A SECOND HETEROTHALLIC *ASPERGILLUS*

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SUMMARY

A new heterothallic fungus, *Neosartorya fennelliae* (asexual state: *Aspergillus fennelliae*), was isolated from the eyeballs of laboratory rabbits. The conidial and ascosporic states resemble those of *Neosartorya fischeri* (*Aspergillus fischeri*). The strains occur in two mating types, *A* and *a*, of an allelic pair. Although interspecific crossing does not occur with any known members of the *N. fischeri* group, stimulation of the growth of *N. fischeri* var. *glaber* by the new species was observed. The fungus survives in mouse tissue for several weeks without evidence of multiplication.

Heterothallism in the genus *Aspergillus* was first reported by Kwon, Fennell, and Raper (1) in *A. heterothallicus*. The ascosporic state of this fungus was later transferred to the genus *Emericella* as *E. heterothallica* (Kwon, Fennell & Raper) Malloch & Cain (7). A second heterothallic species of *Aspergillus* was isolated from the eyeballs of laboratory rabbits by one of the authors (K-C) in April, 1972. Morphological characteristics of the asexual and sexual fruiting structures place the new species in the *A. fischeri* series (11), for which Malloch and Cain have established the new generic name *Neosartorya* (8). This paper provides a description of the species, observations on ascocarp formation, proof of heterothallism, and a discussion of the relationship of the new species to the other species of *Neosartorya*.

MATERIALS AND METHODS

Six original strains, AF1 through AF6, were purified by isolating single conidia. Cultures were grown singly and in all possible combinations upon a variety of agar substrata at different temperatures of incubation. The media used were Czapek's solution (11), malt extract (11), soil extract (4), and alphacel-yeast extract agars (6). Incubation was at 25, 37, and 45 C. The species description is based upon cultural characteristics and details of morphology exhibited on Czapek's

solution and malt extract agars at 25 C. Color references are based upon plates in Ridgway's *Color standards and color nomenclature* (12). Strains AF4 (*a*) and AF5 (*A*) were paired to obtain progeny for proof of heterothallism. An albino mutant (AF4W) was obtained by irradiating (3) a conidial suspension of AF4 with 15-W germicidal UV lamp. The white conidial heads served as a genetic marker. Random isolation of single-ascospore cultures was carried out as described elsewhere (2).

Tester strains, AF4 (*a*) and AF5 (*A*), were crossed with type or representative strains of several members of *A. fischeri* series received from the Northern Regional Research Laboratory (NRRL), Peoria, Illinois, and the American Type Culture Collection, Rockville, Maryland. The strains tested include: *A. fischeri* Wehmer (ATCC 16904); *A. fischeri* var. *glaber* Fennell & Raper (ATCC 16909, 18787); *A. fischeri* var. *spinosus* Raper & Fennell (ATCC 16898); *A fischeri* var. *thermomutatus* Paden (ATCC 18618); *A. aureolus* Fennell & Raper (NRRL 2244); and *A. auratus* Warcup (NRRL 4379). The two tester strains were also paired with several strains of *A. fumigatus* Fres. isolated from a clinical specimen at National Institutes of Health, Bethesda, Maryland.

Mice and rabbits were injected intravenously with conidial suspensions (1×10^6/ml) of AF4 and AF5 to observe the pathogenicity of the fungus. Mice were autopsied at 3 and 6 wk, and the livers, lungs, spleens, and kidneys were processed for recovery of the fungus and histopathologic preparations as described previously (5). The eyes of rabbits were examined periodically by ophthalmoscopy to detect any lesions.

DESCRIPTION

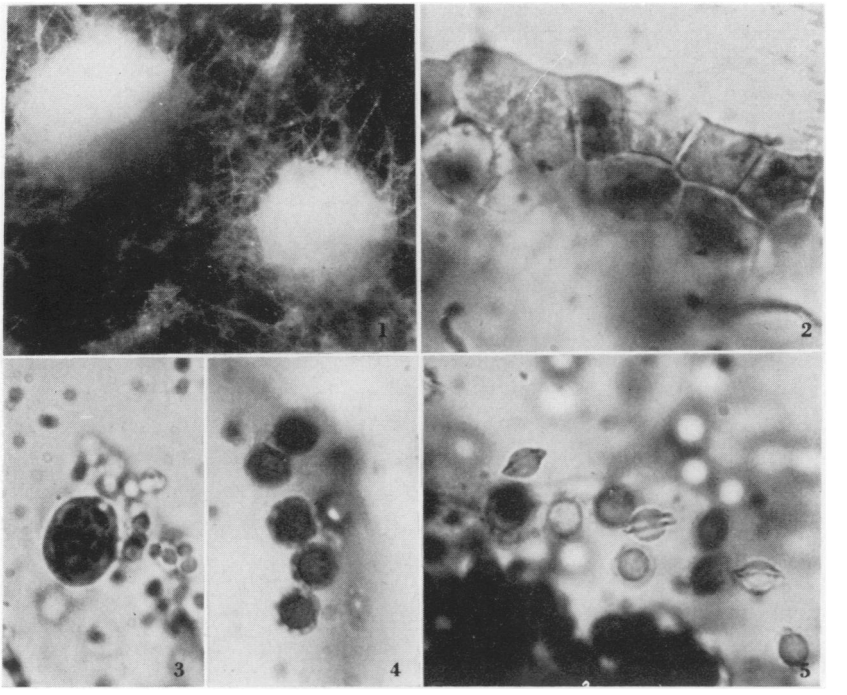
Neosartorya fennelliae Kwon-Chung et Kim, sp. nov. FIGS. 1–18

Fungus heterothallicus. Cleistotheca globosa alba, singulatim vel in caespitibus parvis intra integumentum laxum hyphalicum enata, 150–450 μ diam., parietibus tenuibus fragilibusque, e stratis 2–3 cellularum irregulariter applanatarum compositis; asci subglobosi octospori 10–11.5 × 11.5–13.5 μ; ascosporae biconvexae hyalinae 5.5–7.7 × 3.2–5 μ, cristis duobus ornatae, superficie convexa propter costas tenues anastomosantes subtiliter asperula; capita conidica viridia (12: Pl. XLVII), primum hemisphaerica deinde laxe columnaria, 80–300 × 30–100 μ; conidiophora e mycelio submerso vel ex hyphis aereis enata, glabra, praecipue in parte superiore pallide viridula, 150–250 × 4–6 μ; vesiculae lageniformes 10–17 μ diam. viridula, partibus superioribus dimidiis vel tertiis quartis sterigmata ferentibus; sterigmata uniseriata, pallide vel obscure viridia, 4.5–7 × 2.2–2.8 μ; conidia globosa subglobosa vel elliptica, glabra vel leviter aspera, pallide viridula, globosa 2.2–2.5 μ diam., elliptica usque ad 2.8 μ longa uninucleata.

Status asexualis: *Aspergillus fennelliae*.

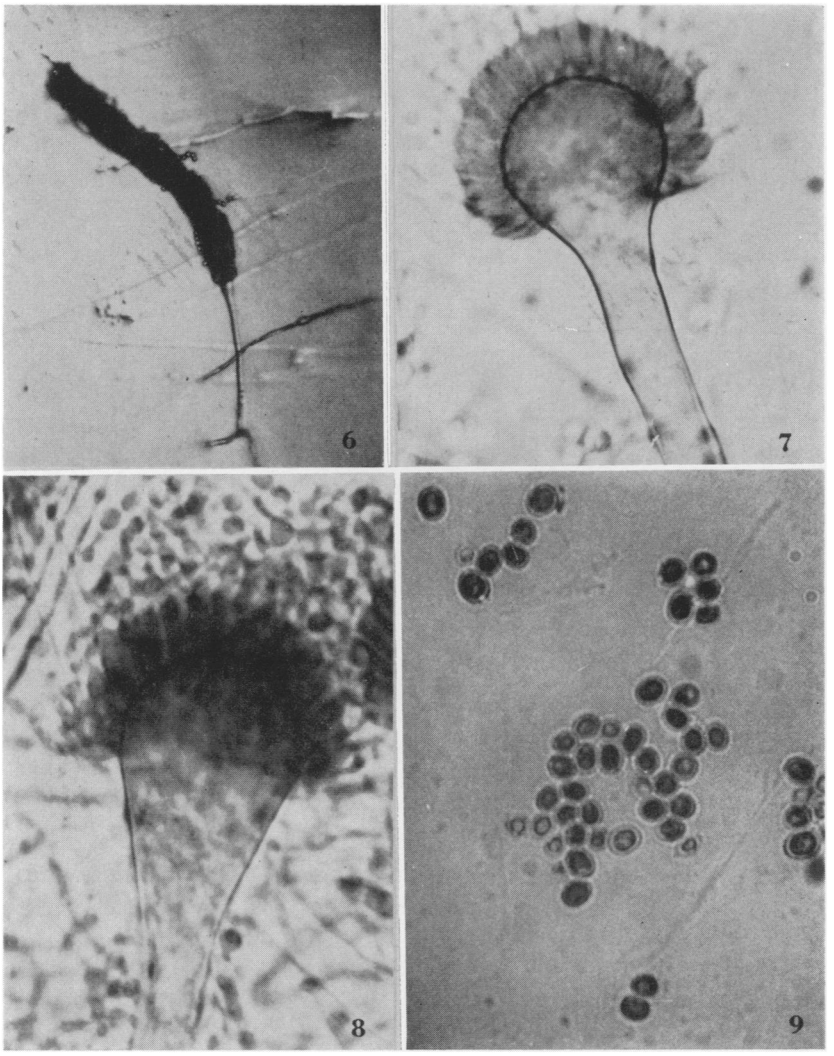
Ex ocullo cuniculi isolata.

Heterothallic fungus. Cleistothecia (FIG. 1) globose, white, borne singly or in small clusters within a loose hyphal envelope, 150–450 μ in diam, with walls thin and fragile, consisting of two to three layers of irregularly flattened cells (FIG. 2); asci subglobose, 8-spored (FIG. 3), 10–11.5 × 11.5–13.5 μ; ascospores biconvex, uncolored, 5.5–7.7 × 3.2–5 μ, with two equatorial crests and convex surfaces delicately roughened due to the anastomosing shallow ridges (FIGS. 4, 5). Conidial heads Gnaphalium Green to Court Gray or Tea Green (12: Pl XLVII), hemispherical at first becoming loosely columnar (FIG. 6), 80–300 ×



FIGS. 1–5. Ascigerous structures of *Neosartorya fennelliae*. 1. White globose cleistothecia, × 130. 2. Irregularly flattened cells of cleistothecial wall, × 1,250. 3. Subglobose ascus with ascospores, × 1,250. 4. Top view of ascospores showing convex surface with anastomosing shallow ridges, × 1,250. 5. Side view of ascospores showing two equatorial crests and finely roughened convex surface, × 1,250.

30–100 μ; conidiophores arising from submerged mycelium or from aerial hyphae, smooth (FIG. 7), faintly colored in greenish shades especially in the upper part, 150–250 × 4–6 μ; vesicles flask shaped (FIGS. 7, 8), 10–17 μ in diam, greenish in color, bearing sterigmata



FIGS. 6–9. Conidial structures of *Aspergillus fennelliae*. 6. Loosely columnar conidial head, × 300. 7–8. Details of the conidial structure, × 1,250. 9. Globose to subglobose or elliptical conidia, × 1,250.

over the upper one half to three fourths; sterigmata in a single series (FIG. 7), pale to dull greenish shades, 4.5–7 × 2.2–2.8 μ; conidia (FIG. 9) globose, subglobose to elliptical, smooth or finely roughened, faintly green, 2.2–2.5 μ in diam when globose and up to 2.8 μ in long axis when elliptical, uninucleate.

Source of isolation.—Eye balls of a rabbit.

Asexual state.—*Aspergillus fennelliae.*

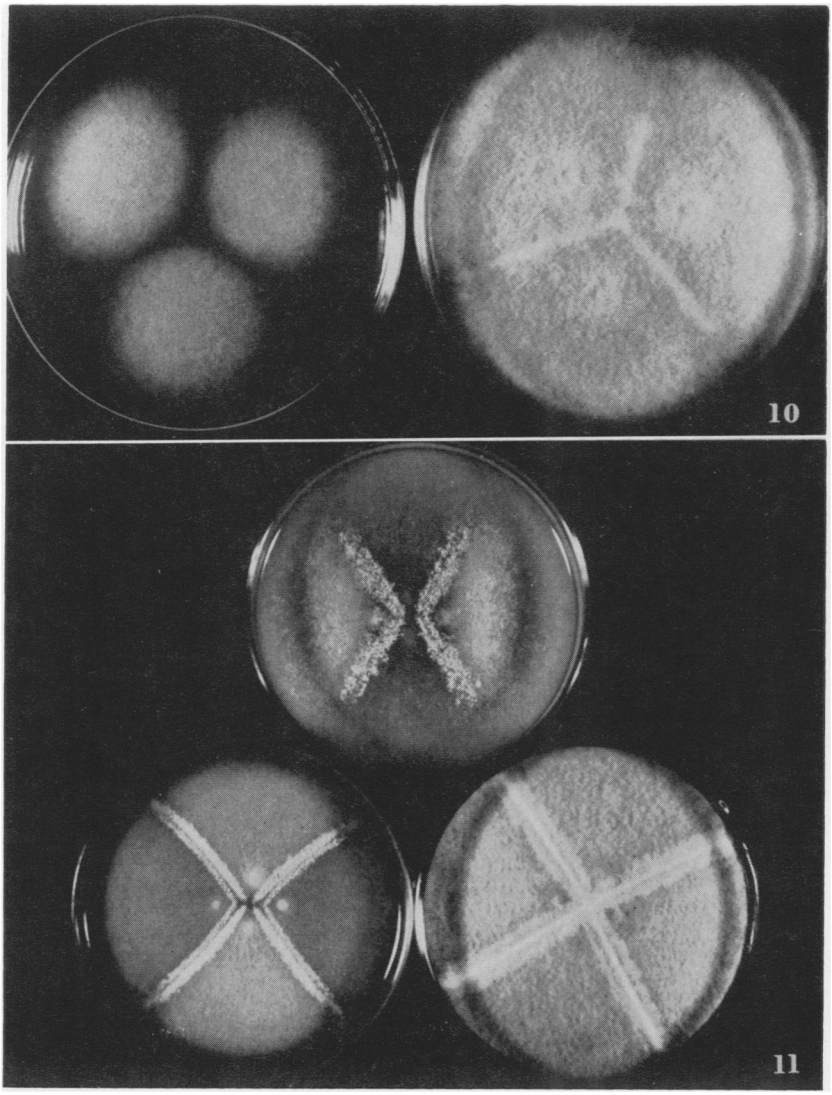
Type cultures.—AF4 (NRRL 5535 = ATCC 24326) and AF5 (NRRL 5534 = ATCC 24325) have been deposited in the Northern Regional Research Laboratory, USDA, Peoria, Illinois, and with the American Type Culture Collection, Rockville, Maryland.

The species epithet *fennelliae* was selected in honor of Miss D. I. Fennell for her contribution to our knowledge of the genus *Aspergillus.*

The pattern of the conidial structures and the character of the cleistothecia of this species are similar to those of *Neosartorya fischeri* and its varieties (**8, 10, 11**). However, strains of *N. fennelliae* are heterothallic and produce ascospores with a delicately rough convex surface due to the shallow ridges; whereas, *Neosartorya fischeri* and its varieties are homothallic and produce ascospores with a convex surface conspicuously echinulate due to deeper ridges in *N. fischeri, N. fischeri* var. *thermomutatus,* and *N. fischeri* var. *spinosus* or smooth as in *N. fischeri* var. *glaber.*

RESULTS

The fungus grows moderately at 25 C and more rapidly at 37 C on Czapek's solution agar. Growth is enhanced on malt extract agar at both 25 C and 37 C, and colonies attain diameters of 3.5 and 6 cm, respectively, within 5 da (FIG. 10). The rate of growth is much decreased at 45 C, and no growth is observed at 47 C. Ascogonial coils are produced uniformly throughout colonies of single strains on malt extract or alphacel-yeast extract agar within 4 da of incubation at either 25 or 37 C. These coils develop into pseudocleistothecia, devoid of ascospores, in 2–3 wk. Fertile cleistothecia are produced only when two compatible strains are paired. When the six original isolates were paired in all possible combinations (TABLE I) on malt extract, soil extract, and alphacel-yeast extract agar, four isolates (AF1, AF2, AF3, and AF4) were found to be compatible with the remaining two (AF5 and AF6) and produced abundant fertile cleistothecia (FIG. 11). The mating type of the first four strains is designated as *a* and the other two as *A*. Forty single ascospores, obtained from the cross AF4 (*a*) × AF5 (*A*), gave rise to 22 *a* and 18 *A* self-sterile cultures (TABLE II). Sixty single-ascospore cultures, isolated from cleistothecia, obtained by crossing AF4 W (*a*) and the wild type, AF5 (*A*), showed: 16 (green *a*), 15 (green, *A*), 16 (white, *a*), and 13 (white, *A*). These results indicate that the mating types segregate independently of the locus for



FIGS. 10–11. *Neosartorya fennelliae.* 10. Five-da-old colonies on malt extract agar at 25 C (left) and 37 C (right). 11. Paired cultures of $a \times A$ on alphacel-yeast extract agar (top) on soil extract agar (left) and on malt extract agar (right) with abundant cleistothecia.

conidial-head pigmentation. Of the total 100 progeny tested, the ratio of a (54) and A (46) was 1: (χ^2 test, $p = 0.43$), indicating that the mating-type factors are an allelic pair.

TABLE I

RESULTS OF CROSSES AMONG THE ORIGINAL SIX ISOLATES OF *Neosartorya fennelliae*

Strain No.	AF1	AF2	AF3	AF4	AF5	AF6
AF1	—	—	—	—	+	+
AF2		—	—	—	+	+
AF3			—	—	+	+
AF4				—	+	+
AF5					—	—
AF6						—

Cleistothecial formation initiates with helical ascogonia (FIGS. 12, 13) and becomes abundant after 3–4 da of growth on either malt extract or alphacel-yeast extract agar. The tip of a young hypha arising from a large hypha in the mycelium begins to coil (FIG. 12) and continues (FIGS. 13, 14) until an average of 4–5 turns is made. At first, the coil is coenocytic (FIGS. 12, 13) but becomes multiseptate and swells when coiling ceases (FIG. 14). At this time, thin hyphal branches, presumably from the opposite mating strain, grow toward the ascogonium and surround it from various points (FIG. 15). The thin hyphae form a dense layer about the ascogonium, and the young cleistothecia are produced (FIG. 16). At maturity, the cleistothecia are enveloped by white loose hyphae as shown in FIG. 1.

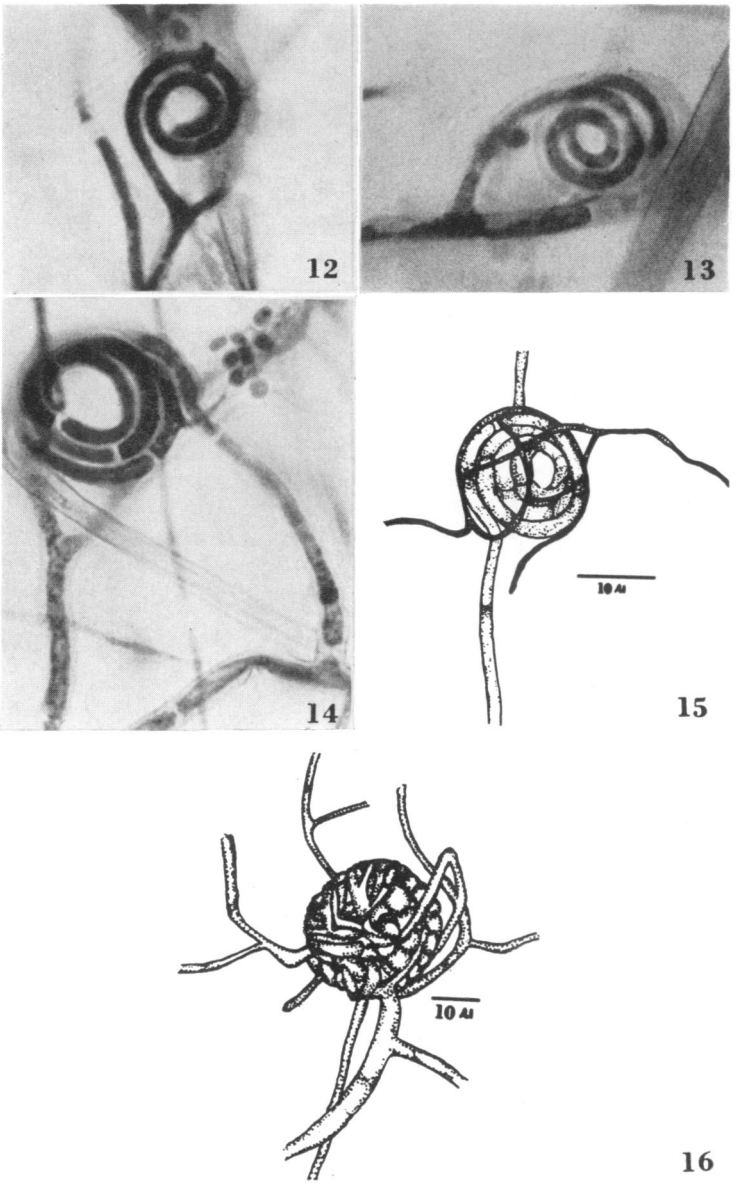
Interspecific pairs not only failed to cross but usually produced clear zones between strains of *N. fennelliae* and other members of the *N. fischeri* series (FIG. 17) or *A. fumigatus*. One exception to this phenomenon was found when *A. fischeri* var. *glaber* and *A. fennelliae* were paired. Colonies of both *a* and *A* mating type of *A. fennelliae*

TABLE II

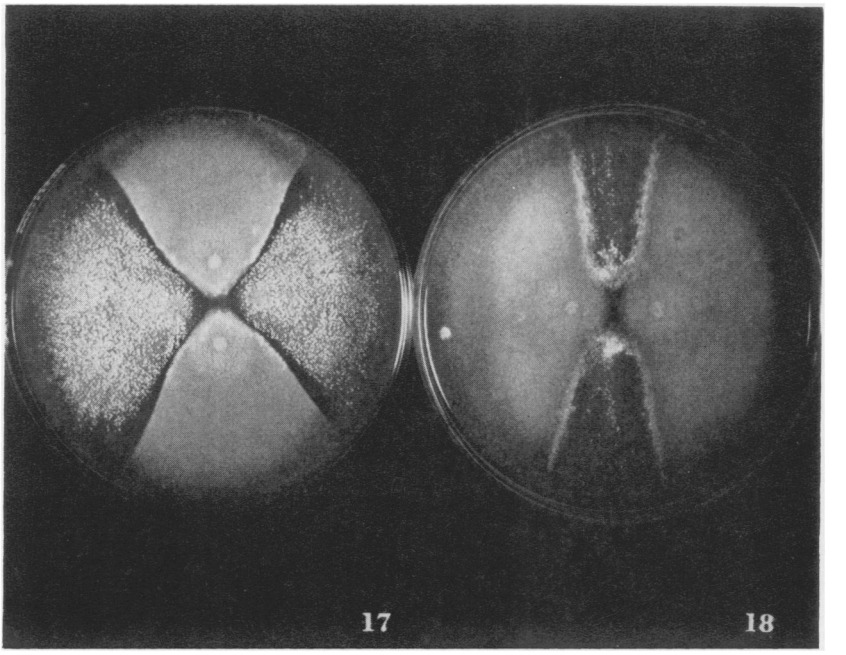
MATING-TYPE TESTS OF PROGENY OF *Neosartorya fennelliae* FROM PAIRINGS OF AF4 (*a*) WILD TYPE AND ALBINO MUTANT WITH AF5 (*A*) WILD TYPE

Crosses of strains	Conidial-head color of progeny	Number of each progeny obtained	Mating type		*P*-value on the basis of estimated 1:1 ratio
			A	*a*	
AF4 × AF5	Wild type	40	18	22	0.66
AF4W* × AF5	Wild type	31	15	16	0.90
	White	29	13	16	0.72
Total		100	46	54	0.43

* W—White.



FIGS. 12–16. *Neosartorya fennelliae*. 12–13. Coenocytic helical ascogonia (stained with cotton blue), × 1,250. 14. Septate ascogonium, later stage than FIG. 13 × 1,250. 15. Drawing of ascogonium surrounded by thin hyphae from opposite mating type, × 1,250. 16. Young cleistothecium.



FIGS. 17–18. Paired cultures of *Neosartorya fennelliae* and two other members of *Neosartorya*. 17. *N. fennelliae a* type × *N. fischeri* ATCC 16904, producing a clear zone between the two species. Cleistothecia are produced only in the two quadrants of homothallic *N. fischeri*. 18. *N. fennelliae a* type × *N. fischeri* var. *glaber* ATCC 16909. Enhanced hyphal growth can be seen at the interfaces.

were found to stimulate hyphal growth of *A. fischeri* var. *glaber* along the line of contact (FIG. 18).

The fungus produced no lesions in mice but could be recovered from mouse tissue at autopsy 3 wk after injection. Sections of stained tissue showed no growth of the fungus. The fungus also failed to produce lesions in the eyes of the rabbits.

DISCUSSION

Heterothallism in *N. fennelliae* was discovered accidentally by one of the authors (K-C) when homogenized eye balls of rabbits were spread on soil extract agar in attempts to recover *Histoplasma capsulatum* Darling. The rabbits had been injected previously with the mycelial form of *H. capsulatum* in a study of experimental ocular histo-

plasmosis. Several colonies of *N. fennelliae* grew out from each eye of two rabbits, and the phenomenon of cross-mating between two adjacent colonies was observed after 1 wk of incubation. Had each eye contained only one mating type, heterothallism would not have been detected, and the cultures probably would have been considered *Aspergillus fumigatus*.

True heterothallism has now been found in two species with *Aspergillus* asexual states. The pattern of sexuality in both *Emericella heterothallica* (*A. heterothallicus*) and *Neosartorya fennelliae* (*A. fennelliae*) belong to the one locus-two allele incompatability system (2), as do the majority of heterothallic species of Ascomycetes.

The ascogonial coil of *N. fennelliae* resembles that of *N. fischeri* (9), but mature cleistothecia are generally smaller than those of *N. fischeri*. Strains of *N. fennelliae* stimulate hyphal growth of *N. fischeri* var. *glaber* but form a clear barrier zone when paired with other members of the genus. Further evidence that *N. fennelliae* is more closely related to *N. fischeri* var. *glaber* than to other members of the genus is found in the ornamentation of their ascospores.

Although the original six strains of *N. fennelliae* were isolated from rabbits' eyeballs, the fungus proved to be nonpathogenic. It neither produced lesions nor multiplied in mouse tissue or rabbit eyes when injected into the animals intravenously. However, since the optimum temperature for growth in vitro was 37 C and the conidia survived for at least 3 wk in mouse tissue, *N. fennelliae* may be a potential pathogen in patients with compromised immune defenses (13).

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