Original Report

Antigenic Relations between Pathogenic *Paecilomyces lilacinus* and *P. variotii* and Other Hyalohyphomycotic Agents

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ABSTRACT

Objective: The antigenic relations between *Paecilomyces variotii* and *Paecilomyces lilacinus* were studied by comparison of their exoantigens.

Methods: Forty-one isolates of hyalohyphomycosis-causing fungi including *P. lilacinus* (n = 15) and *P. variotii* (n = 12), one isolate each of *Paecilomyces inflatus*, *Paecilomyces marquandii*, and *Paecilomyces carneus*, *Fusarium* species (n = 3), *Neosartorya pseudofischeri* (n = 5), and *Penicillium* species (n = 3), were studied using the exoantigen test. Ten-day-old extracts derived from Sabouraud's dextrose agar slants were concentrated (25×), and reacted against rabbit anti-*P. lilacinus* and anti-*P. variotii* sera in the presence of partially purified homologous antigens (20×) prepared from 5-week-old shaken cultures. Extracts of isolates of *P. lilacinus* and *P. variotii* produced one to three lines of identity against the reference homologous antisera. No cross-reactivity or bands of non-identity were observed with other *Paecilomyces* isolates, *N. pseudofischeri*, *Fusarium* species, and *Penicillium* species isolates.

Conclusion: The results of this investigation indicate that *P. lilacinus* and *P. variotii* are antigenically distinct and that they can be identified rapidly and accurately by their exoantigens. Exoantigen extracts of morphologically similar fungi and other species of *Paecilomyces* isolates studied did not possess antigens common to *P. lilacinus* and *P. variotii*.

Key Words: *exoantigens, micro-immunodiffusion,* Paecilomyces lilacinus, Paecilomyces variotii

Int J Infect Dis 1997; 2:37–39.

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Received: November 2, 1996; Accepted: April 12, 1997.

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Paecilomyces species are becoming increasingly important in the infection of diverse patient populations, including cancer patients and transplant recipients. Generally, these fungi occur worldwide as saprophytes in soil and organic debris and as insect and animal pathogens. Since they are ubiquitous and frequently resistant to clinical laboratory cleaning solutions,[1,2] they are also frequently recovered as laboratory contaminants. To date more than 53 cases of hyalohyphomycosis, caused by various species of the genus *Paecilomyces* (such as *P. aerugineus*, *P. javanicus*, *P. lilacinus*, *P. marquandii*, *P. variotii*, and *P. viridis*), have emerged with pathologies including endocarditis, keratitis, onychomycosis, sinusitis, pyelonephritis, endophthalmitis, pulmonary infections, orbital granuloma, and cutaneous manifestations.

Cases of hyalohyphomycosis have been reported in both patients with and those without underlying diseases.[3,4] All cases of *Paecilomyces* species endocarditis and many *Paecilomyces* infections in leukemics have been fatal.[5–7] Castor et al reported the involvement of *P. lilacinus* in a renal transplant patient who developed a cutaneous infection on her forearm.[8] A systemic infection caused by *P. lilacinus* in an armadillo (*Dasypus novemcintus*), in which tissue forms in internal organs exhibiting budding yeasts and cuboidal forms, was reported by Gordon.[9] Of 53 cases recently described, isolates (>10%) were misidentified when the identification of *Paecilomyces* species was based on time-consuming traditional procedures using macro- and micromorphologic characteristics.[3,5] These fungi sometimes resemble other closely related mold species, such as the members of the genera *Aspergillus*, *Fusarium*, and *Penicillium* in their gross morphology and when present in tissue sections.

Exoantigen tests have been used successfully in the rapid and accurate diagnosis of atypical, nonsporulating cultures of a variety of fungi. To minimize misidentifications and improper diagnosis of *Paecilomyces* infections, the authors attempted to develop a cost-effective, simple, rapid, reliable, and specific exoantigen procedure for accurately identifying hyalohyphomycotic infections caused by species of *Paecilomyces*.

MATERIAL AND METHODS

Fungi

Forty-one isolates representing *P. lilacinus* (n = 15), *P. variotii* (n = 12), and *P. marquandii* (n = 1), and nonpathogenic *P. carneus* (n = 1), *P. inflatus* (n = 1), *Fusarium* species (n = 3), *Neosartorya pseudofischeri* (n = 5), and *Penicillium* species (n = 3) were studied.

Inoculum Production

Five milliliters of sterile distilled water were pipetted onto 7-day-old Sabouraud dextrose agar (SDA) (Difco Laboratories, Inc., Detroit, MI) slant cultures. The tubes were shaken vigorously for about 5 minutes. Microscopic examination of the resulting suspensions revealed a mixture of conidia and mycelial fragments.

Antigen Production

Antigens for *P. lilacinus* and *P. variotii* were obtained from shaken Sabouraud dextrose broth cultures.[10] Each Erlenmeyer flask, containing broth (300 mL/flask), received 5 mL of an inoculum suspension, after which, the flasks were placed on a gyratory shaker (150 rpm) at room temperature (25°C) for 5 weeks. At the end of the incubation period, approximately 30 mg of 1% aqueous solution of Merthiolate (Thimerosal, Sigma Chemical Company, St. Louis, MO) was added to each flask to kill the culture. The flasks were then shaken for an additional 72 hours at 25°C. Sterility checks were carried out on all the Merthiolated broth cultures.

Antigen Extraction

The Merthiolated culture filtrates of *Paecilomyces* species were freed of mycelium and other fungal elements by passing them through Whatman filter paper (grade 202) under suction. The clear filtrates were then sterilized by passing them through membrane filters (0.45 μ). The sterile filtrates were then treated with chilled acetone (1:3, v:v), shaken vigorously, and left at 0 to 2°C for approximately 20 hours. The acetone-filtrate mixture was centrifuged at 6000 × g (0°C), the supernatent was discarded and the precipitate was retained at room temperature for 45 minutes, to free it of acetone traces. The precipitate was then dissolved in phosphate buffered saline (0.1M, pH 7.2), equal to approximately one twenty-fifth of the volume of the original sterilized culture filtrate. It was then centrifuged (15,000 rpm/45 min at 0–2°C). This 25×-concentrated antigen, was Merthiolated (1% aqueous) and used for the production of antisera. All antigens were stored in 2-mL aliquots at -70°C following freeze-drying. As required, the freeze-dried antigen was reconstituted in deionized, distilled water (2 mL) and stored at 0 to 2°C. The extraction, preservation and storage procedures were the same as described elsewhere.[10]

Antiserum Production

New Zealand white rabbits were injected intramuscularly at weekly intervals with 0.25 mL of the antigens mixed with equal volumes of Freund's incomplete adjuvant (Difco Laboratories). The immunization schedule and method for the collection of the rabbit antiserum from the animal, its preservation, and storage have been described elsewhere.[10]

Extraction of Exoantigens

Ten-day-old cultures of the isolates of the *Paecilomyces* species, *N. pseudofischeri*, *Fusarium* species, and *Penicillium* species, grown on SDA slants at 25°C, were flooded with 0.02% aqueous Merthiolate (approximately 8–10 mL) and incubated for 24 hours. These extracts were then centrifuged at 18,000 rpm for 10 minutes at 0°C. After centrifugation, extracts were concentrated 25×, using Minicon B-15 concentrators (Amicon Corporation, Oakville, ON).

Performance of Exoantigen Tests

Concentrated exoantigen extracts of the 41 isolates were reacted with anti-*P. lilacinus* and *P. variotii* sera. The *P. lilacinus* and *P. variotii* antisera were also reacted against their homologous antigens (10 μL), in micro-immunodiffusion (MID) tests in 1% noble agar prepared in 0.1M phosphate buffer, pH 7.5. Details for performance of the MID technique have been presented elsewhere.[11-13]

RESULTS

The reactivity of the exoantigen extracts, derived from the 41 isolates belonging to *P. lilacinus*, *P. variotii*, *Paecilomyces* species, *N. pseudofischeri*, *Fusarium* species, and *Penicillium* species, tested against the rabbit anti-*P. lilacinus* and anti-*P. variotii* sera, are summarized in Table 1. Extracts of all the isolates of *P. lilacinus* and *P. variotii*

Table 1. Reactivity of Exoantigen Extracts of Pathogenic and Nonpathogenic Species of *Paecilomyces* and Other Hyalohyphomycotic Agents with Reference Rabbit Anti-*P. lilacinus* and Anti-*P. variotii* Sera

	Number and Type of Bands Produced after Reaction of Antigens with Reference Antisera to	
Culture Extract (Number of Isolates)	*P. lilacinus*	*P. variotii*
P. lilacinus (15)	1–3 ID	NR
P. variotii (12)	NR	1–3 ID
P. carneus (1)	NR	NR
P. inflatus (1)	NR	NR
P. marquandii (1)	NR	NR
Fusarium species (3)	NR	NR
N. pseudofischeri (5)	NR	NR
Penicillium species (3)	NR	NR

ID = Line(s) of identity; NR = nonreactive.

produced one to three lines of identity with their respective homologous reference rabbit serum. None of the extracts of the other species of *Paecilomyces*, *N. pseudofischeri*, *Fusarium* species, and *Penicillium* species demonstrated any reaction when tested against the rabbit anti-*P. lilacinus* and *P. variotii* sera (see Table 1).

DISCUSSION

A specific exoantigen test using unabsorbed *P. lilacinus* and *P. variotii* antisera was developed. Data demonstrated that the pathogenic isolates of *P. lilacinus* and *P. variotii* produced unique exoantigens and are antigenically unrelated. Isolates of each species produced one to three specific antigens. Neither *P. lilacinus* or *P. variotii* appear to share antigens with the morphologically similar *Fusarium* and *Penicillium* species. Results are consistent with findings reported earlier in studies with *Penicillium* and *Fusarium* species.[10,14]

Interestingly, *P. carneus*, *P. inflatus*, *P. marquandii*, *Fusarium* species, *N. pseudofischeri*, and the *Penicillium* species studied were antigenically unrelated to *P. lilacinus* and *P. variotii* sera (see Table 1). Because of their specificity in nonabsorbed *P. lilacinus* and *P. variotii* antisera, the method developed proved suitable for the immunoidentification of *P. lilacinus* and *P. variotii* isolates.

Since the hyphal elements of *Aspergillus*, *Fusarium*, *Paecilomyces*, and *Penicillium* species may look alike in stained histopathologic sections, and many of the pigment-producing isolates of these fungi, particularly those of pathogenic and nonpathogenic *Penicillium*, *Fusarium* and *Paecilomyces* may grossly or morphologically appear similar, it is important that all clinical isolates be identified with specificity and accuracy, using rapid and specific exoantigen tests. The data of the present investigation suggest that the exoantigen test is both rapid and specific (100%). The authors emphasize this point because the isolate of the first case of *P. lilacinus* infection was thought to be an isolate of *Penicillium marneffei*, based on its macro- and micromorphologies.[9] However, the exoantigen investigation carried out in the senior author's laboratory did not support this conclusion, and it was accurately identified later as *P. lilacinus*.[9] Furthermore, the use of rapid and specific tests for identification of emerging fungal species will aid in the cost-effective management of fungal infections.

ACKNOWLEDGMENT

The senior author would like express his deep gratitude to Dr. Libero Ajello, Professor, Eye Research Center, Department of Ophthalmology, Emory University School of Medicine, Atlanta, Georgia, for his critical suggestions and comments on the manuscript.

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