Construction and Characterization of a DNA Probe for Distinguishing Strains of *Aspergillus flavus*

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Repetitive DNA sequences have proven useful and reliable characters in evaluating genetic relatedness of strains at different levels of taxonomic classification. A DNA probe was constructed to distinguish among strains of *Aspergillus flavus* by DNA fingerprinting techniques. Chromosomal DNA of *A. flavus* var. *flavus* NRRL 6541 was partially digested with *Eco*RI and ligated to a Lambda Dash bacteriophage vector. Four lambda clones were identified which displayed multiple and distinct bands when hybridized with chromosomal DNA from seven strains of *A. flavus* var. *flavus* digested with either *Eco*RI or *Pst*I. One of these clones was chosen for further analysis and was subcloned into pUC19. The subclone, pAF28, contained a 6.2-kb chromosomal DNA insert and was able to distinguish among strains characterized by K. E. Papa (Mycologia 78:98–101, 1986) as belonging to 22 different vegetative compatibility groups. The subclone identified unique banding patterns when hybridized to genomic DNA digested with *Pst*I. The cloned probe may be species specific as it hybridized with the DNA of all isolates of *A. flavus* tested in addition to strains recognized as varieties of *A. flavus* (e.g., *A. flavus* var. *oryzae*, *A. flavus* var. *parasiticus*, and *A. flavus* var. *sojae*). pAF28 hybridized to a single band on a Southern blot with *Aspergillus nomius* DNA but did not hybridize with the DNA of other fungal species tested including *Aspergillus ochraceus*, *Aspergillus auricomus*, *Aspergillus alliaceus*, *Fusarium moniliforme*, and *Penicillium thomii*.

Aspergillus flavus Link is a fungal plant pathogen that infects seeds of corn, cotton, peanuts, and tree nuts and may produce aflatoxins, a serious food safety hazard (10). Aflatoxins are recognized as potent hepatotoxins and carcinogens causing mortality or reducing the productivity of farm animals. Aflatoxin-contaminated foodstuffs have also been associated with a high incidence of liver cancer in humans (4, 32). Our laboratory is studying the environmental conditions that contribute to aflatoxin outbreaks in preharvest corn. One of our objectives is to identify the source(s) of the *A. flavus* population that infects the grain (e.g., soil, corn insects, crop residues, wind-borne spores, etc.) and to determine if some strains are selectively favored by an ability to infest preharvest corn (6, 8, 31). In order to accomplish this, it is necessary that we identify *A. flavus* isolates belonging to the same genotype or clone. The ability to characterize and monitor genetically identical strains from *A. flavus* populations should allow one to determine how the disease is spread and which of the subpopulations are associated with aflatoxin-contaminated seeds at harvest. Repetitive DNA sequences have proven useful and reliable characters in evaluating the genetic relatedness of strains at different levels of classification (e.g., species, forma speciales, vegetative compatibility groups [VCGs], clonal isolates) (7, 12, 18, 21–24, 30). In this report, we describe the construction and characterization of a molecular hybridization probe containing a chromosomal DNA insert of *A. flavus* var. *flavus* NRRL 6541 which is able to distinguish among strains of *A. flavus* belonging to different VCGs.

MATERIALS AND METHODS

Fungal strains. The fungal strains used in this research were obtained from the Agricultural Research Service Culture Collection (Northern Regional Research Laboratory). These included *A. flavus* var. *flavus* NRRL 6541 and NRRL 6577, from infected corn kernels, North Carolina; NRRL A-27859, NRRL A-27865, NRRL A-27869, NRRL A-27870, and NRRL A-27885, from agricultural sources, Hungary; 29 strains isolated from agricultural sources, Georgia, which were classified according to their VCG by K. E. Papa (25) (Table 1); *A. flavus* var. *oryzae* (Ahlburg) Kurtzman et al. NRRL 447 and NRRL 1911, from koji, Japan; *A. flavus* var. *parasiticus* (Spear) Kurtzman et al. NRRL 502, from mealy bug, Hawaii, and NRRL 465, from J. Takamine, source unknown; *A. flavus* var. *sojae* (Sakaguchi and Yamada) Kurtzman et al. NRRL 5594 and NRRL 5596, from koji, Japan; *Aspergillus nomius* Kurtzman et al. NRRL 13137, from moldy wheat, culture received from the U.S. Food and Drug Administration; *Aspergillus ochraceus* Wilhelm NRRL 402, source unknown; *Aspergillus auricomus* (Gueguen) Saito NRRL 391, from George Smith, source unknown; *Aspergillus alliaceus* Thom and Church NRRL 315, from blister beetle; *Fusarium moniliforme* Sheldon NRRL A-28160, from P. E. Nelson, source unknown; and *Penicillium thomii* Maire NRRL 6218 (= culture ex type of *Penicillium thomii* var. *flavescens*), from D. Fennell, source unknown.

Media and growth conditions. Fungal strains were maintained as conidial suspensions in 50% (vol/vol) glycerol in deionized water at –20°C. Conidial suspensions were prepared from 4- to 5-day-old (29°C) cultures grown on sporulation medium for *A. flavus* (28). To prepare fungal mycelia for DNA extractions, 500-ml flasks containing 200 ml of yeast extract-peptone-dextrose broth (YEPD) (3% yeast extract, 1% peptone, 2% dextrose) and ampicillin at 75 µg/ml were inoculated with 5 ml of a conidial suspension (10⁶/ml) in YEPD broth and incubated on a rotary shaker (200 rpm) for 22 to 24 h at 32°C. Following incubation, the mycelial suspensions were harvested by filtering through a Buchner funnel with sterile Whatman no. 1 filter paper and rinsed twice with sterile distilled water. The mycelial mat was transferred to a disposable 50-ml conical centrifuge tube (Sarstedt), frozen at –80°C, and lyophilized for 24 h or until the mycelia were completely brittle and dry.

DNA isolation. DNA from fungal strains was isolated and purified by a modification of the method of Raeder and Broda (27). A portion of a lyophilized mycelial mat (0.5 to 1.0 g [dry weight]) was placed in a precooled mortar and pestle, ground to a fine powder, and stored at –80°C. An aliquot (50 mg [dry weight]) was resuspended in 500 µl of extraction buffer (200 mM Tris-HCl [pH 8.5], 250 mM NaCl, 25 mM EDTA, 0.5% [wt/vol] sodium dodecyl sulfate), gently mixed with 350 µl of buffer-saturated phenol and 150 µl of chloroform, and centrifuged for 45 min at 14,000 × g. The aqueous phase was transferred to a new microfuge tube containing 25 µl of RNase A (20 mg/ml), incubated for 15 min at 37°C, and extracted once with an equal volume of chloroform. The DNA

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TABLE 1. VCG designations for *A. flavus* strains as determined by K. E. Papa (25)

K. E. Papa VCG	Lane position in Fig. 3	K. E. Papa strain designation	ARS ^a culture collection no.
1	1	81-1-41	NRRL 19981
1	2	81-15a-55	NRRL 19982
2	3	81-4-96	NRRL 20052
2	4	81-5-152	NRRL 20053
2	5	81-5-154	NRRL 20054
3	6	81-6-160	NRRL 20004
3	7	81-46-193	NRRL 20001
4	8	81-8-129	NRRL 19987
5	9	81-15b-123	NRRL 19985
5	10	81-30a-137	NRRL 19989
6	11	81-37a-191	NRRL 19992
7	12	81-2-67	NRRL 20222
8	13	81-7-133	NRRL 22516
9	14	81-9-107	NRRL 20221
10	15	81-11-118	NRRL 20220
11	16	81-16-91	NRRL 20219
12	17	81-17-58	NRRL 20217
13	18	81-18-92	NRRL 20223
14	19	81-20-23	NRRL 20224
15	20	81-29a	NRRL 20226
16	21	81-29b-200	NRRL 20234
17	22	81-30b	NRRL 20055
18	23	81-32-163	NRRL 20231
19	24	81-36-196	NRRL 20229
19	25	81-36-197	NRRL 20230
20	26	81-37b-187	NRRL 20228
21	27	81-47-211	NRRL 19997
22	28	81-52-102	NRRL 20235
22	29	81-52-103	NRRL 20236

^a ARS, Agricultural Research Service.

was precipitated from the aqueous phase with 0.54 volume of isopropyl alcohol, and the resulting pellet was rinsed once with 300 μ l of 70% ethanol and suspended in 100 μ l of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA). The DNA solution was reprecipitated with 0.25 volume of 7.5% ammonium acetate and 2 volumes of 100% ethanol, and the pellet was rinsed with 70% ethanol as suggested by Manicom et al. (22), in order to enhance digestibility of the DNA by restriction endonucleases. The resulting pellet was dried and dissolved in 100 μ l of TE and stored at -20°C . DNA concentrations were determined spectrophotometrically at 260 nm and by ethidium bromide fluorescent quantitation (29). With good preparations, the DNA concentration values for each method deviated by less than 20%. The quantity of DNA was further assessed visually after electrophoresis on 0.7% ethidium bromide-stained agarose gels.

Construction of DNA library. For cloning purposes, *A. flavus* NRRL 6541 DNA was isolated as described above and chromosomal DNA was separated from mitochondrial and ribosomal DNA in CsCl-bisbenzamide gradients as described by Garber and Yoder (13). Purified *A. flavus* chromosomal DNA was partially digested with *Eco*RI and cloned into Lambda Dash bacteriophage (Lambda Dash II/*Eco*RI Cloning Kit, Stratagene) according to the manufacturer's protocol. *Escherichia coli* P2392 was the host strain. Recombinant plaques were detected by standard colony hybridization techniques (29).

Lambda clones were subcloned into pUC19. DNA isolated from lambda recombinants was digested with *Eco*RI, and the DNA fragments were separated by gel electrophoresis in 0.8% agarose gels. DNA fragments representing *A. flavus* insert DNA were cut from the gel, purified with the Sephaglass Kit (Pharmacia LKB Biotechnology), and ligated to the dephosphorylated *Eco*RI site of pUC19 DNA. Ligated DNA was transformed into competent *E. coli* TG1 and plated on Luria-Bertani agar plates containing ampicillin at 75 μ g/ml. Prior to plating, agar plates were overlaid with 45 μ l of a solution containing 40 μ l of X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) at 20 mg/ml and 5 μ l of IPTG (isopropyl- β -D-thiogalactopyranoside) at 200 mg/ml. Recombinant clones were identified as white colonies.

Enzymes, restriction mapping, and Southern hybridization. Restriction endonucleases (Bethesda Research Laboratories) were used as specified by the manufacturer. Restriction enzyme cleavage maps were constructed with single and multiple digests of plasmids. Restriction fragments were analyzed by electrophoresis in 0.5 to 4.0% agarose gels in TAE buffer (0.04 M Tris-acetate [pH 8.0], 0.001 M EDTA). For Southern blotting, fungal DNAs were digested overnight with restriction enzymes, and 4 μ g of the DNA digests was applied to wells on a

0.7% agarose gel (15 by 20 cm) run at 1.8 V/cm for 20 h. Separated DNAs were transferred to Hybond-N nylon membranes (Amersham International) with a vacuum blotter (Model 785 Vacuum Blotter, Bio-Rad Laboratories) according to the manufacturer's protocol. Probes were labeled with a nonradioactive labeling system (Genius System, Boehringer Mannheim). Membranes were prehybridized, hybridized, and washed in a hybridization oven (Hybaid Minioven MK II, National Labnet Co.). DNA fingerprints were detected with the LumiPhos detection system (Boehringer Mannheim), and membranes were exposed to X-ray film (Kodak) at room temperature for 15 to 30 min.

PCR. A 570-bp DNA segment corresponding to the 5' end of *A. flavus* 18S rRNA was amplified by PCR with fungal primers NS1 (5'-GTAGTCATATGCT TGTCTC-3') and NS2 (5'-GGCTGCTGGCACCAGACTTGC-3') (33). The presence of 5.8S and 26S ribosomal gene sequences in the DNA insert of plasmid pAF28 was checked by using fungal primers ITS3 (5'-GCATCGATGAA GAACGCAGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and primers D2R (5'-TTGGTCCGTGTTCAAGACG-3') and D7R (5'-TTG GAGACCTGCTGCGG-3'). In *A. flavus*, primers ITS3 and ITS4 border a 320-bp DNA segment coding for the 5.8S rRNA gene and ITS2 region (33). Primers D2R and D7R border a 1,500-bp segment of the 3' end of the 26S rRNA gene (26). The PCR was carried out for 35 cycles with annealing at 50°C for 1 min, extension at 72°C for 3 min, and denaturation at 94°C for 45 s. Reaction volume was 100 μ l with 2.5 ng of *A. flavus* or plasmid pAF28 DNA, 50 pmol of each primer, and 2.5 U of *Taq* polymerase (Perkin-Elmer Corp.).

RESULTS

Cloning and characterization of DNA probe. Putative *A. flavus* DNA clones were initially identified from a Lambda Dash library by dot blot hybridization with digoxigenin-labeled *A. flavus* var. *flavus* NRRL 6541 chromosomal DNA as a plaque hybridization probe. Bacteriophage lambda vectors containing *A. flavus* repetitive chromosomal DNA sequences were detected by a modification of the technique described by Hamer et al. (16). Lambda vectors containing fungal DNA with single-copy sequences produced faint hybridization signals, while recombinant clones containing repetitive fungal DNA sequences produced a more intense hybridization signal.

Fifty plaques with strong hybridization signals were recovered and grown separately. Vectors containing fungal chromosomal DNA sequences coding for ribosomal genes were identified by screening plaques with a PCR-generated 570-bp DNA fragment coding for the 5' end of 18S nuclear rRNA. Dot blot hybridization indicated that 36 of the plaques contained ribosomal DNA sequences as shown by positive binding with both the genomic probe and the ribosomal probe. The remaining 14 colonies hybridized only with the whole genomic probe (data not shown). In preliminary tests, all 14 of the latter lambda clones were hybridized against chromosomal DNA from seven strains of *A. flavus* var. *flavus* (NRRL 6541, NRRL 6577, NRRL A-27859, NRRL A-27865, NRRL A-27869, NRRL A-27870, and NRRL A-27885) digested with either *Eco*RI or *Pst*I. Four of the lambda probes produced multiple hybridization signals that clearly distinguished the seven *A. flavus* strains from one another. One of these clones, designated λ AF28, was chosen for further analysis.

λ AF28 contained a 9.4-kb DNA insert composed of two *Eco*RI fragments, 6.2 and 3.2 kb in length. Both of these fragments were subcloned into pUC19. The subclone containing the 6.2-kb DNA insert produced hybridization patterns similar to λ AF28 while the subclone containing the 3.2-kb DNA insert produced a single band with all strains tested. The subclone containing the 6.2-kb DNA insert was designated pAF28. The restriction map of pAF28 is shown in Fig. 1. Plasmid pAF28 was further screened for the presence of 5.8S and 26S ribosomal gene sequences by using it as a substrate in a PCR with either of two primers that generate an internal 5.8S ribosomal gene sequence (320 bp) or two primers that generate the 3' end of the 26S nuclear ribosomal gene sequence (1,500 bp; see Materials and Methods). Control experiments showed that under similar conditions chromosomal DNA from

FIG. 1. Restriction endonuclease map of pAF28. Striped box indicates pUC19 sequences; open box indicates *A. flavus* chromosomal DNA insert. Abbreviations: B, *Bam*HI; E, *Eco*RI; H, *Hind*III; K, *Kpn*I; P, *Pst*I; S, *Sac*I; Sm, *Sma*I; Sp, *Sph*I.

A. flavus NRRL 6577 yielded the expected DNA fragments of 320 and 1,500 bp while plasmid pAF28 yielded no visible DNA fragments (data not shown). The *E. coli* strain containing pAF28 has been deposited in the Agricultural Research Service Culture Collection as NRRL B-21002.

Distribution of RDS in fungal strains. The presence and copy number of the repetitive DNA sequence (RDS) in different fungal strains were examined by Southern blotting with pAF28 to probe chromosomal DNA digested with *Pst*I (Fig. 2). DNA from *A. flavus* var. *flavus* NRRL 6541 and NRRL 6577 (lanes 1 and 2, respectively) showed numerous signals of varying intensities, ranging in size from <2.0 to >23 kb. Careful examination of lane 1 and a number of other hybridizations indicated that as many as 30 signals were generated when pAF28 was hybridized to strain NRRL 6541. The DNA probe also hybridized with the DNA of closely related varieties including *A. flavus* var. *oryzae*, *A. flavus* var. *parasiticus*, *A. flavus*

var. *sojae*, and *A. nomius* (lanes 3 to 9). The DNA of *A. flavus* var. *oryzae* NRRL 447 and NRRL 1191 (lanes 3 and 4, respectively) produced hybridization patterns and signal intensities similar to those of the DNA of *A. flavus* var. *flavus* strains. DNA from *A. flavus* var. *parasiticus* NRRL 502 and NRRL 465 (lanes 5 and 6, respectively) also showed multiple signals but of lower intensity than those of the *A. flavus* var. *flavus* strains. Also, other hybridizations showed that DNA from *A. flavus* var. *parasiticus* NRRL 6433, isolated from corn in North Carolina, produced a hybridization pattern with pAF28 similar but not identical to that of strain NRRL 502 (data not shown). DNA from *A. flavus* var. *sojae* NRRL 5594 and 5596 (lanes 7 and 8, respectively) produced only a few and mostly faint signals even when this hybridization was repeated several times.

A single strong signal was observed when DNA from *A. nomius* NRRL 13137 was probed with pAF28 (lane 9). In another hybridization, a similar single strong signal was observed with DNA from *A. nomius* NRRL 13138 (data not shown). No hybridization was observed when the probe was hybridized with the DNA of other fungal species such as *A. ochraceus* NRRL 402 (lane 10), *A. auricomus* NRRL 391 (lane 11), *A. alliaceus* NRRL 315 (lane 12), *F. moniliforme* NRRL A-28160 (lane 13), and *P. thomii* NRRL 6218 (lane 14).

Differentiation of *A. flavus* strains belonging to different VCGs. The pAF28 probe could distinguish among tester strains of *A. flavus* belonging to the 22 VCGs determined by K. E. Papa (25) and listed in Table 1 (Fig. 3). In Fig. 3, the amount of digested chromosomal DNA in each lane was quantitated to ensure that the amount of DNA among lanes did not differ by more than 20% (see Materials and Methods). pAF28 identified characteristic and distinct hybridization patterns with DNA of strains from all VCGs. Strains belonging to the same VCG yielded similar banding patterns. For example, strains in VCG 1, NRRL 19981 and NRRL 19982 (lanes 1 and 2); VCG 2, NRRL 20052, NRRL 20053, and NRRL 20054 (lanes 3 to 5); VCG 3, NRRL 20001 and NRRL 20004 (lanes 6 and 7); VCG 19, NRRL 20229 and NRRL 20230 (lanes 24 and 25); and VCG 22, NRRL 20235 and NRRL 20236 (lanes 28 and 29) had very similar hybridization patterns when strains within each group were compared. Identical hybridization patterns were obtained when Southern hybridizations were done with new DNA preparations of Papa strains digested with *Pst*I. The signal patterns were repeatable.

Southern hybridizations using pAF28 as a probe produced multiple signals when genomic DNA from strains of different VCGs were digested with a number of different restriction enzymes including *Eco*RI, *Hind*III, *Sal*I, and *Xba*I. *Pst*I produced the best results because genomic digests produced a unique and clearly distinct hybridization pattern for strains of each VCG. When genomic DNA was digested with other restriction endonucleases, hybridizations with probe pAF28 could not distinguish among all of the VCGs or produced hybridization patterns that were not as distinct.

DISCUSSION

RDSs occur in the chromosomes of all eukaryotes studied, and these sequences have been shown to accumulate polymorphisms because of genetic drift and rearrangement (11). Both the specific nucleotide sequences and the number of copies of RDSs found in eukaryotic chromosomes appear to be altered at a high rate such that strains which have only recently diverged can be distinguished by analyzing their chromosomal RDSs. A number of studies have shown that strains belonging to different fungal species and different strains belonging to the

FIG. 2. Hybridization of pAF28 with DNA from various fungal species. Lanes 1 through 14 each contain approximately 4.0 μ g of *Pst*I-digested chromosomal DNA from fungal strains. Lanes: 1, *A. flavus* NRRL 6541; 2, *A. flavus* NRRL 6577; 3, *A. flavus* var. *oryzae* NRRL 447; 4, *A. flavus* var. *oryzae* NRRL 1191; 5, *A. flavus* var. *parasiticus* NRRL 502; 6, *A. flavus* var. *parasiticus* NRRL 465; 7, *A. flavus* var. *sojae* NRRL 5594; 8, *A. flavus* var. *sojae* NRRL 5596; 9, *A. nomius* NRRL 13137; 10, *A. ochraceus* NRRL 402; 11, *A. auricomus* NRRL 391; 12, *A. alliaceus* NRRL 315; 13, *F. moniliforme* NRRL A-28160; 14, *P. thomii* NRRL 6218. Lambda DNA size markers are indicated (in kilobases).

FIG. 3. Southern analysis of *Pst*I-digested chromosomal DNA of *A. flavus* strains from different VCGs hybridized with plasmid pAF28. Lanes 1 through 29 contain DNA from *A. flavus* strains characterized by Papa (25). *A. flavus* strains and corresponding lane numbers are indicated in Table 1. Lambda DNA size markers are indicated in kilobases.

same species can be distinguished by comparing the hybridization patterns produced when a cloned RDS is used as a hybridization probe for restriction enzyme-digested chromosomal DNA of fungal strains. Scherer and Stevens (30) described an RDS used to characterize isolates of *Candida albicans*. Hamer et al. (16) identified a family of dispersed RDSs specific for the rice blast fungus *Magnaporthe grisea* which produced very different hybridization patterns between rice-pathogenic strains of *M. grisea* and grass pathogens. Goodwin et al. (14, 15) characterized randomly cloned species-specific DNA probes for *Phytophthora parasitica* and *Phytophthora citrophthora*. Subsequent studies include those by Kistler et al. (18) for *Fusarium oxysporum* and Milgroom et al. (23) of the chestnut blight fungus *Cryphonectria parasitica*.

In this study, we developed a probe, pAF28, containing a 6.2-kb DNA insert from the chromosome of *A. flavus* NRRL 6541. The probe hybridized to a number of DNA fragments of the parent *A. flavus* NRRL 6541, indicating that the clone contained a repetitive DNA sequence. The DNA insert does not appear to contain DNA sequences coding for ribosomal genes since it did not hybridize with the 570-bp probe for the 5' end of fungal 18S nuclear rRNA, nor did it yield any DNA fragments in a PCR with primers that should generate 5.8S and the 3' end of 26S nuclear ribosomal DNA segments.

pAF28 hybridized to DNA from strains of *A. flavus* var. *oryzae*, *A. flavus* var. *parasiticus*, and *A. flavus* var. *sojae* (Fig. 2). Kurtzman et al. (20) have shown that strains of these species show high DNA homology with *A. flavus* var. *flavus* NRRL 1957 (e.g., DNA homology of 100% for *A. flavus* var. *oryzae* NRRL 447, 70% for *A. flavus* var. *parasiticus* NRRL 502, and 74% for *A. flavus* var. *sojae* NRRL 5595). These researchers concluded that strains of these species are varieties of the same species. Our results support this conclusion. pAF28 appears to be a species-specific probe that hybridizes strongly only to strains of *A. flavus*. pAF28 also hybridized to the DNA of *A. nomius* NRRL 13137 and NRRL 13138. Kurtzman et al. (19) showed between 32 and 47% DNA homology between some strains of *A. flavus* and *A. nomius*. *A. nomius* probably represents a species that has recently separated from *A. flavus*, so that some hybridization would be expected.

The pAF28 probe was able to distinguish among different strains of *A. flavus* belonging to 22 VCGs characterized by Papa (25). The DNA from strains in each VCG produced distinct hybridization patterns. Strains belonging to different VCGs represent genetically different strains and provide a test as to whether a probe can distinguish strains with different genotypes in natural environments. The probe pAF28 should be useful in monitoring individual strains causing a particular crop infestation in the field (unpublished data).

A long-term objective of our research is to identify genotypically distinct strains isolated from separate fields or crop-growing regions that belong to the same VCG. *A. flavus* is known to propagate only asexually, and therefore, genetic exchange must occur through heterokaryosis or vegetative fusions. Barriers to vegetative fusions are widespread among clones of *A. flavus* (2, 3, 25). Asexual genetic exchange under field conditions is probably limited to the same genetically isolated VCG (1). Furthermore, strains arising through asexual propagation from a given parental clone probably belong to the same VCG. As examples, isozymes and/or DNA restriction fragment length polymorphisms have been used to confirm that different isolates of the same VCG belong to the same clone in *F. oxysporum* (5, 17) and *Aspergillus nidulans* (9). In approaching this study, we recognize two problems in using conventional methods of VCG determination. First, to determine the VCG of each new isolate, it is necessary to pair the strain with a representative of each VCG determined for the population. VCG analysis would become increasingly cumbersome in studies calling for genetic analysis of large populations. Consider that Papa (25) found 22 different VCGs in a population of only 32 *A. flavus* isolates. Second, isolates that fail to form complementary nitrate-nonutilizing (Nit⁻) mutants and are incompatible with other VCGs must be excluded from estimates of VCG diversity. Our results indicate that strains belonging to the same VCG yield very similar hybridization patterns. Although slight differences can be observed, the hybridization patterns of related strains from VCGs 1, 2, 3, 5, 19, and 22 (Fig. 3) are clearly similar and different from the patterns of strains from other VCGs. Further research is neces-

sary, but probes such as pAF28 may be useful for quickly and easily identifying VCGs.

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