

## Specific Detection of *Fusarium* Species in Blood and Tissues by a PCR Technique

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***Fusarium* species are opportunistic nosocomial pathogens that often cause fatal invasive mycoses. We designed a primer pair that amplifies by PCR a fragment of a gene coding for the rRNA of *Fusarium* species. The DNAs of the main *Fusarium* species and *Neocosmospora vasinfecta* but not the DNAs from 11 medically important fungi were amplified by these primers. The lower limit of detection of the PCR system was 10 fg of *Fusarium solani* DNA by ethidium bromide staining. To test the ability of this PCR system to detect *Fusarium* DNA in tissues, we developed a mouse model of disseminated fusariosis. Using the PCR, we detected *Fusarium* DNA in mouse tissues and in spiked human blood. Furthermore, *F. solani*, *Fusarium moniliforme*, and *Fusarium oxysporum* were testing by random amplified polymorphic DNA (RAPD) analysis. The bands produced by RAPD analysis were purified, cloned, and sequenced. The information was used to design primer pairs that selectively amplified one or several *Fusarium* species. The method developed may be useful for the rapid detection and identification of *Fusarium* species both from culture and from clinical samples.**

Disseminated fungal infections constitute one of the most difficult challenges for clinicians caring for immunocompromised patients (3). Candidiasis and aspergillosis remain the most common mycoses in neutropenic patients. However, other life-threatening infections caused by new opportunistic pathogens also occur. One of the most frequently occurring of these pathogens is *Fusarium* (15, 19).

Members of the genus *Fusarium* are ubiquitous fungi commonly found in soils and plants (22). *Fusarium* species have long been recognized as a cause of localized infections (11). Because of bone marrow grafts and immunosuppressive therapy, invasive *Fusarium* infections have increased during the last decade. The immunologic status of the host and the extent of the infection are the most important factors for the clinical outcome of *Fusarium* infections (13).

Because an invasive *Fusarium* infection may mimic aspergillosis, patients are usually treated with amphotericin B, an antifungal agent with poor activity against fusariosis (9). Hence, early identification is an important factor for a successful outcome. Furthermore, diagnosis requires the demonstration of hyphae in pathological samples; however, hyphae of *Aspergillus*, *Scedosporium*, and *Fusarium* are difficult to discriminate. Positive culture is thus needed for the identification of a *Fusarium* sp. Currently, the identification of members of the genus *Fusarium* is based on the characteristic colony morphology and the microscopic characters, which include the production of multiseptated sickle-shaped conidia called macroconidia; however, recognition may be difficult when the macroconidia are not produced in culture (11). This usually happens with strains isolated from clinical samples which have been developed in unfavorable conditions. In this case, the isolates can be confused with other genera such as *Acremonium* and *Verticillium*. Furthermore, precise determination of *Fusarium* species remains a prerequisite for studying the spread, host infections, and treatment.

The PCR technique is extremely sensitive and has been used successfully for the specific detection of several fungi (20). We report here on the use of a competitive PCR technique for the detection of *Fusarium* spp. in blood and tissues and PCRs for the identification of the *Fusarium* species. In order to test the PCR system, we also developed a mouse model of fusariosis.

### MATERIALS AND METHODS

**Culture conditions.** DNA was isolated from several *Fusarium* species and a range of medically important fungi. The collection used is listed in Table 1. The *Fusarium* isolates were maintained on potato dextrose agar at 25°C. The other fungi were maintained on Sabouraud-chloramphenicol (SC) at 30°C. *Malassezia furfur* was cultured on Dixon agar at 37°C.

**DNA preparation from fungal cells.** The total cell DNA was extracted from mycelium or yeast grown in YPG liquid medium by the method described by Dellaporta et al. (8). Briefly, the mycelium and the yeast were mechanically disrupted within liquid nitrogen and were mixed with 15 ml of fungal extraction buffer (Tris-HCl, 10 mM; EDTA, 50 mM; NaCl, 500 mM), 1 ml of sodium dodecyl sulfate (10%) was added, and the mixture was incubated for 20 min at 65°C. Then, 5 ml of potassium acetate (3 M) was added, and the homogenate was incubated for 30 min on ice. After centrifugation, the DNA was precipitated with 0.7 volume of isopropanol, and the samples were centrifuged at 9,000 × g for 20 min at 4°C. The pellet was dissolved in 700 µl of Tris (Tris-HCl, 10 mM [pH 8]) and incubated at 65°C, and the DNA was precipitated with 0.7 volume of isopropanol and 0.1 volume of sodium acetate (3 M). The DNA was washed with 70% ethanol, dried, and resuspended in 200 µl of Tris (10 mM; pH 8).

**Oligonucleotide design, internal control, PCR amplification, and detection of PCR products.** The design of oligonucleotides P28SL and P58SL was based on comparison of the sequences of the ribosomal genes (rDNAs) from a large number of isolates belonging to the genus *Fusarium* found in the EMBL/GenBank database (Table 2). The sequences were analyzed with the PILEUP program of the Genetics Computer Group software package as reported earlier (9, 10). Primers P28SL and P58SL (Oligoexpress, Paris, France) amplified a fragment of 329 bp containing ITS2 and a portion of 5.8S and 28S rDNA.

To avoid false-negative results, a positive internal control was made from a part of λ phage DNA (4). We designed two primers, primers C1 and C2 (Table 3), whose 3' ends correspond to the λ DNA and whose 5' ends correspond to the primers used in the PCR amplification. The first PCR generated a fragment of 517 bp which contains the *Fusarium* sequence at the ends. This fragment was amplified with the *Fusarium* primers and was purified with the Qiaquick PCR amplification kit (Qiagen, Courtaboeuf, France). After dilution of the fragments, PCRs were performed. The highest dilution that gave a positive result after electrophoresis was chosen as the internal control. One microliter of internal control was added to each PCR mixture.

The PCRs were performed according to the manufacturer's instructions (Ready to go kit; Pharmacia Biotech, Uppsala, Sweden), with the following temperatures cycles: initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 68°C for 1 min, and extension at

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TABLE 1. Yeast and filamentous fungi screened by PCR

Species	Strains/Sources
<i>Fusarium moniliforme</i> .....	IP <sup>a</sup> 1090.74, IP 1550.84, IP 1579.85, IP 1685.87, IP 2333.95
<i>Fusarium solani</i> .....	IP 1195.79, IP 1272.81, IP 1684.87, IP 2330.95, IP 2447.97, IP 2451.98
<i>Fusarium oxysporum</i> .....	IP 625.72, IP 95.82, 9026
<i>Fusarium proliferatum</i> .....	IP 2240.94, IP 2290.94, IP 2295.95, 68G
<i>Fusarium dimerum</i> .....	IP 1516.83, IP 2356.96
<i>Fusarium semitectum</i> .....	2239
<i>Fusarium subglutinans</i> .....	2241, 2378
<i>Fusarium chlamydosporum</i> .....	IP 1542.84
<i>Fusarium nivale</i> .....	2238
<i>Fusarium equiseti</i> .....	IP 1707.87
<i>Fusarium anthophilum</i> .....	2367.96
<i>Acremonium strictum</i> .....	IP 2334.936
<i>Alternaria alternata</i> .....	CBS 1545-31
<i>Aspergillus flavus</i> .....	IP 2452.98
<i>Aspergillus fumigatus</i>	
<i>Aspergillus niger</i> .....	IP 1431.83 (ATCC 16404)
<i>Candida albicans</i> .....	IP 48.72 (ATCC 10231)
<i>Cryptococcus neoformans</i> .....	IP 960.67 (CDC B 236)
<i>Exophiala jeanselmei</i> .....	IP 69.52
<i>Malassezia furfur</i> .....	IP 2386.96
<i>Neocosmospora vasinfecta</i> .....	IP 2450.97
<i>Paecilomyces variotii</i> .....	IP 1024.70
<i>Penicillium purpurogenum</i> .....	9701
<i>Pseudallescheria boydii</i> .....	IP 1448.83
<i>Trichosporon cutaneum</i> .....	IP 2217.94

<sup>a</sup> IP, Collection of Fungi, Institut Pasteur, Paris, France.

72°C for 1 min. The thermal cycles were terminated by a final extension of 10 min at 72°C. One nanogram of template DNA was used per reaction mixture.

To ensure that no contaminating DNA could give a positive result, one sample without DNA was included in each series of reactions. To examine the specificity of the P58SL and P28SL primer pair, the samples of genomic DNA extracted from fungi and from human blood were tested to verify whether the primer pair selectively amplified the DNA from *Fusarium* (Table 1). To determine the lower limit of detection, PCRs were performed with water samples containing 1 ng, 100 pg, 10 pg, 1 pg, 100 fg, 10 fg, 1 fg, and 0.1 fg of genomic *Fusarium solani* DNA.

Aliquots (10 µl) of the PCR products were analyzed on a 2% agarose gel after electrophoresis in Tris-acetate-EDTA buffer. A 100-bp DNA Ladder (Boehringer Mannheim GmbH, Mannheim, Germany) was used as a molecular size marker. The gels were stained with ethidium bromide (1 µg/ml), visualized under UV light, and photographed with Polaroid 667 film.

**Preparation of PCR template from *F. solani*-containing blood.** Human blood (obtained from healthy volunteers after they provided appropriate written consent) was collected in tubes containing EDTA as an anticoagulant and was inoculated with 10-fold dilutions of *Fusarium* mycelium in sterile saline. The cells in the *Fusarium*-spiked blood samples were lysed (GFX genomic blood DNA purification kit; Pharmacia Biotech) and mixed with 200 µl of enzyme buffer (sorbitol, 0.9 M; Tris, 0.1 M; EDTA, 0.1 M) and 20 µl of Lyticase (Sigma, St. Louis, Mo.). Following incubation at 37°C for 90 min, proteinase K (10 µl, 20 mg/ml; Sigma) was added, and each sample was incubated at 55°C for 30 min, followed by the addition of 5 µl of RNase (Boehringer Mannheim GmbH). After incubation at 37°C for 30 min, the DNA was extracted by the use of the GFX genomic blood DNA purification kit, according to the manufacturer's instructions. PCR was performed by using these extracts as templates (2 µl). Aliquots (10 µl) of the PCR products were analyzed by electrophoresis on a 2% agarose gel as described above.

TABLE 2. GenBank accession numbers for the *Fusarium* DNAs used

Strain	GenBank accession no(s)
<i>F. oxysporum</i> .....	U28159, U28160, U28161
<i>F. solani</i> .....	L236629, L28682
<i>F. subglutinans</i> .....	U38554, U38555
<i>F. anthophilum</i> .....	U61645, U61671
<i>F. napiforme</i> .....	U34512
<i>F. nygamai</i> .....	U34539
<i>F. proliferatum</i> .....	U34500
<i>F. verticillioides</i> .....	U34526, U34555

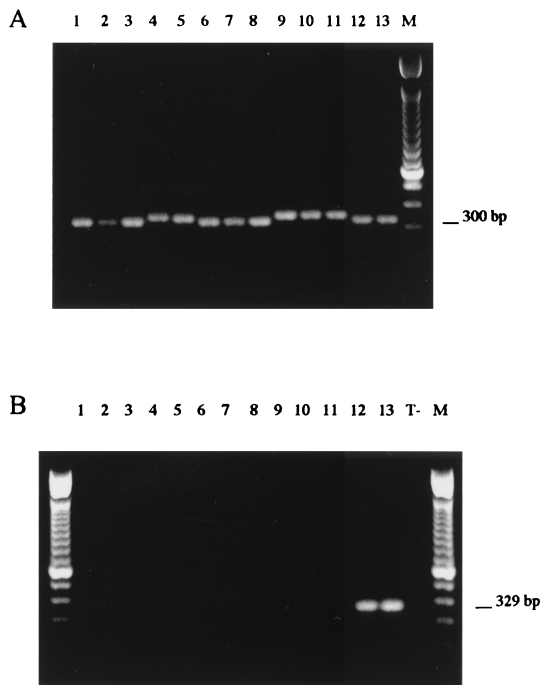


FIG. 1. Specificity of PCR with primer pair P58SL and P28SL. An ethidium bromide-stained agarose gel of PCR products amplified from 1 ng of genomic DNA templates from various fungi is shown. (A) Lanes: 1, *F. moniliforme* IP 1579.85; 2, *F. moniliforme* IP 233.95; 3, *F. solani* IP 2330.95; 4, *F. solani* IP 2447.97; 5, *F. solani* IP 2451.98; 6, *F. oxysporum* 9582; 7, *F. oxysporum* IP 625.72; 8, *F. oxysporum* 9026; 9, *F. proliferatum* 68G; 10, *F. dimerum* IP 1516.83; 11, *F. semitectum* 2239; 12, *F. subglutinans* 2241; 13, *F. nivale* 2238. (B) Lanes: 1, *Candida albicans*; 2, *Cryptococcus neoformans*; 3, *Penicillium purpurogenum*; 4, *Aspergillus fumigatus*; 5, *Acremonium strictum*; 6, *Trichosporon cutaneum*; 7, *Malassezia furfur*; 8, *Exophiala jeanselmei*; 9, *Trichophyton rubrum*; 10, *Alternaria alternata*; 11, *Aspergillus flavus*; 12, *Neocosmospora vasinfecta*; 13, *F. solani* IP 1681.87; lane T-, negative control. Lanes M, 100-bp DNA ladder.

TABLE 3. Nucleotide sequences of the primers

System and primer	Sequence
<b>Fusarium PCR</b>	
P58SL.....	5'-AGT ATT CTG GCG GGC ATG CCT GT-3'
P28SL.....	5'-ACA AAT TAC AAC TCG GGC CCG AGA-3'
<b>Internal control PCR</b>	
C1.....	5'-ACA AAT TAC AAC TCG GGC CCG AGA CCA CAG CGC-3'
C2.....	5'-AGT ATT CTG GCG GGC ATG CCT GTG TAC AAC TGG-3'

**Mouse model of invasive fusariosis.** Four male BALB/c mice (Charles River, Saint Aubin Les Elbeuf, France) weighing between 20 g and 25 g were immunosuppressed with three intraperitoneal injections of triamcinolone (Kenacort; Bristol-Myers Squibb, Paris, France) at a dose of 2 mg/kg of body weight on days -2, 0, and +3. On day 0, they were also inoculated in the lateral tail veins with  $10^6$  conidia of *F. solani* IP 1684.87 in a 200- $\mu$ l volume of sterile saline. One control mouse was treated in the same way and received 200  $\mu$ l of sterile saline on day 0. When the mice died, portions of organs and blood were removed. A total of 50  $\mu$ l of each blood sample was cultured on SC at 25°C. Various organs (spleen, lung, heart, kidney, and liver) were cultured on SC at 25°C. A small piece of each organ was retained for histology. These sections were stained with hematoxylin-eosin-safran and Gomori methenamine silver stains. The remainder of each organ was ground in a 1.5-ml microcentrifuge tube and was stored at -20°C. DNA from both blood and tissues was extracted by the protocol described above.

**Differentiation of *Fusarium* species.** For random amplified polymorphic DNA (RAPD) analysis, DNA fragments were amplified with primers F 24 and A8 (12), as follows: one cycle of 5 min at 92°C, followed by 36 cycles of denaturation at 92°C for 30 s, annealing at 36°C for 1 min, and extension at 72°C for 2 min, with a final cycle of 2 min at 72°C. The DNAs of the three main *Fusarium* species (*F. solani*, *Fusarium moniliforme*, and *Fusarium oxysporum*) were tested. Three high-intensity bands produced by RAPD analysis for each species were purified from agarose and were cloned into the vector pUC 18. The ends of the cloned fragments were sequenced according to the manufacturer's instructions (Thermosequenase Cycle Sequencing; Amersham Life Science, Buckinghamshire, United Kingdom), and the sequences obtained were used to design the primer pairs. The PCR amplifications and the analysis of the DNA were performed as described above.

## RESULTS

**Oligonucleotide design.** After alignment and visual assessment of the *Fusarium* and non-*Fusarium* sequences, primers pair P58SL and P28SL, which amplified a 329-bp fragment, was selected (Table 3).

**Specificity and sensitivity studies.** On the basis of the sequences of the 5.8S and 28S regions of the rDNA complex, a product of 329 bp was amplified by PCR with primer pair P58SL and P28SL from all 27 strains of 11 medically important *Fusarium* species and from *Neocosmospora vasinfecta* but not from any of the other fungi tested (Fig. 1). The specificity of

the 329-bp fragment was verified with the restriction enzymes *HincII* and *XbaI* (Gibco Life Technologies, Cergy-Pontoise, France); as expected, the 329-bp fragment was not digested by *XbaI* and gave two fragments of 200 and 129 bp upon *HincII* digestion. No amplification was observed with human DNA.

The sensitivity allowed detection of as little as 10 fg of *F. solani* DNA by visualization under UV light. There was no amplified product from the negative control (sample without *Fusarium* DNA).

**PCR template from *Fusarium*-containing blood.** As shown in Fig. 2, with primer pair P58SL and P28SL the PCR system was able to amplify the *Fusarium* DNA from blood artificially inoculated with mycelium. Amplified products were not detected from control samples that were devoid of *Fusarium* DNA.

**Mouse model.** A summary of mouse survival and the culture, histology, and PCR results is given in Table 4. Fifteen samples had positive results by culture. Seven culture-positive samples were also positive by PCR (with primer pair P58SL and

TABLE 4. Murine model of *F. solani* infection

Mouse no. (day of death)	Sample no.	Sample	Culture result	Histology result	PCR result
1 (+9)	1	Brain	+	-	-
	2	Heart	+	-	-
	3	Liver	-	-	+
	4	Lung	+	-	ND <sup>a</sup>
	5	Spleen	+	-	-
	6	Kidney	+	+	+
	7	Blood	-	-	-
2 (+10)	8	Brain	+	-	+
	9	Heart	+	-	ND
	10	Liver	-	-	-
	11	Lung	-	-	-
	12	Spleen	-	-	-
	13	Kidney	+	+	+
	14	Blood	-	-	-
3 (+10)	15	Brain	-	-	+
	16	Heart	+	-	ND
	17	Liver	-	-	+
	18	Lung	+	-	-
	19	Spleen	+	-	-
	20	Kidney	+	+	+
	21	Blood	-	-	-
4 (+10)	22	Heart	-	-	ND
	23	Liver	+	-	+
	24	Lung	-	-	NC <sup>b</sup>
	25	Spleen	+	-	+
	26	Kidney	+	+	+
	27	Blood	-	-	-

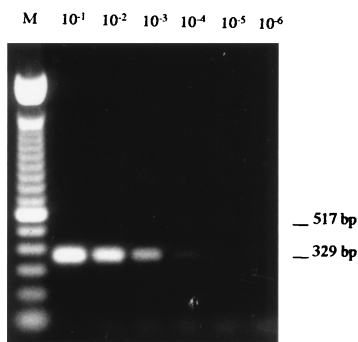


FIG. 2. Ethidium bromide-stained agarose gel of products from *F. solani* IP 1684.87-spiked blood samples in serial 10-fold dilutions. The fragment of 517 bp corresponds to the internal control. The fragment of 329 bp corresponds to amplified *F. solani* DNA. Lane M, 100-bp DNA ladder.

<sup>a</sup> ND, not done.

<sup>b</sup> NC, nonconclusive.

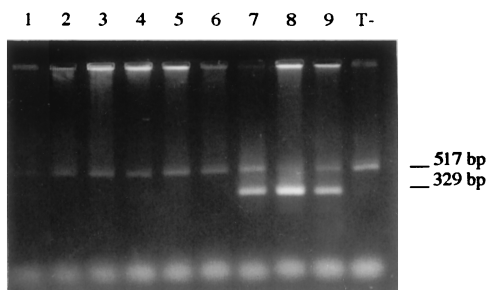


FIG. 3. Three possible results expected by competitive PCR with primers P28SL and P58SL. The results of gel electrophoresis of PCR products amplified from genomic DNA extracted from mouse organs are shown. The fragment of 517 bp corresponds to the internal control. The fragment of 329 bp corresponds to amplified *F. solani* DNA. Lane 1, inconclusive PCR result; lanes 2, 3, 4, 5, and 6, PCR-negative results; lanes 7, 8, and 9, PCR-positive results; lane T-, negative control.

P28SL), and 3 of 13 culture-negative samples also had positive PCR results. The result for sample 24 was nonconclusive: the fragment corresponding to the internal control was not observed (Fig. 3). Despite the use of proteinase K and a column extraction, amplification inhibitor factors may not have been eliminated. All PCR products led to the amplification of a single fragment. The result of PCR, culture, and histology were all negative for uninfected mice.

**Differentiation of the *Fusarium* species.** Table 5 shows the primer pairs that were selected and their characteristics. These primer pairs were used to perform new PCR assays. The use in the same PCR mixture of the two primer pairs OX 31–OX 32 and OX 41–OX 42 allowed the amplification of the *F. oxysporum* DNAs tested (Fig. 4). Although primer pair SOL 31–SOL



FIG. 4. Specificity of the primer pairs OX 31–OX 32 and OX 41–OX 42. An ethidium bromide-stained agarose gel of PCR products amplified from 1 ng of genomic templates from various fungi is shown. Lane 1, *F. oxysporum* IP 625.72; lane 2, *F. oxysporum* 9582; lane 3, *F. oxysporum* 9026; lane 4, *F. moniliforme* IP 1579.85; lane 5, *F. solani* IP 2330.95; lane 6, *F. proliferatum* IP 2240.94; lane 7, *F. dimerum* IP 1516.83; lane 8, *F. semitectum* 2239; lane 9, *F. subglutinans* 2241; lane 10, *F. chlamydosporum* IP 1542.84; lane 11, *Penicillium purpurogenum* 9701; lane 12, *Aspergillus fumigatus*; lane 13, *Acremonium strictum* IP 2234.96; lane 14, *Candida albicans* IP 48.72; lane T-, negative control. Lane M, 100-bp DNA ladder. pb, base pairs.

32 was specific for *F. solani*, only four of five strains tested were amplified (Table 5).

**DISCUSSION**

The results obtained in the present study demonstrate that the rDNA-based PCR method has high degrees of sensitivity and specificity for the detection of a wide range of medically important *Fusarium* species from spiked blood and from tissue from an animal model.

Several previous studies have used PCR technology to detect fungi (20). The primers used target three kinds of sequences: (i) sequences coding for an antifungal target, (ii) fungus-specific regions of conserved proteins such as actin, and (iii) re-

TABLE 5. Specificity and characteristics of species-specific primer pairs

Primer	Sequence (5'-3')	Specificity		Annealing temp (°C)
		Positive PCR result	Negative PCR result	
OX 31 OX 32	TGA CTT GGA TGA GAC CTT GGC G CAG GAT TTA CCG ACA CAG CTT TTG	<i>F. oxysporum</i> 95, 82, and 9026	<i>F. moniliforme</i> , <i>F. solani</i> , <i>F. oxysporum</i> IP 625.72, <i>F. proliferatum</i> , <i>F. dimerum</i> , <i>F. subglutinans</i> , <i>F. semitectum</i> , and <i>F. chlamydosporum</i>	66
OX 41 OX 42	GTA GGA AAA ACA ATT GCT CAG TCG AAG AGA GTG TGT AGT GGT GTG G	<i>F. oxysporum</i> 9582 and IP 625.72	<i>F. moniliforme</i> , <i>F. solani</i> , <i>F. oxysporum</i> 9026, <i>F. proliferatum</i> , <i>F. dimerum</i> , <i>F. subglutinans</i> , <i>F. semitectum</i> , and <i>F. chlamydosporum</i>	66
OX 1 OX 2	GTC ACG ACA TTT TCA CAA GCT G GGT CTC TCG ATA ACT TTG ACA G	<i>F. moniliforme</i> , <i>F. oxysporum</i> , <i>F. proliferatum</i> IP 2240.94, and <i>F. subglutinans</i>	<i>F. solani</i> , <i>F. proliferatum</i> IP 2290.94, IP 2296.95, and 68G, <i>F. dimerum</i> , <i>F. chlamydosporum</i> , and <i>F. semitectum</i>	63
SOL 31 SOL 32	GCT ACC GAG GCC ATC AAT TCA TG TGA TGT TGT ACT TCT CCT TGC CC	<i>F. solani</i> IP 2451.98, IP 1195.79, and IP 2330.95	<i>F. moniliforme</i> , <i>F. solani</i> IP 1984.87, <i>F. oxysporum</i> , <i>F. proliferatum</i> , <i>F. subglutinans</i> , <i>F. semitectum</i> , <i>F. dimerum</i> , and <i>F. chlamydosporum</i>	62
MON 1 MON 2	GAG AGC TGG ATG TAC GAA TG CAC AGA GAT GGT TCA CTG AG	<i>F. moniliforme</i> , <i>F. oxysporum</i> , <i>F. solani</i> , <i>F. subglutinans</i> , and <i>F. proliferatum</i>	<i>F. semitectum</i> , <i>F. dimerum</i> , and <i>F. chlamydosporum</i>	60



peated sequences such as those of rDNA and mitochondrial DNA. For diagnostic purposes, it is essential that repeated sequences be used as targets to ensure good sensitivity, which justifies our choice of primers. We reported that the binding sites for primer pair P58SL and P28SL, within the 5.8S and the 28S regions, were conserved among *Fusarium* species. They amplified DNAs from all 11 different *Fusarium* species tested and also from *N. vasinflecta*, which is a sexual form of an *Acremonium* sp. whose species name is not yet defined. Only two cases of infection with the latter species have been reported in humans (7).

One of the limitations of the molecular methods of diagnosis of microbial infections, especially PCR amplification, are the false positivity and the false negativity of the test results. These issues are being addressed in several ways: standardization of the technical procedure (one-step procedure) and the use of an internal control (2). Impurities in nucleic acid preparations (e.g., ethanol and isopropanol) or in biological samples (e.g., hemoglobin, EDTA, and heparin) can inhibit or reduce the sensitivity of PCR amplification (16). Thus, the internal control is necessary both for clinical diagnosis and for the development of a DNA extraction procedure.

Although many studies regarding the PCR detection of yeast (5, 6, 17, 18, 21, 24) and *Aspergillus fumigatus* (25) have been reported, few PCR systems for the detection of other fungi have been developed (1, 26). Moreover, the PCRs described for the latter case have not been tested with blood or tissues. For this reason, we thought it desirable to test the PCR with primers P28SL and P58SL for the detection of *Fusarium* both in spiked blood and in tissues and blood from experimentally infected mice. In the mouse model, the correlation between PCR results and culture results was not high (46%); however, 23% of the samples with culture-negative results were positive by PCR amplification. One explanation is the poor efficiency of the extraction protocol. Another explanation is the existence of a localized necrotic abscess. Indeed, before extraction histology and culture, each sample was divided into three portions, but not all portions may have been infected.

Different molecular methods are used to differentiate species of fungi: restriction fragment length polymorphism (RFLP) analysis (14, 16) or RAPD analysis (23). RFLP analysis is long and laborious and often requires the use of radioactive probes. RAPD analysis is poorly reproducible, and results are difficult to interpret. Furthermore, RAPD and RFLP analyses require axenic cultures of the fungus, whereas PCR can be performed with tissue samples. PCR should be considered as complementary method for the identification of *Fusarium* species when microscopic characterization cannot distinguish between two species.

Further experiments will be necessary to determine whether specificity and sensitivity similar to those obtained in vitro and with spiked blood samples can be obtained with clinical samples. Larger prospective studies that focus on immunocompromised patients at high risk of invasive fusariosis should evaluate both the utility and the sensitivity of this PCR assay in a routine setup in comparison to those of other available methods (culture and histology).

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