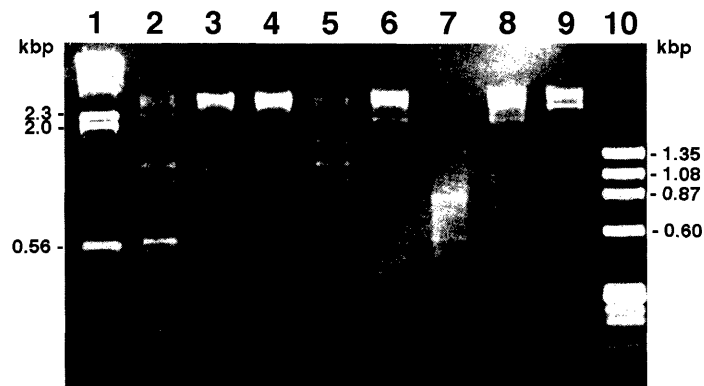


Fig. 2. Electrophoretic analysis of restriction fragments of PE-42 plasmid DNA digested with *AccI* (lane 2), *Bam*HI (lane 3), *Eco*RI (lane 4), *Hinc*II (lane 5), *Hind*III (lane 6), *Msp*I (lane 7), *Pst*I (lane 8), and *Sac*I (lane 9). Lane 1 is λ DNA digested with *Hind*III. Lane 10 is ϕ X174 DNA digested with *Hae*III.

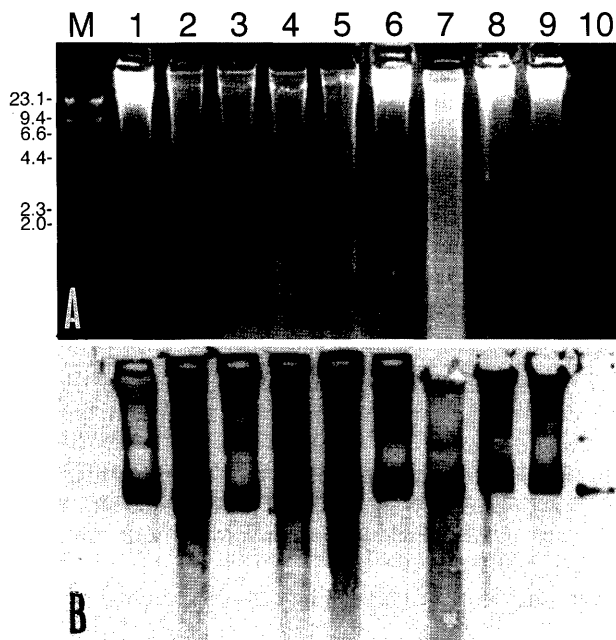


Fig. 3. Electrophoretic (A) and Southern hybridization (B) analyses of total DNAs from nine large patch isolates. Total DNAs (5–20 μ g) from large patch isolates were separated by electrophoresis and stained with ethidium bromide. Total DNAs in the gel were blotted onto nylon membrane and probed with PE-42 plasmid DNA. Lane 1, PE-42; 2, R-K-6; 3, R-K-43; 4, R-K-48; 5, R-K-50; 6, R-K-62; 7, 38-R; 8, 126-2; 9, SI-6; 10, PE-42 plasmid DNA (Control); M, λ DNA digested with *Hind*III.

using PE-42 plasmid DNA as a probe. Distinctive hybridization signals were observed in all 9 isolates including isolates R-K-50 and 38-R (Fig. 3B). Indistinct hybridization signals were also observed at 6.0 kb and chromosomal DNA sites. Positions of the bands varied somewhat depending on lanes, probably because excess amounts of DNAs were applied.

In similar experiment using total DNAs extracted from binucleate *Rhizoctonia* AG-D, *P. graminicola* and a *Curvularia* sp., the plasmid DNA band was detected by

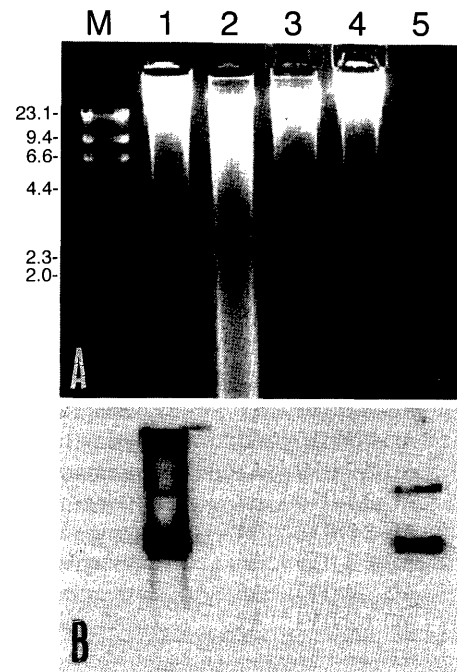


Fig. 4. Electrophoretic (A) and Southern hybridization (B) analyses of total DNA from isolates of zoysiagrass pathogens. Total DNA (10–20 μ g) from isolates of zoysiagrass pathogen was separated by electrophoresis and stained with ethidium bromide. Total DNAs in the gel were blotted onto nylon membrane and probed with PE-42 plasmid DNA. Lane 1, *Rhizoctonia solani* PE-42 (Large patch fungus); 2, *Pythium graminicola* UOP380 (Pythium blight fungus); 3, *Curvularia* sp. TGH-3c (Curvularia leaf blight fungus); 4, Binucleate *Rhizoctonia* AG-D R-K-57 (Yellow patch fungus); 5, PE-42 plasmid DNA (Control); M, λ DNA digested with *Hind*III.

EtBr staining in the large patch isolate PE-42 used as a positive control, but not in the other three fungi (Fig. 4A). Southern blot analysis also showed a distinctive signal only in PE-42, but not in the three other fungi (Fig. 4B).

Table 1. Isolates of *Rhizoctonia solani* and other fungi used in this study and results of hybridization using PE-42 plasmid DNA as a probe

Species	ISG ^{a)}	Isolate	Origin	3.0-kb signal ^{b)}
<i>Rhizoctonia solani</i>	AG-1-1 (A)	Cs-Ka	Rice	—
	AG-1-1 (A)	Cs-a	Rice	—
	AG-1-1 (B)	B-19	Sugarbeet	—
	AG-1-1 (B)	shiba-2	Soil	—
	AG-1-1 (C)	BV-7	Sugarbeet	—
	AG-1-1 (C)	F-2	Unknown	—
	AG-2-1	Ps-4	Pea	—
	AG-2-1	TG-1	Tulip	—
	AG-2-2 (IIIB)	C-96	Unknown	—
	AG-2-2 (IIIB)	C-116	Mat-rush	—
	AG-2-2 (IV)	RI64	Sugarbeet	—
	AG-2-2 (IV)	B-70	Sugarbeet	—
	AG-2-2 (IV)	PE-42	Zoysiagrass	+
	AG-2-2 (IV)	R-K-6	Zoysiagrass	+
	AG-2-2 (IV)	R-K-43	Zoysiagrass	+
	AG-2-2 (IV)	R-K-48	Zoysiagrass	+
	AG-2-2 (IV)	R-K-50	Zoysiagrass	+
	AG-2-2 (IV)	R-K-62	Zoysiagrass	+
	AG-2-2 (IV)	38-R	Zoysiagrass	+
	AG-2-2 (IV)	126-2	Zoysiagrass	+
	AG-2-2 (IV)	SI-6	Zoysiagrass	+
	AG-2-2 (IV)	S1	Zoysiagrass	+
	AG-2-2 (IV)	S2	Zoysiagrass	+
	AG-2-2 (IV)	S3	Zoysiagrass	+
	AG-2-2 (IV)	S4	Zoysiagrass	+
	AG-2-2 (IV)	A1	Zoysiagrass	+
	AG-2-2 (IV)	A2	Zoysiagrass	+
	AG-2-2 (IV)	A3	Zoysiagrass	+
	AG-2-2 (IV)	A4	Zoysiagrass	+
	AG-2-2 (IV)	A5	Zoysiagrass	+
	AG-2-2 (IV)	A6	Zoysiagrass	+
	AG-2-2 (IV)	A7	Zoysiagrass	+

Table 1 (continued)

Species	ISG ^{a)}	Isolate	Origin	3.0-kb signal ^{b)}
	AG-2-2 (IV)	A8	Zoysiagrass	+
	AG-2-2 (IV)	A9	Zoysiagrass	+
	AG-3	ST11-6	Potato	—
	AG-3	ST-9	Potato	—
	AG-4 (HG-I)	AH-1	Peanut	—
	AG-4 (HG-I)	RH-74	Sugarbeet	—
	AG-4 (HG-II)	Rh-165	Sugarbeet	—
	AG-4 (HG-II)	HI521-21	Soil	—
	AG-5	GM-10	Soybean	—
	AG-5	ST6-1	Potato	—
	AG-6 (HG-I)	OHT1-1	Soil	—
	AG-6 (HG-I)	NAT3-1	Soil	—
	AG-6 (HG-II)	NKN2-1	Soil	—
	AG-6 (HG-II)	HN1-1	Soil	—
	AG-7	1535	Soil	—
	AG-7	1529	Soil	—
	AG-BI	TE2-4S	Soil	—
	AG-BI	SN1-2	Soil	—
<i>Rhizoctonia</i>	AG-D	R-K-57	Zoysiagrass	—
sp.	AG-D	S5	Zoysiagrass	—
	AG-D	S6	Zoysiagrass	—
<i>Pythium</i>		UOP380	Zoysiagrass	—
<i>graminicola</i>				
<i>Curvularia</i>		TGH-3c	Zoysiagrass	—
sp.				

a) Intraspecific group (ISG) nomenclature after Ogoshi²³⁾ denotes anastomosis group (AG) along with subgroup where known.

b) Total DNAs extracted from the respective fungi were fractionated by agarose gel electrophoresis, blotted onto nylon membranes, then probed with the PE-42 plasmid DNA.

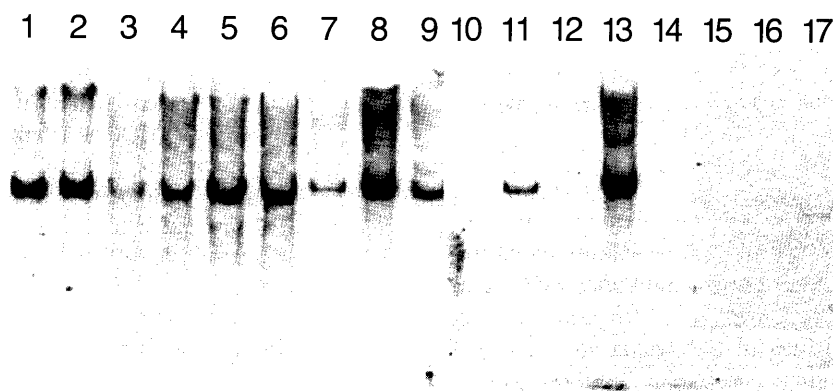


Fig. 5. Detection of the PE-42 plasmid DNA in DNA (5–10 μ g) from zoysiagrass with various stages of large patch and yellow patch, and having no disease symptoms. Lanes 1–9, typical large patch symptoms; 10 and 11, early stage symptoms of large patch; 12 and 13, laterally infected by large patch; 14 and 15, yellow patch symptoms; 16 and 17, no symptoms (healthy).

Specificity of the plasmid DNA sequence in intra-specific groups of *Rhizoctonia*

Twenty-eight isolates of *R. solani* covering 14 intra-specific groups were also analyzed by hybridization using PE-42 plasmid DNA as a probe. EtBr staining showed plasmid-like bands in three of 28 isolates used. Band sizes were 2.4 kb in AG-1-1 C isolate BV-7, 3.5 kb in AG-2-2 IIIB isolate C-116, and 2.7 kb in AG-4 HG-II isolate Rh-165 (data not shown). However, the analysis showed no 3.0-kb signals in all these isolates (Table 1). Nor was a 3.0-kb signal observed in AG-2-2 IV isolates RI64 and B-70 (both came from sugarbeet) which have been considered as members of the same intraspecific group as the large patch isolate.

Survey of the plasmid DNA sequence in field isolates of large patch fungus

The PE-42 plasmid DNA sequence was surveyed in 13 additional large patch isolates (S1, S2, S3, S4, A1, A2, A3, A4, A5, A6, A7, A8, and A9 in Table 1) and two yellow patch isolates (S5 and S6 in Table 1) obtained from zoysiagrass on golf courses in Mie and Shiga Prefectures in 1994. These isolates were identified according to cultural characters and anastomosis analysis. EtBr staining of the total DNA showed 3.0-kb bands in all the large patch isolates, but not in the yellow patch isolates. Hybridization experiments generated distinct signals in the large patch isolates but not in the yellow patch isolates (Table 1).

Detection of large patch fungus in diseased zoysiagrass

Turfgrass showing various stages of large patch and yellow patch, and from healthy turf areas was collected from golf courses in Mie and Shiga Prefectures in the spring and autumn of 1994. The diseases were diagnosed based on isolation of pathogens and the respective symptoms. Total DNAs were extracted from infected leaves by the CTAB method, and subjected to Southern hybridization using PE-42 plasmid DNA as a probe. Leaves of healthy plants were treated similarly for the controls. Hybridization signals of 3.0-kb were observed in all 13 large patch samples regardless of the disease stage, although signal strengths were variable depending on the samples (Fig. 5). No signal was found in two yellow patch samples and two healthy samples.

DISCUSSION

The molecular diagnosis method requires an adequate DNA sequence specific to the target pathogen. We found a specific 3.0-kb band when the total DNA extracted from the large patch isolate PE-42 was subjected to agarose gel electrophoresis and stained with EtBr. A double-stranded RNA^{1,2)} and plasmid DNA^{16,17)} were found in mycelia of *R. solani*. The 3.0-kb band was digested by several restriction enzymes to examine whether it was a double-stranded RNA or plasmid DNA, because RNA is not cleaved by any restriction enzymes. The band was cleaved by *AccI*, *HincII*, *HindIII*, *MspI*,

PstI, and *SacI* although it was not cleaved by *BamHI* and *EcoRI*. This result indicates that the band was composed of DNA having a double-strand at least in its main part. We thus designated the band as a PE-42 plasmid fragment. While *HindIII*, *PstI* and *SacI* sites were on the PE-42 plasmid DNA, an uncleaved band was always observed when these enzymes were used. Furthermore, the total length of the band generated by these restriction enzymes was usually more than 3.0 kb which corresponded to the size of the plasmid. These results suggest that the PE-42 plasmid fragment is composed of more than one plasmid having similar sizes and somewhat different sequences. Miyashita *et al.*¹⁷⁾ reported three plasmid DNAs having the same size but different restriction sites in a *R. solani* AG-4 isolate.

A DNA sequence used for molecular diagnosis must commonly exist in the target pathogen, but never in other fungi. We extracted the total DNAs from the yellow patch fungus, binucleate *Rhizoctonia* AG-D, the Pythium blight fungus, *P. graminicola*, the Curvularia leaf blight fungus, *Curvularia* sp., and nine isolates of the large patch fungus. These were subjected to Southern hybridization using the PE-42 plasmid DNA as a probe. A distinct hybridization signal was observed in all nine isolates of the large patch fungus but not in the other fungi. A similar result was obtained with 13 field isolates of the large patch fungus and two isolates of the yellow patch fungus. These results confirmed the common presence of DNA homologous to the PE-42 plasmid fragment in the large patch fungus, but not in the other zoysiagrass pathogens. Thus, we propose that the PE-42 plasmid fragment is an adequate probe for molecular diagnosis of the large patch disease.

R. solani has been regarded as a species complex which has a large diversity of pathogenicity, sclerotial morphology, mycelial features on media, and physiological and molecular characteristics^{13,14,22,23,26,33,34)}. *R. solani* is known to be a common inhabitant of plant debris and soil, because of its strong saprophytic ability^{23,32)}. By using the plasmid DNA as a probe for molecular diagnosis, the large patch fungus may be distinguished from other *R. solani*. We extracted total DNA from 28 isolates of *R. solani* including intraspecific groups known in Japan and subjected them to Southern hybridization using the PE-42 plasmid DNA as a probe. Because the hybridization signal was found only in the large patch isolates, but not in other isolates, the PE-42 plasmid can distinguish the large patch fungus from other *R. solani*. Note that the hybridization signal was not found in *R. solani* AG-2-2 IV sugarbeet isolates which have been regarded within the same intraspecific group as the large patch fungus. Hyakumachi *et al.*⁹⁾ and Musika *et al.*¹⁹⁾ reported that the large patch fungus was different from the sugarbeet fungus in its colony appearance, hyphal growth rate and rDNA sequence, and proposed to designate the intraspecific group of the large patch fungus as AG-2-2 LP. In addition, Liu and Sinclair¹³⁾ studied the genetic diversity of *R. solani* anastomosis

group AG-2 based on PCR-RFLP analysis of rDNA ITS region; the group had five distinct intraspecific groups. Turfgrass isolates were all in their own intraspecific group which was different from that of the sugarbeet isolates. Our results may offer new evidence to support these previous reports. However, Miyasaka *et al.*¹⁶⁾ reported that the 4.7-kb plasmid DNA of AG-2-2 IV H-16 isolate, which was derived from sugarbeet, hybridized to the 3.0-kb plasmid band of C-616 isolate derived from zoysiagrass. Further experiments using some other AG-2-2 IV isolates are, therefore, necessary to confirm our results.

To examine whether the PE-42 plasmid fragment can be used for molecular diagnosis of the large patch, total DNAs were extracted from zoysiagrass infected with the large patch fungus or yellow patch fungus and also from healthy plants for Southern hybridization. Distinct hybridization signals were observed in the DNAs from all samples having typical symptoms of large patch and from half of the samples having early symptoms and latently infected plants. No signal was detected in the DNAs from samples having yellow patch symptoms or from healthy plants. These results indicate that the large patch fungus can be specifically detected by the present procedure. However, sensitivity of the method should be improved; the probe sometimes could not detect low levels of the fungus.

In addition to the 3.0-kb plasmid signal, the *ca.* 6.0-kb signal was usually detected in DNAs from the large patch isolates which were hybridized with the PE-42 plasmid DNA. A similar signal was also observed when the PE-42 plasmid DNA recovered from agarose gel was hybridized with the homologous DNA. Miyashita *et al.*¹⁷⁾ found a similar signal in plasmid pRS64 extracted from a *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 could be the dimeric form of PE-42 plasmid cannot be ruled out.

Molecular diagnosis has been recently developed for a variety of fungal diseases of plants. In most cases, spacer regions of rDNA^{18,21,31)} or DNA fragments isolated from chromosomal or mitochondrial DNA^{4,6,11)} are used as probes or primers. Plasmid DNAs rarely have been used for the purpose, probably because plasmids are fragile and sensitive to various treatments and not always detected in fungal cells. However, the PE-42 plasmid DNA was common in the large patch isolates in this study. Thus, this plasmid is adequate for diagnosis of large patch. As far as we know, this study is the first report to use plasmid DNA for molecular diagnosis of fungal plant diseases.

For practical use of this technique the following should be improved: 1) cloned fragments of the plasmid DNA should be used for the procedure because preparation of the entire plasmid is time- and labor-consuming, 2) sensitivity, and 3) the protocol should be simplified.

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和 文 摘 要

高松 進・中野真奈美・横田英之・久能 均：プラスミド DNA をプローブとして用いた日本芝ラージパッチ菌 *Rhizoctonia solani* AG-2-2 IV の特異的検出

ラージパッチ菌 *Rhizoctonia solani* AG-2-2 IV PE-42 菌株から全 DNA を抽出し電気泳動を行ったところ、染色体 DNA 以外に 3.0 kb のバンドが認められた。このバンドは制限酵素によって切断されることから 2 本鎖 DNA プラスミドであると考えられたので、PE-42 プラスミドと命名した。アガロースゲルから回収した PE-42 プラスミド DNA をプローブとして、ラージパッチ菌およびその他の日本芝の病原菌から抽出した DNA とハイブリダイゼーションを行ったところ、ラージパッチ菌ではすべての菌株で明瞭なシグナルが検出されたが、その他の病原菌ではシグナルは全く検出されなかった。また、さまざまな菌株融合群および培養型を含む *R. solani* 菌株から抽出した DNA を用いて PE-42 プラスミド DNA とのハイブリダイゼーションを行ったところ、ラージパッチ菌株からのみシグナルが認められ、その他の菌株ではシグナルは全く認められなかった。さまざまな発病段階のラージパッチ罹病芝、イエローパッチ罹病芝および健全な芝から DNA を抽出し、PE-42 プラスミド DNA とハイブリダイズさせたところ、ラージパッチ罹病芝から抽出した DNA のみでシグナルが検出された。以上の結果から、PE-42 プラスミド DNA はラージパッチの遺伝子診断のためのプローブとして有用であると考えられた。