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Detection and Species Identification of Wood-Decaying Fungi by Hybridization of Immobilized Sequence-Specific Oligonucleotide Probes with PCR-Amplified Fungal Ribosomal DNA Internal Transcribed Spacers

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Keywords

Fungal DNA identification
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Summary

We developed an effective detection method for wood-decaying fungi by hybridization of immobilized Sequence-Specific Oligonucleotide Probes with florescent-labeled PCR-amplified fungal rDNA internal transcribed spacer sequences. This method takes advantage of both the sequence specificity of Southern blot hybridization and the sensitivity of the previously reported PCR-based fungal species identification methods. Both *in vitro* cultured fungal strains and naturally decaying wood samples were used to demonstrate that this method is robust and practical for detection of incipient wood-decaying fungi. It can be a useful tool for microbial ecology, plant pathology, protection of wood products in service, preservation efforts for high-value furniture and wood-based art and DNA fingerprinting for tracking the source of contamination of wood decay fungi.

Introduction

Microbiological deterioration of wood by brown-rot fungi can cause structural failure due to the loss of structural integrity (Illman and Dowd 1999). Even a relatively minor 3% loss of wood due to decay can result in a 50% loss in strength measured as toughness (Kennedy 1958). Strength loss can occur in the early decay process, so the best way to prevent structural failure is to identify and control the decay at the early stages of the process when it is not yet visible. Early detection of decay is crucial for proper remedial treatments prior to structural compromise that precedes morphological changes.

Contemporary techniques used for detecting wood incipient decay processes include wood acoustic testing, isolation and culture of fungi and chemical stains, as well as radiography and visual or microscopic inspection of samples. Molecular techniques, such as SDS-PAGE of intracellular proteins and immunological methods, have been used to identify and characterize various fungal species (Palfreyman *et al.* 1988; Schmidt and Kebernik 1989; Vigrow *et al.* 1991; Schmidt and Moreth 1995; Schoknecht 1999). These methods are useful for detecting advanced decay, but not suitable for the detection of early decay for in-service wood because they require large volume samples in order to identify the wood-decaying fungi. A recent US patent (No. 5,563,040) describes an immunodiagonostic method for detecting early stage fungal decay in 'in-service' wood, in which labeled antibodies immobilized in a defined substrate are used to detect the fungal antigens in the wood extract (Clausen and Green III 1996). However, the method requires destructive sampling and can only detect the wood decay fungi that produce specific antigens to the antibodies used. Furthermore, its low sensitivity can limit its use.

DNA polymerase chain reaction (PCR)-based methods circumvent the need for isolation of fungi and/or destructive sampling from infected wood, while offering extreme sensitivity for the detection of fungal decay agents at an incipient stage. The variable sequences of the internal transcribed spacer (ITS) regions of fungal rDNA have been successfully used for species identification by PCR amplification coupled to restriction enzyme analysis (Johnston and Aust 1994; Annamalai *et al.* 1995; Schmidt and Moreth 1999; Jasalavich *et al.* 2000). However, these methods are time-consuming because they require restriction enzyme digestions and gel electrophoresis of PCR-amplified products. Consequently, only a small number of samples can be assayed at a time. In addition, these techniques are sensitive to contamination and are often inconclusive, for example, small fragments of PCR products or restriction enzyme digestion products are often not easily detected in agarose electrophoresis analysis (Lovic *et al.* 1995; Schmidt and Moreth 1999).

Differential hybridization with Sequence-Specific Oligonucleotide Probes (SSOP) is one of the conventional methods for detection of genetic mutation and polymorphisms (Conner *et al.* 1983). The short oligomers (usually 15–25 bases in length) will hybridize to their complementary target sequences in the sample DNA

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only when they are perfectly matched. In the SSOP analysis, the target DNA segment is first PCR-amplified and fixed onto a series of nylon membranes. Each membrane is then hybridized with one of the labeled oligonucleotide probes. Conversely, in an appropriate hybridization condition, the destabilizing effect of a single base-pair mismatch prevents the formation of a stable probe-target duplex. In a reciprocal situation where the oligonucleotides are fixed onto nylon membrane and hybridized with the amplified sample, a sample can be analyzed by a large number of probes. The oligonucleotides are given homopolymer tails with terminal deoxyribonucleotidyl transferases. The tailed oligonucleotides are fixed and covalently bound on the nylon membrane, and then hybridized with one of the labeled DNA samples which was PCR-amplified as a complementary target sequences (Saiki and Erlich 1998). The tailed oligonucleotides act as a probe and labeled DNA as a target in the reciprocal SSOP method. This method allows a large number of samples to be analyzed by a probe in a single hybridization experiment. Although the SSOP method may need much initial effort to construct a system, the final result is a simple, robust and potentially automatable system that can be completed within a short experimental time. In addition, many samples, especially filters containing tailed different oligonucleotides, can be prepared at one time and stored until time of use. This convenience would increase the efficiency of identification process. This SSOP method has been used to determine the HLA-DQA locus genotype of >300 unknown samples in disease-susceptibility studies (Saiki *et al.* 1989).

Here, we describe a new method for the identification of wood-decaying fungi that combines the sensitivity of PCR-based analysis of rDNA gene diversity and the versatility of the SSOP differential hybridization method. We used eleven different wood-decaying fungi to prove the feasibility of this method in the identification of wood-decaying fungi. Fungus specific oligonucleotides in the ITS region of rDNA were immobilized onto membrane filters individually and the membrane filters were then hybridized to test whether each fungal target DNA amplified with ITS 1 and ITS 4 primer pairs can detect its own oligonucleotides on the membrane filter. This diagnostic method offers the sensitivity and reliability needed for early detection of wood-decaying fungi in wood, for species and/or isolate identification of pathogens, and for the study of microbial ecology.

Materials and Methods

Fungal species and culture conditions

The 11 different wood-decaying fungal species used in this study were *Antrodia carbinca* (AC), *Antrodia xantha* (AX), *Aureobasidium pullulans* (AUP), *Chaetomium globosum* (CG), *Gloeophyllum trabeum* (GT), *Irpex lacteus* (IL), *Phoma sp.* (PH), *Pleurotus ostreatus* (PO), *Postia placenta* (PP), *Rhinocladella atrovirens* (RA) and *Leptodontium elatius* (LE) (Table 1). They were grown on 5 % malt extract liquid medium (Difco) for 7–10 days at 30 °C with shaking at 150 rpm as described by Pfeifer and Khachatourians (1993).

Fungal DNA isolation and PCR amplification of ITS region

The fungal DNA was prepared from frozen mycelia using a modified phenol-chloroform extraction method (Zeller and Levy 1995). The resulting DNA was used as a template to amplify the ribosomal DNA ITS regions. The ITS primers used were ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.* 1990). PCR was carried out using 1X PCR buffer, 0.5 µl Taq polymerase (Promega, Madison, WI, USA) and 100 pmol each of the ITS1 and ITS4 primers. The PCR reaction was carried out for 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min using a Perkin-Elmer 2400 PCR machine. The PCR-amplified fragments were cloned into the pGEM® –T vector (Promega) and sequenced at the Genomics Technology Support Facility (GTSF) at Michigan State University.

Sequence-Specific Oligonucleotide Probes (SSOP)

Multiple sequence alignment among the rDNA sequences from the 11 fungal strains was carried out using the Cluster W algorithm (Thompson *et al.* 1994) to design strain-specific oligonucleotides. Oligonucleotides (14–25 nucleotides in length) were

Table 1. The list of the names of eleven wood-inhabiting fungi, accession numbers of ITS sequences, and sequences of oligonucleotides used for this study

Species (Abbreviation)	Accession Number	Oligonucleotide Sequence (5'→3')
Antrodia carbinca (AC)	AF423113	CTTCTTATGAGGACTTGTTG
Antrodia xantha (AX)	AF423115	GGTCGGTTTGAGTGGTTCTGTC
Aureobasidium pullulans (AUP)	AF423114	ACCAAATCGAACGTCTTATAAGCTT
Chaetomium globosum (CG)	AF423116	ACCAACCAAAACTCTTATT
Gloeophyllum trabeum (GT)	AF423117	TGATAATTGTCTACGCCGGGTC
Irpex lacteus (IL)	AF423118	GAGGGCTTCGGTTGA
Leptodontidium elatius (LE)	AF475152	CCAGGCTACCGGCT
Phoma sp. (PH)	AF423119	TATCAGCAAAGGTCTAGCATCCC
Pleurotus ostreatus (PO)	AF423120	GTGTGCCCCGGATC
Postia placenta (PP)	AF423121	GTAGCTAACACCTCGCGACTGG
Rhinocladella atrovirens (RA)	AF423122	GGTTACACCTGCCAACAACCC

designed based on the sequences of the hyper-variable region about each fungal ITS region identified from the multiple alignment and GeneRunner program (Hastings Software, Hudson, NY, USA). The oligonucleotides for SSOP were synthesized at the Macromolecular Structure Facility at Michigan State University. A deoxyribothymidine homopolymer tail, poly (dT), was added to the 3' end of each oligonucleotide by terminal deoxy nucleotidyl transferase (TdT) reaction as described by Saiki *et al.* (1989). The lengths of the homopolymer tails were controlled by limiting dTTP. After the tailing reaction, the sizes of incorporated dTTP (about 400 bp) were monitored by EtBr agarose gel electrophoresis.

Preparation of immobilized SSOP filters

The tailed oligonucleotides were diluted into 45 µl of TE (10mM Tris·HCl/0.1 mM EDTA, pH 8.0) and spotted onto a nylon membrane (Dall Biodyne B, 45 mm) as an array of dots using a Bio-Dot microfiltration apparatus (Bio-rad, Hercules, CA, USA). The membranes were then placed in a Stratalinker® UV cross linker (Stratagen, La Jolla, CA, USA) and irradiated at 254 nm. Membranes were rinsed with water, air-dried and stored at room temperature until needed.

Target DNA synthesis and hybridization

The ITS region of fungal rDNA was amplified and labeled in 100 µl PCR-reaction solution containing genomic DNA (5 to 30 ng) as template, 100 pmol each ITS1 and ITS4 primers, 2 mM dNTP, 20 µM fluorescein-11-dUTP (Amersham, Piscataway, NJ, USA), and 1 µl Taq polymerase. The labeled ITS region of fungal rDNA was used as a target DNA. The PCR reaction was carried out as described above. The synthesized probes were confirmed by Et-Br agarose gel electrophoresis. Filters with each bound fungus SSOP were pre-hybridized in hybridization solution containing 5X SSC (760 mM NaCl, 70 mM Sodium Citrate/l, PH 7.0), 0.5 % sodium dodecylsulfate (SDS), 1/20 diluted liquid block (Amersham). The PCR-amplified target DNA was denatured by heating for 5 min at 94 °C and added immediately to the hybridization solution, which was then incubated overnight at either 40 °C (for AX, GT, PH, PP, AUP and RA), or at 53 °C (for AC, CG, PO, IL and LE). After hybridization, the filters were washed twice for 10 min in wash solution containing 2X SSC and 0.1 % SDS at the hybridization temperature. The signals were developed with the Anti-FITC alkaline phosphatase conjugate (Sigma, St. Louis, MO, USA) in DSP-Start Reaction solution (Amersham) and visualized on a Kodak X-ray film after 1–5 min exposure. Briefly, the hybridized membrane was rinsed in 1X buffer A (100mM Tris-HCl, 300mM NaCl, PH 7.5) and incubated with blocking solution (1X buffer A, 1/10 diluted liquid block) at room temperature for 30 min as a blocking procedure. The diluted (5000 fold) Anti-FITC alkaline phosphatase conjugate was prepared with 0.5 % (W/V) bovine serum albumin in 1X buffer A and incubated (0.3 ml/cm^2 of membrane) with gentle agitation at room temperature for 1 h. After incubation, the unbound conjugate was removed by washing for 3 × 10 minutes in 0.3 % (V/V) Tween 20 in 1X buffer A. Signal generation and detection was carried out using DSP Star ™ detection reagent and X-ray film. The DSP Star ™ detection reagent was spread evenly over the blot. After applying the reagent, the blot was incubated for 5 min at room temperature and then exposed to X-ray film.

Wood samples from field tests

About 1 g of decayed sample was collected from each of 5 wood stakes from fungal decay field tests and used for extraction of fungal DNA. The 5 wood samples were individually incubated with fungal DNA extraction buffer described above and stirred for 10 min at room temperature. DNA extraction followed the modified phenol-chloroform method outlined previously. The DNA isolated from each wood sample was used for PCR amplification of the fungal ribosomal ITS region using ITS 1 and ITS 4 primers as described above. The amplified fungal DNA PCR products were used for target DNA synthesis. The florescent-labeled target DNA was used for hybridization with immobilized SSOP filters which had species-specific oligonucleotides of the 11 fungal wood-decaying fungi as target DNA. Hybridization and signal development were carried out as described above. The PCR products showing sequence-specific hybridization to the filter were cloned into pGEM®–T vector (Promega) and sequenced at the GTSF. The sequence of the individual clones were compared with the ITS regions of 11 fungal species and BLAST search (http://www.ncbi.nlm.nih.gov/BLAST) was performed.

Results

PCR amplification of fungal ribosomal ITS region

Genomic DNA was successfully isolated from the mycelia of the eleven fungal species and the five decayed wood samples using the phenol-chloroform extraction method. These genomic DNAs were subjected to PCR-amplification of the ITS region of the rDNA using ITS1 and ITS4 primers. The amplified PCR products of the ITS region were size fractionated by agarose gel electrophoresis and are approximately 650 bp in length (Figs. 1 and 4). There were slight size-differences among the PCR products of each fungal ITS region. The DNA sequences of the individual PCR products of the eleven different fungal strains have been deposited into the GenBank (Table 1).

Preparation of immobilized SSOP filters

Multiple sequence alignment analysis of the PCR-amplified ITS regions showed two 'hyper-variable' regions (ITS I and ITS II) separated by the 5.8 S rDNA region (data not shown). Eleven SSOPs were designed from the ITS I or ITS II regions of the eleven fungal strains following the guidelines for SSOP design outlined by Saiki and Erlich (1998). First, the oligonucleotides should have a uniform length and one melting temperature. Second, the region showing maximum specificity against the other species should be selected. Third, any sequences with potential for the formation of secondary

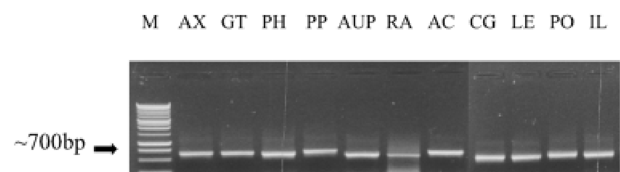

Fig. 1. Amplification of wood-decaying fungal ITS I and ITS II region by using the ITS 1 and ITS 4 primer pair. Fungi genomic DNA was used as a template, and the PCR products were separated with 1 % EtBr-agarose gel. M; Marker.

structure (*e.g.*, hairpin loops, dimers) should be avoided because secondary structures inhibit the hybridization with target DNA. Although all of our initial SSOPs were designed to have one melting temperature at approximately 53 °C and to be used at one hybridization stringency at 40 °C, only the probes from the fungal strains AC, CG, PO, IL and LE showed sequence-specific hybridization at the hybridization temperature. The initial probes from the AX, GT, PH, PP, AUP and RA strains showed no specific hybridization at 37 °C, 40 °C, 42 °C or 45 °C. The reason for this failure remains to be studied. A new set of SSOPs was designed for those six fungal strains (AX, GT, PH, PP, AUP and RA). The melting temperature of the new oligonuclotides was raised to 68 °C by adding several nucleotides to the existing sequences. Sequence-specific hybridization was achieved when the new SSOPs filters were hybridized at 53 °C. The sequence of the final eleven SSOPs is shown in Table 1. The two-stringency SSOP filter system worked for the eleven fungal species tested in this study. Consequently, two sets of SSOP filters (high- and low-stringency) were developed and used to test our hypothesis that SSOP can be designed based on the ITS sequences of fungal rDNA genes and used for detection of incipient fungal contamination of wood products. Along with these SSOPs, an oligonucleotide (5'-CATCGATGAAGAACGCAGCG-3') designed from a highly conserved internal region of 5.8 S rDNA fragment was blotted to the filters as a positive control.

Before being covalently bound to the nylon membrane filters, homopolymer poly(T) tails were added to the synthetic oligonucleotides by terminal deoxyribonucleotidyl transferase reaction. The negatively charged poly(T) tail facilitates binding of the probes onto the positively charged filter while leaving the oligonucleotides available for sequence-specific hybridization. Therefore, the length and uniform size of the homopolymer tailing reaction is critical. It appears that a 400bp-tail gives optimal sequence-specific hybridization (Saiki *et al.* 1989). When the tails were substantially shorter or longer than 400 bp, the sequence-specific hybridization did not occur (data not shown). The size of the tailed-oligomer was monitored by EtBr-agarose gel electrophoresis. A uniform size of poly(T) tailing was achieved by controlling the concentration of dTTP in the reaction mixture. With the addition of 80 nmol dTTP to the terminal transferase reaction mixture, we obtained oligonucleotides with about 400 bp tails (data not shown).

Labeled target DNA synthesis and hybridization

The PCR-amplified ITS regions of the eleven different fungal species were fluorescent-labeled and used as target DNAs in the hybridization experiments. The hybridized SSOP filters were exposed to X-ray film for signal development (Figs. 2 and 3). Figures 2A and 3A show that the SSOPs were specifically identified with their own labeled-target DNAs. In order to further confirm the sequence specificity of the immobilized SSOPs, multiple detection ability of the target DNAs was evaluated using two, three or four target-DNA combinations (Fig. 2B, C and D for AX, GT, PH, PP, AUP and RA, Fig. 3B and C for AC, CG, LE, PO and IL). The results showed that the SSOP method is very sensitive at discriminating between the different fungus species when tested using combinations of fungal target DNAs. The results from these multiple target DNA hybridization experiments proved that the immobilized SSOPs had high sequence specificity and ability to discriminate the target species from mixed fungal strains. The ability to distinguish specific fungal strains is especially important for this SSOPs-based protocol to be practical in detecting and identifying the incipient wood-decaying fungi in wood products, which are usually infected with multiple fungal strains.

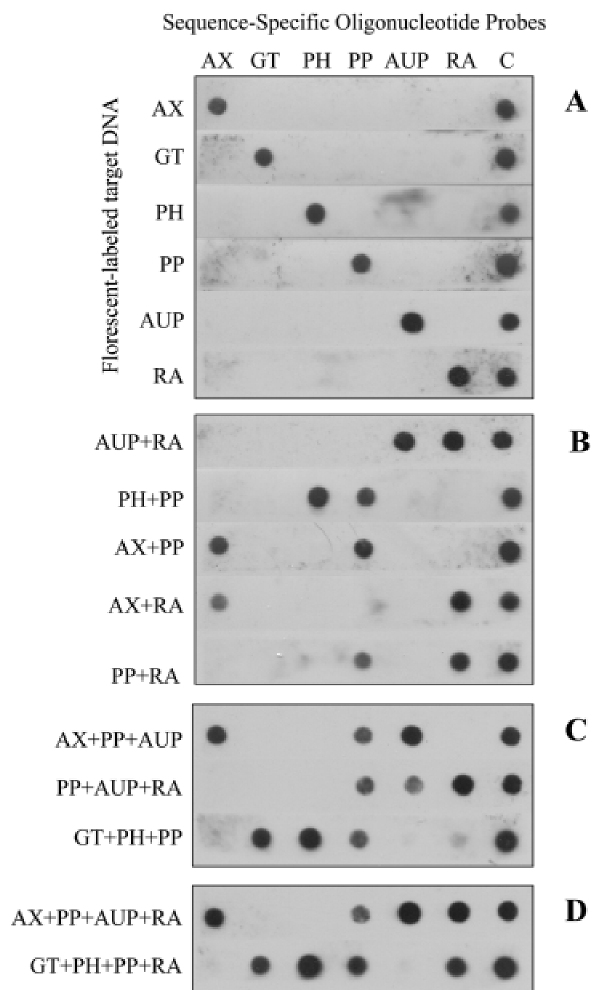

Fig. 2. Hybridization of six SSOP membrane filters with their labeled target DNA, AX, GT, PH, PP, AUP and RA . The hybridizations were carried out at 53 °C for overnight. Each florescent-labeled target DNA was hybridized with its complementary immobilized SSOP membrane filter (A). Multiple combinations of two target DNA (B), three target DNA (C) and four target DNA (D) were carried out. C; positive control.

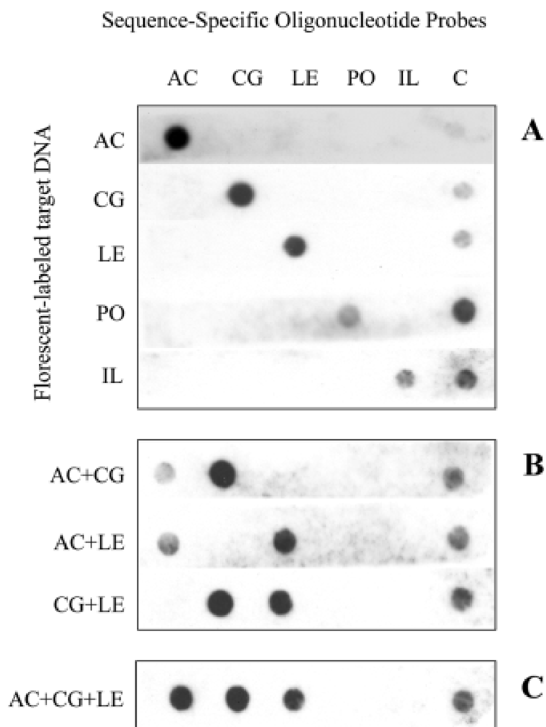

Fig. 3. Hybridization of five SSOP membrane filters with their labeled target DNA, AC, CG, LE, PO and IL. The hybridizations were carried out at 40 °C overnight. Each florescent-labeled target DNA was hybridized with its complementary immobilized SSOP membrane filter (A). Multiple combinations of two target DNA (B) and three target DNA (C) were carried out. C; positive control.

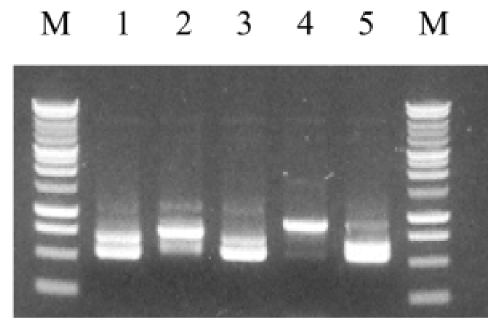

Fig. 4. Fungal genomic DNA from decayed samples (No.1 through No.5) was isolated and used for PCR-amplification of rDNA ITS I and ITS II region with ITS 1 and ITS 4 primer pair. All PCR-products of No. 2 were cloned into the pGEM-T vector (Promega) and sequenced. M; 1 kb DNA molecular marker (Promega).

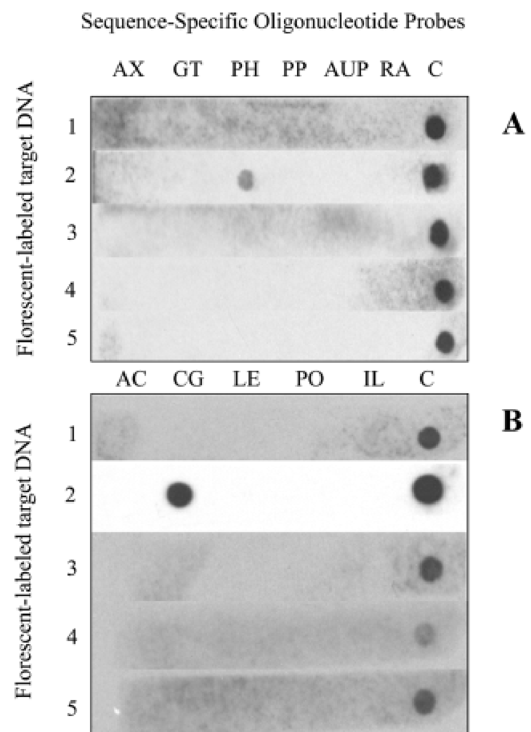

Fig. 5. Hybridization of eleven SSOP membrane filters with five florescent-labeled target DNA from five decayed wood samples, No. 1 through No. 5. Only one target DNA from No. 2 wood sample was hybridized with two SSOP, CG and PH. C; positive control.

Tests with decayed wood samples from field test

In addition to the *in vitro* cultures of the eleven fungal species, five naturally decaying wood samples from field tests were used to prove the utility of the fungal DNA diagnosis method. The wood samples showed severe natural decay (about 50 % biomass loss). Therefore, although the samples were not artificially inoculated, it was assumed that all five wood samples were infected with various wood decay fungi. Genomic DNA was successfully isolated from the naturally decaying wood samples. The fungal ribosomal DNA ITS region was PCR-amplified from the genomic DNAs using the ITS 1 and ITS 4 primer pair. Several different sizes of PCR products were generated from each wood sample, indicating the presence of multiple species of wood-decaying fungi (Fig. 4). The entire PCR products were used as a template to synthesize labeled-target DNA. When the SSOP filters were hybridized with these florescent labeled target DNAs, only one (wood sample #2) out of the five wood samples developed a positive signal with the SSOP probes derived from the fungal species CG and PH (Fig. 5), suggesting that the wood sample #2 was infected with the fungal species CG and PH along with several other fungal species that are not represented among the 11 SSOPs used in this study. We then attempted to determine the sequences of the ITS regions of the other fungal species. The PCR products from the sample #2 were successfully cloned into pGEM®–T vector, which was later transformed into *E. coli* cells. The sequences of the inserts (*i.e.*, ITS regions) from 17 randomly selected clones were determined and compared with the ITS sequences of the 11 fungal species used in the SSOP filters. Of the 17 clones, two matched with the

ITS sequences of CG and one with PH, confirming that the sample was infected with CG and PH. None of the other sequences matched with any of the ITS sequences from the eleven fungal species. With future representation of additional fungal species on the SSOP filters, we expect to identify more fungi that caused wood decay in the samples. Our data clearly demonstrate that the SSOP-based detection method described here is robust in detecting and identifying fungal species in naturally decaying wood samples. In order for this method to be practical, the filters will have represent a wide range of wood decay fungal species. Recent advancement in gene microarray technology makes it technically feasible to design a large number of sequence-specific oligonucleotides as demonstrated in Affymetrix gene chip technology.

Discussion

Many researchers have tried to develop a reliable detection method for wood decay fungi (Henrion *et al.* 1992; Schulze *et al.* 1997). Morphology-based identification methods have traditionally been used for detection of wood-decaying fungi. However, it often takes up to two months (Kim *et al.* 1999). PCR-based detection approaches have been introduced as alternatives to the time-consuming morphology-based method. Random amplified polymorphic DNA (RAPD) of the ITS region of the rDNA has been used to detect white rot fungus species, *Ganoderma lucidum* (Hsen *et al.* 1996) and internal wood decay fungi (Schmidt and Moreth 1998). While this offers a sensitive method for identifying a group of fungal species that share DNA sequence homology, it is not possible to distinguish individual species within the group. An improved version of ITS-RAPD, called Amplified Ribosomal DNA Restriction Analysis (ARDRA), has been developed and used to identify many wood-decaying fungi (Annamalai *et al.* 1995; Schmidt and Moreth 1999; Jasalavich *et al.* 2000). The ARDRA is based on the differential restriction enzyme digestion patterns due to the repetitive units of the nuclear rDNA consisting of conserved coding and variable non-coding regions (*i.e.*, ITS regions). The two ITS regions of an rDNA can be PCR-amplified using universal primers derived from the conserved regions (White *et al.* 1990) and subsequently analyzed by restriction enzyme digestion (ARDRA-ITS) to generate fungal strain-specific restriction patterns that can be used as a marker for strain identification (Nho *et al.* 1997). However, this ARDRA-ITS suffers from several weaknesses. First, the number of restriction enzymes that have recognition sites within an ITS-sequence are limited. Second, some enzymes generate restriction fragments whose size difference is not distinguishable in a subsequent gel analysis. For example, identical ARDRA-ITS patterns have been reported for *Serpula* spp. (Schmidt and Moreth 1999) and the ascomycetes *Monosporascus* spp. (Lovic *et al.* 1995). Later, restriction enzyme digestion differentiated between the fungal species tested. Third, ribosomal DNA is generally one of the heavily methylated regions of the genome and the methylation pattern can be changed during fungal growth. Therefore, any restriction enzymes that are methylation sensitive can cause misleading variation in the restriction patterns. The fungi detection method we report here overcomes the problems associated with ARDRA by using sequence-specific hybridization of immobilized species-specific probes with PCR-amplified rDNA ITS regions of fungal species in question. The SSOP method is sensitive and robust for identification of wood-decaying fungi.

A robust DNA isolation protocol is critical for any DNA-based diagnostic system to be successfully applied to detect and identify wood decay fungi from various types of samples. Kim *et al.* (1999) reported that the use of microwave oven reduced the time required to complete fungal DNA isolation. The protocol we describe in this report was effective in obtaining high quality DNA for PCR amplification not only from fungal mycelia, but also from naturally decaying wood samples.

Hybridization and wash temperature are important factors for determining stringency during SSOP design (Saiki and Erlich 1998). Salt concentration in the hybridization buffer is another important factor that needs to be optimized. While we did not determine the optimum salt concentration in the current study, we highly recommend that the salt concentration be optimized in future development of any SSOP-based fugal DNA diagnosis protocol. Alternatively, separate SSOP filters with different melting temperatures of the oligonucleotides and stringency can be produced and used.

Sequence-specific oligonucleotides have been used as a probe in various Southern/northern hybridization experiments or PCR primers for the taxon-specific priming PCR (Moreth and Schmidt 2001). The oligonucleotides were used as a probe after the end labeling reaction in that method. Saiki *et al.* (1989) used SSOPs in a reverse Southern blot analysis, where the probes were immobilized onto a nylon membrane and hybridized with genomic DNAs. By adding oligo-(dT) tailing to the SSOPs, they increased the immobilization and hybridization efficiency. In this study, we combined this “reverse Southern blot” concept with the ARDRA-ITS to develop a robust method for identification of wood-decaying fungi. This method takes advantage of both the sequence specificity of Southern blot hybridization and the sensitivity of the PCR-based fungal species identification methods. We demonstrated with both *in vitro* cultured fungal species and naturally decaying wood samples that the technique is robust and practical for detection of incipient wood-decaying fungi. It can be useful tool in microbial ecology, plant pathology, protection of wood products in service, preservation efforts for high-value furniture and wood-based art and DNA fingerprinting for keeping track of the source of contamination of wood decay fungi.

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