DNA Fingerprinting Analysis of Vegetative Compatibility Groups in Aspergillus flavus from a Peanut Field in Georgia

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ABSTRACT

The ability of species-specific DNA probe pAF28 to correctly match 75 strains of Aspergillus flavus isolated from a peanut field in Georgia with 1 of 44 distinct vegetative compatibility groupings (VCGs) was assessed. Multiple strains belonging to the same VCG typically produced identical DNA fingerprints, with the exception of VCG 17 and VCG 24, which contained strains that showed 83 and 87% similarity, respectively. A. flavus isolates sharing more than 80% of the fragments are recognized as belonging to the same DNA fingerprint group. Each VCG represented by a single isolate produced unique DNA fingerprints. The results provide further evidence that the pAF28 probe is able to distinguish A. flavus VCGs based on DNA fingerprints and can be used to predict the approximate number of VCGs in a sample population. The DNA probe also hybridized strongly and displayed multiple and distinct bands with other species in Aspergillus section Flavi: A. bombycis, A. caelatus, A. nomius, A. pseudotamarii, and A. tamarii. Although individual strains representing Aspergillus spp. in section Flavi produced DNA fingerprints with multiple bands, the banding patterns could not be used to classify these strains according to species.

Additional keywords: Aspergillus bombycis, A. caelatus, A. nomius, A. pseudotamarii, A. tamarii, cluster analysis, corn, DNA profile, restriction fragments

Aspergillus flavus Link:Fr. may infect seed of corn, cotton, peanut, and tree nuts and contaminate them with aflatoxins, which are potent hepatotoxic, carcinogenic metabolites that pose a significant health hazard (3,4). Members of Aspergillus section Flavi are widespread in crop fields, particularly in subtropical to tropical latitudes (5,11,17,19). Population biologists seek to understand the genetic structure and diversity of A. flavus populations in crop fields and the distribution of relevant phenotypic characters (e.g., aflatoxin and cyclopiazonic acid production, enzyme production, sclerotium formation, pathogenicity or virulence, competitive ability, and so on) among genetically isolated clonal populations. Vegetative compatibility groupings (VCGs), based on complementation tests between nitrate-nonutiliz-
32°C, the mycelium was harvested by filtering through a Whatman No. 1 filter paper in a Buchner funnel and rinsed two to three times with sterile distilled water. The mycelial mat was placed in Sarstedt tubes, frozen overnight, and lyophilized for 24 h. DNA was isolated using the method by Raeder and Broda (16) as modified by McAlpin and Mannarelli (12).

**DNA hybridization and detection.** Total DNA was digested to completion with PST 1 (Roche Molecular Biochemicals, Indianapolis, IN) according to the manufacturer’s recommendations. PST 1 was selected as the single enzyme for DNA digestion because it proved superior among five enzymes tested by McAlpin and Mannarelli (12) in distinguishing *A. flavus* VCGs as determined by Papa (14). Eight microgram of the digested DNA was dispensed in each lane on 0.8% agarose gel in TAE buffer (0.04 M Tris-acetate, pH 8.00; 0.001 M EDTA ) at 1.5 V cm⁻¹ for 22 h and visualized with UV light after staining with ethidium bromide. Southern blots were performed according to the manufacturer’s protocol by transferring restriction fragments from agarose gels to nylon membranes (Nytran N, Schleicher and Schuell, Keene, NH) using a vacuum blower (Model 785; BioRad Laboratories, Hercules, CA). Probes were labeled by random primed labeling and were detected by using the Nucleic Acid Nonradioactive Hybridization System (Roche Molecular Biochemicals). Membranes were exposed to X-ray film (Eastman Kodak, Rochester, NY) at room temperature for 30 to 60 min. Several film exposures were made to identify bands of varying intensity.

**DNA fingerprint analyses.** DNA bands were recorded on the basis of the presence or absence of fragments at a specific position. DNA fragments within each strain were compared by designating and re-
Fig. 2. Phenogram based on cluster analysis of 75 isolates of *Aspergillus flavus* from a Georgia peanut field and 3 isolates from corn using the Dice similarity coefficient of individual DNA bands produced by hybridization with pAF28 repetitive sequence (12). All isolates belonging to the same vegetative compatibility group are found in the same DNA fingerprint group (C > 80%). The phenogram was generated using the NTSYS-pc ver. 1.8 (18).
cording 55 fragment positions, representing different molecular weights, with an equidistant marker. Banding patterns produced by different strains were then compared to determine similarity or dissimilarity of fingerprints. Dice similarity coefficients \( (C) \) were used to calculate pairwise matching similarity values for each pair of isolates according to the equation \( C = 2N_{xy}/(N_{x} + N_{y}) \), in which \( N_{xy} \) is the number of hybridizing DNA bands shared by the isolates \( x \) and \( y \), and \( N_{x} \) and \( N_{y} \) refer to the number of DNA bands in isolates \( x \) and \( y \), respectively. The similarity coefficients were used to generate the cluster analysis with SIMQUAL and SAHN programs. The SAHN program determines which strains share identical fingerprints or identifies those belonging to different molecular weights, with an equidistant marker. Banding patterns produced nearly identical DNA fingerprints among 75 peanut field isolates from the same fingerprint group, but does not imply phylogenetic relationships. A program was constructed using the Numerical Taxonomy and Multivariate Analysis System (18; NTYSYS-pc, ver. 2.01; Exeter Software, Setauket, NY) as generated by the unweighted pair-group arithmetic average (UPGMA) method.

RESULTS AND DISCUSSION

The species-specific DNA probe pAF28 \( (12) \) was able to successfully distinguish among 75 \( A. flavus \) strains representing the 44 VCGs reported by Horn and Greene \( (7) \) using \( nit \) complementation tests. Strains belonging to the same VCG typically produced nearly identical DNA fingerprints (Fig. 1). VCG 6 (lanes 1 to 6) is represented by five peanut field isolates and NRRL 20032 from corn, VCG 23 (lanes 8 to 14) is represented by seven peanut field isolates, and VCG 14 (lanes 15 to 23) is represented by nine peanut field isolates.

Two fragments of isolate NRRL 29467 (lane 18) showed a slight shift in position. These fragments had strong homology with the probe, judging from the greater band intensity compared with the hybridization patterns of the other isolates in the same VCG. The shift could not be attributed to gel differences because repeated trials showed the same shift in all the gels, but most likely was due to the intensity (strong signal) of the bands.

Several of the peanut isolates produced DNA fingerprints with only one or two major bands while other isolates produced only very faint bands that were difficult to match with other fingerprints. This necessitated repeating the gels, placing isolates adjacent to each other in various combinations, and using longer film exposures for accurate comparisons. Each VCG represented by a single isolate produced unique DNA fingerprints. Two VCGs were represented by isolates with similar, but not identical, DNA profiles sharing more than 80% of the fragments. These isolates belonged to the same “DNA fingerprint group” according to previously published criteria \( (20,21) \). In VCG-17, a peanut field isolate (NRRL 29473) showed 83% similarity to NRRL 20050 from corn (lanes 25 and 26); and, in VCG 24, two peanut field isolates (NRRL 29481 and NRRL 29483) showed 87% similarity (lanes 27 and 28) (Fig. 1). Horn and Greene \( (7) \) performed two independent experimental pairings of isolates representing VCG 17, recording a weak but definite line of heterokaryotic heads in seven of the eight plates and a moderately heavy line of heterokaryons in one plate (B. W. Horn, personal communication). The experimental pairing of isolates representing VCG 24 produced a strong and obvious line of heterokaryons and was not repeated. The differences in fingerprints detected for isolates within a VCG could be the result of genetic changes in clonal lineages. Leslie \( (10) \) noted that isolates from the same VCG are not always members of the same clone.

The phenetic analysis and phenogram (Fig. 2) compares the DNA banding patterns for 75 peanut field isolates of \( A. flavus \) \( (7) \) and three \( A. flavus \) isolates from corn \( (14) \). The phenogram shows that strains having identical or similar \( (C > 80\%) \) DNA fingerprint profiles belong to the same VCG. In our previous effort to characterize \( A. flavus \) and \( A. parasiticus \) populations from a single corn field in Illinois \( (13,20) \), we applied the index of genetic similarity for RFLP comparisons after Xia et al. \( (21) \). The present results suggest that strains assigned to the same “genotype” or “fingerprint group” in the Illinois populations would also belong to the same VCG.

The DNA probe also hybridized strongly and displayed multiple and distinct bands (Fig. 3), with strains representing other species in \textit{Aspergillus} section \textit{Flavi} that have been isolated from peanut fields \( (e.g., \textit{A. caelatus} and \textit{A. tamarri}) \) or shown to produce aflatoxin \( (e.g., \textit{A. bombycis}, \textit{A. nomius}, and \textit{A. pseudotamarii}) \). The DNA fingerprints of \( \textit{A. bombycis}, \textit{A. caelatus}, \textit{A. nomius}, \textit{A. pseudotamarii}, \) and \( \textit{A. tamarri} \) all differed from one another. Within each species, identical DNA fingerprints were

Fig. 3. DNA fingerprints of \textit{Aspergillus pseudotamarii} (NRRL 443, NRRL 25517 ex type), \textit{A. tamarri} (NRRL 13139, NRRL 20818 ex type), \textit{A. bombycis} (NRRL 25593, NRRL 26010 ex type), \textit{A. flavus} (NRRL 19997 reference strain), \textit{A. caelatus} (NRRL 25528 ex type, NRRL 26015), and \textit{A. nomius} (NRRL 13137 ex type, NRRL 13138).
produced by each pair of strains, with the exception of A. tamarii, in which the two strains differed in their fingerprint patterns. Interestingly, the two A. bombycis strains selected for this study were isolated from different silk worm rearing houses in Tokushima prefecture (NRRL 25593) and Kagawa prefecture (NRRL 26010). This genotype may have spread among silk worm rearing facilities from a common source. Individual strains representing Aspergillus spp. in section Flavi produced DNA fingerprints with multiple bands; therefore, the banding patterns could not be used to classify these strains according to species.

The results of this study and those of McAlpin and Mannarelli (12) have shown that DNA fingerprinting using the repetitive DNA probe pAF28 is specific for Aspergillus section Flavi and provides an independent confirmation of the genetic structure and diversity of A. flavus populations where all the isolates were initially grouped by VCG analysis (7,14). McAlpin and Mannarelli (12) noted that VCG analysis becomes increasingly cumbersome in studies calling for genetic analysis of large populations because it is necessary to pair each new strain with a representative of each VCG determined for the population. DNA fingerprinting of A. flavus isolates allows us to readily compare new DNA fingerprints with those stored electronically in our database. It is now possible to more accurately predict that A. flavus isolates belonging to the same pAF28 DNA fingerprint group are also vegetatively compatible.

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LITERATURE CITED