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Differentiation of *Aspergillus ustus* Strains by Random Amplification of Polymorphic DNA

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The sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of water-soluble proteins and the randomly amplified polymorphic DNA (RAPD) patterns of whole-cell lysates from 21 *Aspergillus ustus* isolates, including 11 reference strains and 10 patient and environmental strains from one hospital, were investigated. All isolates showed identical protein patterns. The RAPD assay discriminated between all reference strains. Comparison of hospital isolates showed identical RAPD patterns in some of the patient and environmental isolates. The data indicate that the RAPD technique is useful for fingerprinting *A. ustus*.

Aspergillus ustus is a rare cause of infections in humans. To date, only nine cases have been reported (5, 14). However, most cases were published after 1990, which indicates that this species might be an emerging pathogen. A large number of studies of the molecular epidemiology of *Aspergillus fumigatus* have been published (for a review, see reference 7). In contrast, data on the phenotypical and genotypical characteristics of other *Aspergillus* spp. are scant. Recently, a study of *Aspergillus terreus* infections in immunosuppressed patients showed that environmental and some patient isolates had identical band patterns by randomly amplified polymorphic DNA (RAPD) analysis (6). However, fingerprinting techniques to study *A. ustus* have not been established. Therefore, we chose to study the usefulness of two fingerprinting techniques (sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE] and the RAPD assay) for analyzing *A. ustus* isolates.

Eleven reference strains (Table 1) were obtained from international culture collections (Centraalbureau voor Schimmelcultures [CBS], Utrecht, The Netherlands; Biomedical Fungi and Yeast Collection [IHEM], Brussels, Belgium; National Collection of Pathogenic Fungi [NCPF], Bristol, United Kingdom). The remaining 10 isolates (AZN) were from the University Medical Center, Nijmegen, The Netherlands. A detailed description of these isolates and their origins (Table 1, isolates 12 to 21) is given elsewhere (14). An analysis of the protein patterns of water-soluble proteins of whole-cell lysates in Coomassie blue-stained SDS-PAGE gels (7.5%) was performed as described previously (11).

For the RAPD assay, extraction of DNA was performed as previously described (12). Briefly, mycelial cells (1 g [wet weight]) from 24-h-old broth cultures were disrupted by using glass beads and DNA was isolated by using phenol-chloroform, ammonium acetate, isopropanol, and RNase A.

Amplification was carried out with four single primers that have already been successfully used with other *Aspergillus* spp. (6, 10–12): primer A, 5′-GTA TTG CCC T-3′ (1); primer B, 5′-GCT GGT GG-3′ (8); primer C, 5′-TCA CCC TGG A-3′ (12); and primer D, 5′-$(GATA)_4$-3′ (13). The primers were run under the same conditions (master mix containing 3 mM $MgCl_2$, 200 pmol of each primer, and 5 ng of DNA; final volume, 100 μl; 45 cycles at 94°C for 1 min, 35°C for 2 min, and 72°C for 2 min in a Minicycler [MJ Research, Watertown, Mass.]). For primer E (5′-GAG GGT GGC GGT TCT-3′), which was used to fingerprint *Pseudallescheria boydii* and yeasts (9, 15), 4 mM $MgCl_2$ and an annealing temperature of 50°C were used. After electrophoretic separation in 1.6% agarose gels, the ethidium bromide-stained band patterns were analyzed by using Imagemaster 1D Elite software (Amersham Pharmacia Biotech, Freiburg, Germany). The similarity of band patterns was estimated by means of the Jaccard comparison, and the clustering was determined by the unweighted-pair-group method with averages. Band patterns of >90% similarity were classified as identical (3). Reproducibility of band patterns was demonstrated by analysis of two to three different subcultures of each strain.

By SDS-PAGE, all 21 *A. ustus* isolates showed numerous bands in the range of 115 to 20 kDa. Major bands were seen at 61, 57 to 55, 53, 47, 43, and 37 kDa (data not shown) but the patterns of the isolates did not differ. This method is obviously not useful for fingerprinting *A. ustus*. These same results were found for *A. fumigatus*, *Aspergillus niger* (10; our unpublished results), and *A. terreus* (11). In *Aspergillus flavus* and *Aspergillus* (*Emericella*) *nidulans*, however, different protein patterns were found within the species (10). The basis for the different phenotypic features of the species of *Aspergillus* is not known but may indicate genetic recombination or different subspecies.

In contrast to the phenotypic method, the RAPD analysis showed a high degree of discriminatory power. Amplification

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TABLE 1. Sources and RAPD types of 21 *A. ustus* isolates

Isolate[a]	Designation	Source (yr of isolation)	Type of RAPD pattern produced by primer[b]:					Combined type[c]
			A	B	C	D	E	
1	CBS 596.65	Sugar; Louisiana (1965)	A-1	B-1	C-1	D-1	E-1	A/D-1
3	IHEM 1139	Air; Brussels, Belgium (1982)	A-2	B-2	C-2	D-2	E-2	A/D-2
4	IHEM 9696	Indoor air (hospital); Marseille, France (1991)	A-3	B-3	C-3	D-3	E-3	A/D-3
5	CBS 209.92	Soils; La Palma, Spain (1992)	A-4	B-4	C-4	D-4	E-3	A/D-4
6	CBS 133.55	Textile buried in soil; Utrecht, The Netherlands (1955)	A-5	B-5	C-5	D-5	E-4	A/D-5
2	CBS 102278	Human, subcutaneous infection; Utrecht, The Netherlands (1999)	A-6	B-6	C-6	D-6	E-5	A/D-6
7	NCPF 2848	Human, brain; Bristol, United Kingdom (1991)	A-3	B-3	C-3	D-7	E-3	A/D-7
8	NCPF 2951	Human, sputum; Bristol, United Kingdom (1993)	A-3	B-3	C-3	D-8	E-3	A/D-8
9	NCPF 2922	Human, ethmoid sinus; Bristol, United Kingdom (1993)	A-7	B-3	C-3	D-3	E-3	A/D-9
10	NCPF 2744	Human, sputum; Bristol, United Kingdom (1990)	A-8	B-7	C-3	D-9	E-6	A/D-10
11	NCPF 2766	Human, mitral valve prosthesis; Bristol, United Kingdom (1990)	A-9	B-8	C-3	D-10	E-3	A/D-11
12	AZN 677	Bronchoalveolar lavage specimen, patient 1; University Medical Center, Nijmegen, The Netherlands (1992)	A-10	B-9	C-7	D-11	E-3	A/D-12
13	AZN 678	Sputum, patient 1; University Medical Center, Nijmegen, The Netherlands (1992)	A-10	B-9	C-7	D-11	E-3	A/D-12
14	AZN 682	Autopsy specimen, patient 1; University Medical Center, Nijmegen, The Netherlands (1992)	A-10	B-9	C-7	D-11	E-3	A/D-12
15	AZN 741	Hematology ward, room; University Medical Center, Nijmegen, The Netherlands (1993)	A-10	B-9	C-7	D-11	E-3	A/D-12
16	AZN 924	Hematology ward, room; University Medical Center, Nijmegen, The Netherlands (1993)	A-11	B-10	C-3	D-12	E-3	A/D-13
17	AZN 943	Laboratory contaminant; University Medical Center, Nijmegen, The Netherlands (1993)	A-12	B-10	C-8	D-13	E-3	A/D-14
18	AZN 2725	Sputum, patient 2; University Medical Center, Nijmegen, The Netherlands (1995)	A-13	B-10	C-8	D-12	E-3	A/D-15
19	AZN 3297	Feces, patient 3; University Medical Center, Nijmegen, The Netherlands (1995)	A-10	B-9	C-7	D-11	E-3	A/D-12
20	AZN 6989	Ascites fluid, patient 4; University Medical Center, Nijmegen, The Netherlands (1997)	A-11	B-10	C-3	D-12	E-3	A/D-13
21	AZN 7134	Hematology ward, room; University Medical Center, Nijmegen, The Netherlands (1997)	A-10	B-9	C-7	D-11	E-3	A/D-12

[a] For a detailed description of isolates 12 to 21, see reference 14.
[b] Numbers of different RAPD types for the following primers: A, 13; B, 10; C, 8; D, 13; E, 6.
[c] Results of the combined analysis of the RAPD patterns produced by primers A and D. The number of different RAPD types for the combined type was 15.

with primers A and D resulted in 13 different RAPD types of patterns each, whereas amplification with primers B, C, and E yielded 10, 8, and 6 different patterns, respectively. In Fig. 1, the cluster analyses of the band patterns of the 21 strains produced by primers A and D are depicted. The combined analyses of the patterns of these two primers resulted in complete discrimination of the 11 reference strains (Table 1, combined types A/D-1 to A/D-11). When investigating the isolates from patients and the environment from the hospital in Nijmegen, The Netherlands (isolates 12 to 21), we found that primer A showed the highest degree of discriminatory power with four different types (A-10 to A-13). Combined analysis of patterns produced by different primers resulted in no further discrimination (A/D-12 to A/D-15). As expected, all three of the isolates obtained from one patient (Table 1, isolates 12 to 14) showed identical patterns (RAPD combined type A/D-12). However, the same pattern was found in two environmental isolates obtained from the ward (isolates 15 and 21) and in one isolate obtained from a patient (isolate 19) from a different ward. Furthermore, two other isolates (isolate 16 from the environment and isolate 20 from a patient) showed an identical RAPD type (combined type A/D-13). This finding indicates that at least some RAPD combined types (A/D-12 and A/D-13) persisted over long periods (up to 6 years) in the environment. Similar results were found for *A. fumigatus* (4) and *A. terreus* (6).

The present data verify that RAPD analysis is useful for fingerprinting not only *A. fumigatus* (1, 7, 8, 10, 12), *A. flavus* (10), *A. nidulans* (10), and *A. terreus* (6, 11) but also *A. ustus* when the same primers (A and D) are used. Primer E (core sequence of phage M13), which was successfully used for fingerpinting *P. boydii* (9) and yeasts (15), showed a minor degree of discriminatory power in the present study.

Since other published techniques for fingerprinting aspergilli, e.g., the microsatellite PCR described by Bart-Delabesse et al. (2) or the Southern hybridization probe described by Debeaupuis et al. (4), work only with *A. fumigatus* (4; our unpublished results), the RAPD technique is at present

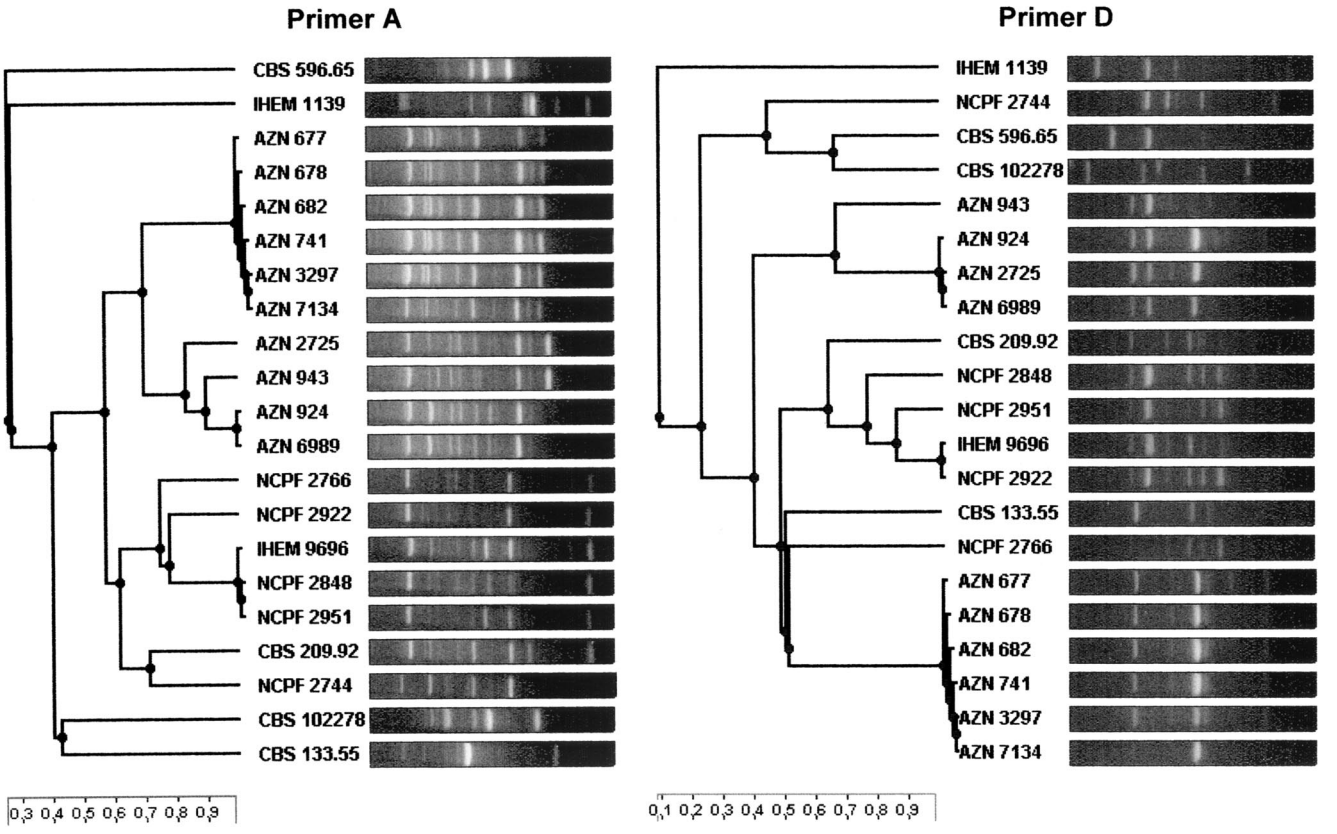

FIG. 1. Cluster analysis of RAPD patterns of 21 *A. ustus* strains (11 reference strains and 10 patient and environmental isolates from Nijmegen, The Netherlands) produced by primers A and D. The scales at the bottom represent the similarity index. AZN, isolates from Nijmegen, The Netherlands; CBS, NCPF, and IHEM, reference strains from culture collections.

the sole method which is generally applicable for the typing of various *Aspergillus* spp., including *A. ustus*.

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