APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1995, p. 1378–1383 Vol. 61, No. 4
0099-2240/95/$04.00+0

Molecular Characterization of the Food-Borne Fungus *Neosartorya fischeri* (Malloch and Cain)

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Received 4 August 1994/Accepted 5 February 1995

The food-borne fungus *Neosartorya fischeri*, which is phenotypically related to the human opportunistic pathogen *Aspergillus fumigatus*, causes spoilage of heat-processed fruit products. Genomic methods were used to type *N. fischeri* strains and identify the genomic relationship between *A. fumigatus* and *N. fischeri* and between the different varieties of *N. fischeri*. *Eco*RI restriction fragment length polymorphism (RFLP) patterns obtained after ethidium bromide staining could differentiate most of *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa* strains. On the contrary, all *N. fischeri* var. *fischeri* strains tested exhibit the same RFLP pattern, which was similar to the *A. fumigatus* pattern. Similarly, Southern hybridization with a ribosomal probe showed some polymorphism between *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa* strains but could not distinguish between *N. fischeri* var. *fischeri* and *A. fumigatus* strains. By using the endonucleases *Eco*RI, *Hin*dIII, and *Bgl*II to generate Southern blot patterns with a fragment of the *A. fumigatus* gene coding for a 33-kDa protease, it was possible to differentiate *N. fischeri* var. *fischeri* from *A. fumigatus*. The difference between *N. fischeri* and *A. fumigatus* was confirmed by the use of moderately repetitive nonribosomal *A. fumigatus* sequences. These results are in agreement with previous studies that showed important infraspecific polymorphism within *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa* and, in contrast, the homogeneity of *N. fischeri* var. *fischeri* strains. A unique Southern blot pattern was seen for each strain of *N. fischeri* fingerprinted with the *A. fumigatus* repetitive sequence. In the future, such typing systems may contribute to localization of the origins and the routes of fungal contaminations in food-industrial environments.

Neosartorya fischeri (22) is a ubiquitous fungus which commonly grows in damp environments, such as soil, decaying vegetation, and organic debris (26), where it produces high numbers of spores and particularly ascospores (6). Consequently, ascospores of *N. fischeri* are frequently isolated from fruits that grow or are harvested at the ground level (apples, strawberries, grapes, pineapples, and others) (15, 24, 32). Ascospores of *N. fischeri* are thermoresistant (2, 23, 30, 33) and are the cause of food spoilages of heat-processed fruit products in food industries (12, 14, 16, 28–31).

Within the species *N. fischeri*, three varieties (*N. fischeri* var. *fischeri*, *N. fischeri* var. *glabra*, and *N. fischeri* var. *spinosa*) have been identified on the basis of the ascospore ornamentation (26, 27). Recent studies based on phenotypic methods (i.e., scanning electron microscopic observations of conidium and ascospore morphology, secondary metabolite patterns, heat resistance, and sodium dodecyl sulfate [SDS]-polyacrylamide gel electrophoresis protein and antigen patterns) as well as genomic methods (i.e., DNA complementation experiments) divided the species *N. fischeri* into very different groups (7, 11, 18, 23, 25, 27). On the basis of morphological observations, Kozakiewicz considered that the differences between the three varieties of *N. fischeri* were sufficient to raise them to species level (18). However, distinguishing between the three varieties of *N. fischeri* remains difficult (26a). And the taxonomic status of *N. fischeri* has not been officially ratified by the International Code of Botanical Nomenclature.

In addition, strains of *N. fischeri* cannot be efficiently discriminated from the human pathogenic fungus *Aspergillus fumigatus* (7, 11, 27). Indeed, the asexual stage of *N. fischeri* is morphologically very similar to *A. fumigatus* (26), and *N. fischeri* has been considered by some taxonomists to be the putative sexual stage or at least to be identical to an ancestor of *A. fumigatus*.

Several genomic methods have recently been developed to estimate the variability within the species *A. fumigatus*. These methods include restriction fragment length polymorphism (RFLP) after ethidium bromide staining (4, 5) or after Southern hybridization with ribosomal (34) or nonribosomal (8) repeated sequences and randomly amplified polymorphic DNA (1, 21).

In the study presented here, we used different genomic techniques to type *N. fischeri* strains and identify the genomic relationships between *A. fumigatus* and *N. fischeri* and between the different varieties of *N. fischeri*. First, *Eco*RI RFLP patterns of total genomic DNA were stained with ethidium bromide after electrophoresis. Alternatively, Southern blot patterns of endonuclease-digested total DNA were probed either with sequences from a single *A. fumigatus* gene coding for a 33-kDa protease (13) or with repeated sequences of ribosomal or nonribosomal origin (8).

MATERIALS AND METHODS

Strains. The collection numbers and the abbreviations of the *Aspergillus* and *N. fischeri* strains used in this study are listed in Table 1. All strains were maintained on 2% malt extract agar at room temperature.

Monospore isolates of *N. fischeri*. Cleistothecia were harvested from 3-week-old slants of *N. fischeri* var. *spinosa* S7 on malt extract agar. Free ascospores were obtained by manual disruption of cleistothecia in 500 μl of distilled water with a glass pestle fitted to 2-ml microcentrifuge tubes. Monoascospore isolates were obtained by the limiting dilution technique. Before being plated on 2% malt

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TABLE 1. Strains of *Aspergillus* and *N. fischeri* used in this study

Species and variety	Strain[a]	Abbreviation
A. fumigatus	CBS 143-89	AF1
	CHUV 5	AF2
A. flavus	CBS 569-65	AFl1
N. fischeri var. *fischeri*	IBT 3003	F1
	IBT 3007	F3
	IBT 3008	F4
	IBT 3009	F5
	CBS 832-88	F13
N. fischeri var. *glabra*	IBT 3006	G2
	CBS 112-55	G5
	CBS 165-63	G6
	LCP 87.3513	G8
	LCP 88.3577	G9
	CBS 585-90	G10
N. fischeri var. *spinosa*	CBS 297-67	S3
	IMI 16061	S4
	CBS 161-88	S7
	LCP 76-3116	S8

[a] CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; CHUV, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; IBT, Department of Biotechnology, Technical University of Denmark, Lyngby, Denmark; IMI, International Mycological Institute, Kew, United Kingdom; LCP, Laboratoire de Cryptogamie-Mycologie, Museum d'Histoire Naturelle, Paris, France.

extract agar plates, ascospore suspensions were heated at 80°C for 20 min to inhibit the growth of any contaminating mycelial or conidial propagules.

Three successive sexual cycles were obtained by repeated transfers of 1- to 2-week-old cultures after heating of ascospore suspensions used for inoculating the slants to ensure that the new culture originated from ascospores.

DNA extraction. DNA was extracted from a sample of *N. fischeri* that had been cultured in a 2% glucose–1% peptone medium at 37°C and shaken for 16 to 24 h. DNA was purified and quantified as previously described (10).

Ethidium bromide RFLP. After complete digestion by the endonuclease *Eco*RI, DNA electrophoresis was conducted as described in reference 10. DNA fragments were stained with a 0.5% ethidium bromide solution.

Southern blot hybridizations with repetitive sequences. *Eco*RI-digested DNA fragments were transferred to a nylon membrane (Hybond N^+; Amersham) and hybridized to the molecular probes at 65°C in a solution containing 5× SSPE (1× SSPE is 10 mM sodium phosphate [pH 7.5], 1 mM EDTA [pH 7.5], and 0.18 M NaCl), 5% dextran sulfate, 150 μg of sheared denatured salmon sperm DNA per ml, and 0.3% SDS (8). The moderately repetitive sequence λAF 3.19 (designated 3.19 in reference 8) and the 4.6-kb *Bam*HI fragment of the ribosomal complex of *Aspergillus nidulans* (20) (kindly provided by W. T. Timberlake) were labelled either with [α-^{32}P]dCTP or with fluoresceinylated dCTP with, respectively, the random priming kit Megaprime (Amersham) or the ECL labelling kit (Amersham). The membranes were washed and revealed as previously described (8).

Southern blot hybridizations with single-copy *A. fumigatus* sequences. DNA digested with *Eco*RI, *Bgl*II, or *Hin*dIII was blotted onto a GeneScreen nylon membrane (Dupont). The filters were prehybridized at 42°C for 30 min in a solution containing 5× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate [pH 7.0]), 10% dextran sulfate, 20% formamide, 1% SDS, and 100 μg of denatured salmon sperm DNA per ml. The 1.5-kb *Bgl*II-*Hin*dIII fragment of the *A. fumigatus alp* gene encoding a 33-kDa protease (13) was labelled with [α-^{32}P]dATP with a random-primed DNA-labelling kit (Boehringer, Mannheim, Germany). The labelled probes were added to the prehybridization solution and incubated overnight with the blots. The membranes were exposed to X-ray films after two washes in 3× SSC–1% SDS at 40°C.

RESULTS

Ethidium bromide-stained RFLP patterns of *Eco*RI-digested total DNA. RFLP patterns of *Eco*RI-digested DNA from *N. fischeri* and *A. fumigatus* strains are shown in Fig. 1. The 11 strains of *N. fischeri* tested generated 9 different patterns (Fig. 1). The patterns of the three strains of *N. fischeri* var. *fischeri* were all identical (Fig. 1, lanes F1, F3, and F5) and

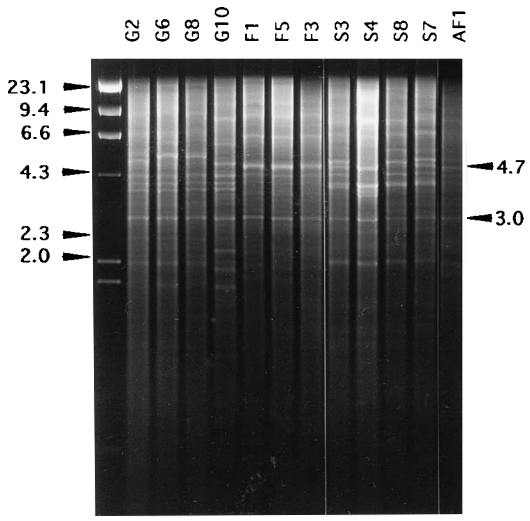

FIG. 1. Ethidium bromide RFLP patterns of *N. fischeri* and *A. fumigatus* strains. AF, *A. fumigatus*; F, *N. fischeri* var. *fischeri*; G, *N. fischeri* var. *glabra*; S, *N. fischeri* var. *spinosa* (Table 1). Total genomic DNA of each strain was digested with *Eco*RI. Molecular sizes are expressed in kilobases.

similar to the pattern seen with *A. fumigatus* (Fig. 1, lane AF1), with two major bands at 3.0 and 4.7 kb. In contrast, the RFLP patterns from each strain belonging to *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa* were different. All RFLP patterns showed a major band at 3.0 kb, but the variability of the patterns was too great to cluster together the strains either from *N. fischeri* var. *glabra* or from *N. fischeri* var. *spinosa*.

Southern blot hybridization patterns with a ribosomal sequence. Southern blots of *N. fischeri* strains with a ribosomal DNA probe showed two major bands (Fig. 2). The band at 3.0 kb seen in ethidium bromide-stained gels (Fig. 1) was invariant

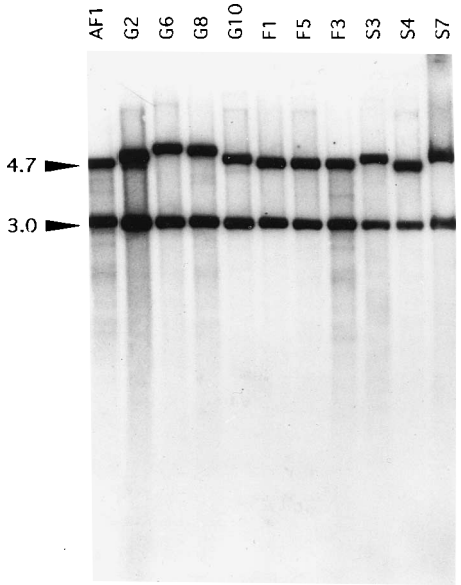

FIG. 2. Southern blot hybridization patterns with a ribosomal probe of *N. fischeri* and *A. fumigatus* strains. AF, *A. fumigatus*; F, *N. fischeri* var. *fischeri*; G, *N. fischeri* var. *glabra*; S, *N. fischeri* var. *spinosa* (Table 1). *Eco*RI-digested DNA fragments were probed with a ^{32}P-radiolabelled ribosomal sequence from *A. nidulans*. Molecular sizes are expressed in kilobases.

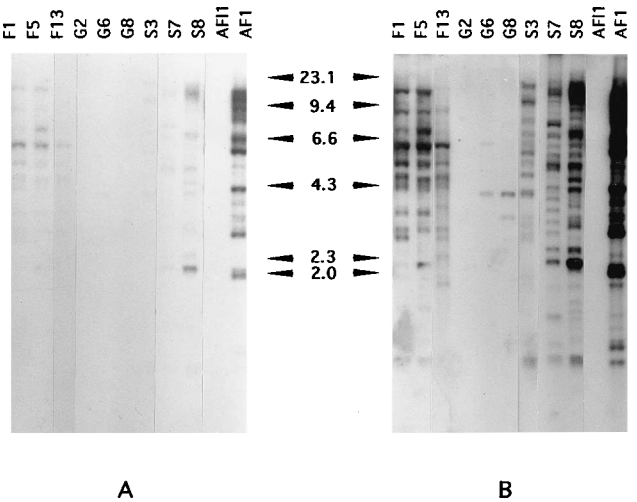

FIG. 3. Differential hybridizations of the moderately repetitive *A. fumigatus* sequence λAF 3.19 probe with *A. fumigatus*, *A. flavus*, and *N. fischeri* DNAs. AF, *A. fumigatus*; Afl, *A. flavus*; F, *N. fischeri* var. *fischeri*; G, *N. fischeri* var. *glabra*; S, *N. fischeri* var. *spinosa* (Table 1). *Eco*RI-digested restriction fragments were probed with the radiolabelled λAF 3.19 probe under conditions described in Materials and Methods. Hybridization bands were revealed by autoradiography after 6 h (A) or 72 h (B) of exposure at −80°C. Molecular sizes are expressed in kilobases.

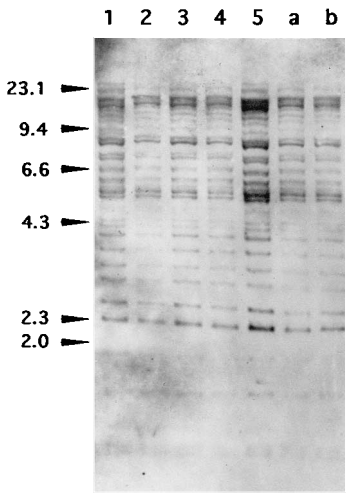

FIG. 4. Stability of the Southern blot hybridization patterns, generated by the moderately repetitive *A. fumigatus* sequence λAF 3.19, of five different monoascospore isolates of *N. fischeri* var. *spinosa* S7 (lanes 1 to 5) and of two cultures of *N. fischeri* var. *spinosa* S7 separated by three sexual sporulating cycles (lanes a and b). The probe was labelled with fluoresceinylated dCTP. Visualization was performed by the indirect chemiluminescence method (Amersham) after a 30-min exposure in a cassette with an intensifying screen. Molecular sizes are expressed in kilobases.

for all strains. The size of the highest-molecular-weight band varied from 4.7 to 5.8 kb depending on the strain. The band at 4.7 kb seen for all strains of *N. fischeri* var. *fischeri* (Fig. 2, lanes F1, F3, and F5) was also found in *A. fumigatus* (lane AF1). The size of the high-molecular-weight band was variable within strains of *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa.*

Neither *Eco*RI RFLP patterns nor Southern hybridization patterns obtained with a ribosomal sequence provide the level of discrimination sufficient to separate *A. fumigatus* from *N. fischeri* var. *fischeri* or to cluster together the strains belonging to *N. fischeri* var. *glabra* or *N. fischeri* var. *spinosa.*

Southern blot patterns with moderately repetitive *A. fumigatus* sequences. Several nonribosomal repeated sequences isolated from *A. fumigatus* genomic DNA (8) have been successfully used to fingerprint *A. fumigatus* strains (8–10). Because of the taxonomic closeness between *A. fumigatus* and *N. fischeri*, it was expected that the same probes could be used to fingerprint *N. fischeri* strains. Two of the moderately repetitive *A. fumigatus* sequences, λAF 3.9 and λAF 3.19 (designated 3.9 and 3.19 in reference 8), were therefore tested on *N. fischeri* DNA. The λAF 3.9 probe generated only one band of hybridization with one of the *N. fischeri* strains tested (*N. fischeri* var. *fischeri* F5) (data not shown). The λAF 3.19 probe gave significant hybridization signals with DNA from *N. fischeri* strains (Fig. 3). With a short film exposure time (4 to 6 h), weak hybridization signals were observed on *N. fischeri* Southern blots with the moderately repetitive sequence λAF 3.19 (Fig. 3A). Conversely, a strong hybridization banding signal was seen with the same probe when *A. fumigatus* DNA was probed (Fig. 3A, lane AF1). Increasing the exposure time from between 4 and 6 h to 72 h was necessary in order to detect signals in *N. fischeri* blots (Fig. 3B). Under these conditions, *N. fischeri* var. *fischeri* and *N. fischeri* var. *spinosa* Southern patterns showed between 20 and 30 hybridization bands of different intensities and sizes (Fig. 3B). The same DNA probe generated very few hybridization bands, of extremely weak intensity, on *N. fischeri* var. *glabra* blots (Fig. 3B).

The specificity of the hybridization with the moderately repetitive *A. fumigatus* sequence λAF 3.19 was demonstrated by the negative results observed with other *Aspergillus* species, such as *Aspergillus nidulans* (data not shown) and *Aspergillus flavus* (Fig. 3), even after a 72-h exposure of the blots to radiographic films.

Each strain of *N. fischeri* fingerprinted by the moderately repetitive sequence λAF 3.19 showed a unique Southern blot pattern. These results suggested that moderately repetitive sequences of *A. fumigatus* could be used to fingerprint strains of *N. fischeri*. However, before this could be ascertained it was essential to check that the hybridization pattern was stable over time after several sexual cycles and that monoascospore isolates that issued from a single strain displayed the same pattern. As an example to test the stability of the fingerprints, the strain *N. fischeri* var. *spinosa* S7 (Table 1) was used. Five monoascospore isolates randomly chosen from the same original slant were probed with the moderately repetitive sequence λAF 3.19. The Southern hybridization patterns obtained were all identical (Fig. 4, lanes 1 to 5). Similarly, the banding pattern of strain S7 remained unchanged over several successive sexual sporulating cycles (Fig. 4, lanes a and b). These results indicate that homothallic sexual cycles did not affect the Southern hybridization pattern of an individual strain of *N. fischeri.*

Southern blots with the *A. fumigatus* 33-kDa protease gene. A fragment of the gene encoding the 33-kDa protease of *A. fumigatus* (13) was probed with *N. fischeri* DNA. Southern blots generated by the 33-kDa protease genomic probe are presented in Fig. 5. When digested by *Hin*dIII, all strains of *N. fischeri* presented a major hybridization band at 2.0 kb. In contrast, *A. fumigatus* patterns showed a 5.0-kb band (Fig. 5, strain AF2). An invariant major hybridization band of 2.1 kb was visible on *Bgl*II Southern patterns of *A. fumigatus*, *N. fischeri* var. *fischeri*, and *N. fischeri* var. *spinosa*, whereas *N. fischeri* var. *glabra* patterns showed different hybridization bands, one at 4.0 and one at 3.0 kb, for the two respective strains tested. The 33-kDa protease genomic probe hybridized to *Eco*RI-digested DNA fragments of *N. fischeri* var. *fischeri*,

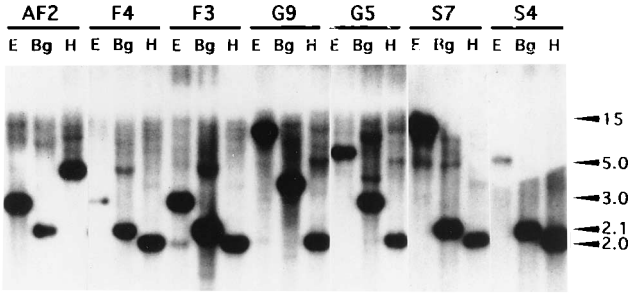

FIG. 5. Hybridization of the *A. fumigatus* 33-kDa protease gene fragment to *A. fumigatus* and *N. fischeri* DNAs. AF, *A. fumigatus*; F, *N. fischeri* var. *fischeri*; G, *N. fischeri* var. *glabra*; S, *N. fischeri* var. *spinosa* (Table 1). Endonuclease-digested restriction fragments were probed with the ^{32}P-radiolabelled probe under conditions described in Materials and Methods. E, *Eco*RI; Bg, *Bgl*II; H, *Hin*dIII. Molecular sizes are expressed in kilobases.

and *A. fumigatus* strains generated identical *Eco*RI Southern patterns with a unique band at 3.0 kb.

The use of several endonucleases to generate different Southern blot patterns with the 33-kDa protease probe from *A. fumigatus* was an efficient way to discriminate between the three varieties of *N. fischeri* and to differentiate *N. fischeri* var. *fischeri* from *A. fumigatus* strains.

DISCUSSION

Relationship between *A. fumigatus* and *N. fischeri*. The most important difference between *N. fischeri* and *A. fumigatus* is the presence of ascospores in *N. fischeri* cultures (26). A study of the relationship between these two species cannot be undertaken on the basis of mycelial phenotypic characters such as protein and antigenic patterns, as well as secondary metabolite profiles, because of the high infraspecific variations within the species *N. fischeri* and *A. fumigatus* (7, 11, 23, 27).

Genomic tools have been very useful for investigation of the relationships between *N. fischeri* and *A. fumigatus*. On the basis of nuclear DNA complementarity measurements, Peterson (25) showed that *A. fumigatus* and *N. fischeri* species are closely related. These two species exhibited 40 to 70% DNA complementarity, whereas genomic similarities of 0 to 9% were observed between *N. fischeri* and taxonomically distant *Aspergillus* species such as *A. flavus*. In addition, genomic probes originating from *A. fumigatus*, such as the 33-kDa and the 18-kDa protease genes (19) and the midrepeat sequence λAF 3.19, which were positive with *N. fischeri*, are negative with other *Aspergillus* species, such as *A. nidulans*, *A. flavus*, *Aspergillus terreus*, and *Aspergillus niger* (reference 8 and data not shown). Nevertheless, DNA complementarity experiments demonstrate that *A. fumigatus* represents a distinct taxonomic entity. The four strains of *A. fumigatus* tested by Peterson had similarities to each other of 92 to 100% and similarities to the most closely related strain of *N. fischeri* of less than 70% (25). Similarly, the reactivity of the moderately repetitive sequence λAF 3.19 was lower with *N. fischeri* DNA than with *A. fumigatus* DNA (Fig. 3). In addition, the moderately repetitive probe λAF 3.9, which efficiently hybridizes with *A. fumigatus* DNA, gave one weak band with only one of the *N. fischeri* strains. These results of hybridization of *A. fumigatus* sequences to *N. fischeri* DNA suggest that *N. fischeri* and *A. fumigatus* are indeed two distinct taxa.

N. fischeri var. *fischeri* appeared to be the variety most closely related to *A. fumigatus*. This variety shares four major groups of mycotoxins with *A. fumigatus* (fumitremorgins A, B, and C and tryptoquivalins) (27). In addition, ethidium bromide-stained RFLP patterns of *Eco*RI-digested DNA from *A. fumigatus* and *N. fischeri* var. *fischeri* are identical (Fig. 1). Moreover, similar Southern hybridization patterns were seen when *Eco*RI-, *Hin*dIII-, or *Bgl*II-digested DNAs from *A. fumigatus* and *N. fischeri* var. *fischeri* were hybridized, respectively, with the 1.5-kb *Bgl*II-*Hin*dIII fragment of the 33-kDa protease gene (Fig. 5) or the 1.3-kb *Pst*I fragment of the 18-kDa ribotoxin gene (reference 19 and data not shown).

In contrast, *N. fischeri* var. *glabra* appeared to be the variety most genetically different from *A. fumigatus*. For example, *N. fischeri* var. *glabra* was the only variety which gave a weak reactivity with the moderately repetitive sequence of *A. fumigatus* λAF 3.19 (Fig. 3). In addition, *Hin*dIII-digested DNA probed with the 18-kDa probe (data not shown), as well as *Eco*RI-, *Hin*dIII-, and *Bgl*II-digested DNA probed with the 33-kDa probe (Fig. 5), showed Southern hybridization patterns that differed between *N. fischeri* var. *glabra* and *A. fumigatus* strains.

Variability within *N. fischeri* species. Ascospore and conidium morphology and physiological characters (secondary metabolites and heat resistance), as well as DNA complementation experiments, allow the division of *N. fischeri* strains into different taxa (18, 23, 27). The three varieties of *N. fischeri* were raised to the species level by Kozakiewicz (18) on the basis of differences in conidial and ascospore morphologies. On the basis of DNA complementation experiments, Peterson (25) showed that all three varieties of *N. fischeri* represent separate taxa. However, a certain level of heterogeneity was observed: depending on the strains tested, DNA complementarity varied from 58 to 66% between *N. fischeri* var. *fischeri* and *N. fischeri* var. *spinosa*, from 23 to 44% between *N. fischeri* var. *fischeri* and *N. fischeri* var. *glabra*, and from 30 to 52% between *N. fischeri* var. *spinosa* and *N. fischeri* var. *glabra.*

Among all *N. fischeri* varieties, *N. fischeri* var. *fischeri* is homogenous and is well separated from the two others. Scanning electron microscopy showed that *N. fischeri* var. *fischeri* ascospores are reticulate and differ markedly from *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa* ascospores, which are typically roughened (25, 27). Besides, *N. fischeri* var. *fischeri* ascospores were less thermoresistant (the decimal reduction time at 85°C [$D_{85°C}$] was 6 to 10 min, and the *z* value, the elevation of temperature necessary to reduce the D value to 1/10 its former value, was 9 to 10°C) than those of *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa* ($D_{85°C}$, 10 to 96 min; *z*, 5 to 14°C) (23). High-pressure liquid chromatography (HPLC) analysis showed that the majority of *N. fischeri* var. *fischeri* strains produced the same secondary metabolites (27). Ethidium bromide-stained RFLP (Fig. 1) and Southern blot hybridization patterns obtained with a ribosomal probe (Fig. 2) or the 33-kDa protease gene (Fig. 5) were identical for all the *N. fischeri* var. *fischeri* strains tested. These results are in agreement with previous studies that recommend the raising of *N. fischeri* var. *fischeri* to species rank (18, 27).

On the contrary, *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa* exhibit important infraspecific variations. HPLC analysis of secondary metabolites showed marked differences among strains of *N. fischeri* var. *glabra* and among strains of *N. fischeri* var. *spinosa* (23, 27). In particular, *N. fischeri* var. *glabra* could be divided into three very different chemotypes, each of them being characterized by the presence of a particular metabolite. The genomic characterizations presented herein also reflect significant heterogeneities within the varieties *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa*. In most cases, ethidium bromide-stained RFLP patterns (Fig. 1) and Southern blot patterns obtained with a ribosomal sequence (Fig. 2) or an *A.*

fumigatus gene (Fig. 5) exhibit fragments of variable molecular weights for the different strains of *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa* tested (Fig. 1, 2, and 5 and reference 8). So far, different ribosomal Southern blot patterns have been observed only between various species of *Aspergillus* and not at the strain level (8, 34). Therefore, the heterogeneity of *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa* ribosomal Southern blot patterns (Fig. 2) suggests the putative presence of different taxonomic entities within these taxa. On the basis of DNA complementarity, mating reactions with other species of *Neosartorya*, and the ascospore morphology, Peterson (25) showed that one of the strains classified in the variety *N. fischeri* var. *glabra* could indeed represent a different species. The use of other taxonomic methods, such as the sequencing of internal transcript spacers of the ribosomal complex (3), could contribute to clarification of the taxonomic relationships among *N. fischeri* varieties.

Fingerprinting of *N. fischeri* strains. Food spoilages are caused mainly by *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa* (23, 29). The use of fingerprinting methods to type these food-borne fungi could allow the routes of contamination of food products in industrial environments to be traced.

RFLP patterns of *Eco*RI-digested DNA did not adequately discriminate among strains of *A. fumigatus* (4, 8). Conversely, *Eco*RI RFLP patterns of *N. fischeri* exhibited sufficient polymorphism on major restriction bands to distinguish most of the *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa* strains tested. RFLP can then be used for typing strains of the two varieties *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa*. Nevertheless, ethidium bromide-stained RFLP patterns are always more difficult to analyze than Southern hybridization blots because of the high number and poor definition of most restriction bands.

The moderately repetitive sequence λAF 3.19 generated discrete and unique Southern hybridization patterns with all the unrelated strains of *N. fischeri* tested. Banding patterns were stable within a single strain of *N. fischeri* var. *spinosa* even through several homothallic sexual cycles (Fig. 4). A similar stability of Southern hybridization patterns with moderately repetitive sequences was also shown for the fungal species *Sclerotinia sclerotiorum*, which propagates through homothallic, sexual reproduction (17). Thus, the λAF 3.19 DNA probe appeared to fulfill all of the requisites for effective fingerprinting of food-borne strains of *N. fischeri*. Moderately repetitive *A. fumigatus* sequences have demonstrated their efficiency during the course of epidemiological studies of several aspergillosis syndromes (including aspergilloma [9] and invasive aspergillosis [10]). By comparison of *N. fischeri* strains isolated from soils where fruits were harvested with strains from fruits used in the industrial process and strains from contaminated processed products, it may be possible to localize the origins of contaminations. An extensive epidemiological study may also allow a clustering of the strains depending on the geographical locations or on the thermoresistance, which is known to vary significantly among isolates (23), of the isolates.

ACKNOWLEDGMENTS

We thank M. F. Roquebert, Laboratoire de Cryptogamie-Mycologie, Museum National d'Histoire Naturelle, Paris, France, and R. A. Samson, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, for providing the *N. fischeri* isolates used in this study.

REFERENCES

1. **Aufauvre-Brown, A., J. Cohen, and D. W. Holden.** 1992. Use of randomly amplified polymorphic DNA markers to distinguish isolates of *Aspergillus fumigatus*. J. Clin. Microbiol. **30:**2991–2993.
2. **Beuchat, L. R.** 1986. Extraordinary heat resistance of *Talaromyces flavus* and *Neosartorya fischeri* ascospores in fruit products. J. Food Sci. **516:**1506–1510.
3. **Bruns, T. D., J. J. White, and J. W. Taylor.** 1991. Fungal molecular systematics. Annu. Rev. Ecol. Syst. **22:**525–564.
4. **Burnie, J. P., A. Coke, and R. C. Matthews.** 1992. Restriction endonuclease analysis of *Aspergillus fumigatus* DNA. J. Clin. Pathol. **45:**324–327.
5. **Denning, D. W., K. V. Clemons, L. H. Hanson, and D. A. Stevens.** 1990. Restriction endonuclease analysis of total cellular DNA of *Aspergillus fumigatus* isolates of geographically and epidemiologically diverse origins. J. Infect. Dis. **162:**1151–1158.
6. **Domsch, K. H., W. Gams, and T. H. Anderson (ed.).** 1980. Compendium of soil fungi, vol. 1, p. 511–512. Academic Press, London.
7. **Girardin, H., and J. P. Latgé.** 1992. Comparison of *Neosartorya fischeri* varieties based on protein profiles and immunoactivities, p. 177–188. *In* R. A. Samson, A. D. Hocking, J. I. Pitt, and A. D. King (ed.), Modern methods in food mycology. Elsevier, Amsterdam.
8. **Girardin, H., J.-P. Latgé, T. Srikantha, B. Morrow, and D. R. Soll.** 1993. Development of DNA probes for fingerprinting *Aspergillus fumigatus*. J. Clin. Microbiol. **31:**1547–1554.
9. **Girardin, H., J. Sarfati, H. Kobayashi, J.-P. Bouchara, and J.-P. Latgé.** 1994. Use of DNA moderately repetitive sequence to type *Aspergillus fumigatus* isolates from aspergilloma patients. J. Infect. Dis. **169:**683–685.
10. **Girardin, H., J. Sarfati, F. Traoré, J. Dupouy Camet, F. Derouin, and J.-P. Latgé.** 1994. Molecular epidemiology of nosocomial invasive aspergillosis. J. Clin. Microbiol. **32:**684–690.
11. **Hearn, V. H., M. Moutaoukil, and J.-P. Latgé.** 1990. Analysis of components of *Aspergillus* and *Neosartorya* mycelial preparations by gel electrophoresis and Western blotting procedures, p. 235–248. *In* R. A. Samson and J. I. Pitt (ed.), Modern concepts in *Penicillium* and *Aspergillus* classification. Plenum Press, London.
12. **Hocking, A. D., and J. I. Pitt.** 1984. Food spoilage fungi. II. Heat-resistant. CSIRO Food Res. Q. **44:**73–82.
13. **Jaton-Ogay, K., M. Suter, R. Crameri, R. Falchetto, A. Fatih, and M. Monod.** 1992. Nucleotide sequence of a genomic and a cDNA clone encoding an extracellular alkaline protease of *Aspergillus fumigatus*. FEMS Microbiol. Lett. **92:**163–168.
14. **Jesenska, Z., D. Havranekova, and I. Sajbidorova.** 1984. On some problems of moulds on some products of canning industry. Cesk. Hyg. **24:**102–109.
15. **Jesenska, Z., E. Pieckova, and J. Sepitkova.** 1991. Thermoresistant propagules of *Neosartorya fischeri*: some ecologic considerations. J. Food Prot. **54:**582–584.
16. **Kavanagh, J., N. Larchet, and M. Stuart.** 1963. Occurrence of a heat-resistant species of *Aspergillus* in canned strawberries. Nature (London) **198:**1322.
17. **Kohn, L. M., E. Stasovski, I. Carbone, J. Royer, and J. B. Anderson.** 1991. Mycelial incompatibility and molecular markers identify genetic variabili ty in field populations of *Sclerotinia sclerotiorum*. Phytopathology. **81:**480–485.
18. **Kozakiewicz, Z.** 1989. *Aspergillus* species on stored products. Mycol. Papers **161:**48–59.
19. **Lamy, B., and J. Davies.** 1991. Isolation and nucleotide sequence of the *Aspergillus restrictus* gene coding for the ribonucleic toxin restrictocin and its expression in *Aspergillus nidulans*: the leader sequence protects producing strains from suicide. Nucleic Acids Res. **19:**1001–1006.
20. **Lockington, R. A., G. G. Taylor, M. Winther, C. Scazzocchio, and R. W. Davies.** 1982. A physical map of the ribosomal DNA repeat unit of *Aspergillus nidulans*. Gene **20:**135–137.
21. **Loudon, K. W., J. P. Burnie, A. P. Coke, and R. C. Matthews.** 1993. Application of polymerase chain reaction to fingerprinting *Aspergillus fumigatus* by random amplification of polymorphic DNA. J. Clin. Microbiol. **31:**1117–1121.
22. **Malloch, D., and R. F. Cain.** 1972. Trichocomataceae: ascomycetes with *Aspergillus*, *Paecilomyces* and *Penicillium* imperfect states. Can. J. Bot. **50:** 2613–2628.
23. **Nielsen, P. V., and R. A. Samson.** 1992. Differentiation of food-borne taxa of *Neosartorya*, p. 159–168. *In* R. A. Samson, A. D. Hocking, J. I. Pitt, and A. D. King (ed.), Modern methods in food mycology. Elsevier, Amsterdam.
24. **Patterson, D. S. P., B. J. Shreeve, B. A. Roberts, and S. M. MacDonald.** 1981. Verruculogen produced by soil fungi in England and Wales. Appl. Environ. Microbiol. **42:**916–917.
25. **Peterson, S. W.** 1992. *Neosartorya pseudofischeri* sp. nov. and its relationship to other species in *Aspergillus* section *Fumigati*. Mycol. Res. **96:**547–554.
26. **Raper, K. B., and D. I. Fennell.** 1965. *Aspergillus fumigatus* group, p. 238–268. *In* K. B. Raper and D. I. Fennell (ed.), The genus *Aspergillus*. Williams & Wilkins, Baltimore.

26a. **Samson, R. A.** Personal communication.

27. **Samson, R. A., P. V. Nielsen, and J. C. Frisvad.** 1990. The genus *Neosartorya*: differentiation by scanning electron microscopy and mycotoxin profiles, p. 455–467. *In* R. A. Samson and J. I. Pitt (ed.), Modern concepts in *Penicillium* and *Aspergillus* classification. Plenum Press, London.
28. **Samson, R. A., and E. S. van Reenen-Hoekstra (ed.).** 1988. Introduction to food-borne fungi, 2nd ed. Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

29. **Samson, R. A., E. S. van Reenen-Hoekstra, and B. J. Hartog.** 1992. Influence of pretreatment of raspberry pulp on the detection of heat resistant moulds, p. 155–158. *In* R. A. Samson, A. D. Hocking, J. I. Pitt, and A. D. King (ed.), Modern methods in food mycology. Elsevier, Amsterdam.
30. **Scott, V. N., and D. T. Bernard.** 1987. Heat resistance of *Talaromyces flavus* and *Neosartorya fischeri* isolated from commercial fruit juices. J. Food Prot. **50:**18–20.
31. **Splittstoesser, D. F.** 1991. Fungi of importance in processed fruits, p. 201–219. *In* K. D. Arora, K. G. Mukerji, and E. H. Marth (ed.), Handbook of applied mycology, vol. 3. Foods and feeds. Marcel Dekker, New York.
32. **Splittstoesser, D. F., F. R. Kuss, W. Harrison, and D. B. Prest.** 1971. Incidence of heat-resistant molds in eastern orchards and vineyards. Appl. Microbiol. **21:**335–337.
33. **Splittstoesser, D. F., and C. M. Splittstoesser.** 1977. Ascospores of *Byssochlamys fulva* compared with those of a heat-resistant *Aspergillus*. J. Food Sci. **42:**685–688.
34. **Spreadbury, C. L., B. W. Bainbridge, and J. Cohen.** 1990. Restriction fragment length polymorphisms in isolates of *Aspergillus fumigatus* probed with part of the intergenic spacer region from the ribosomal RNA gene complex of *Aspergillus nidulans*. J. Gen. Microbiol. **136:**1991–1994.