A NEW PATHOGENIC SPECIES OF *ASPERGILLUS* IN THE *ASPERGILLUS FUMIGATUS* SERIES

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SUMMARY

A new pathogenic species, *Aspergillus phialiseptus,* based on three isolates from clinical specimens is described. The species belongs to the *Aspergillus fumigatus* series and is characterized by conidial heads with uniseriate but septate phialides bearing short chains of subglobose to pyriform, smooth to finely roughened conidia. When the last conidium in a chain is liberated, a terminal fragment of the phialide remains attached. Strains of *A. phialiseptus* and *A. fumigatus* appear to be equally pathogenic in mice.

In 1973, we received a culture of an *Aspergillus* (ATCC 26606) isolated from biopsy material from the sphenoid sinus of a patient at the University of California, San Francisco Medical Center. The morphological characteristics of the fungus closely resembled the published description of two unusual clinical isolates of *Aspergillus fumigatus* Fresenius, WB 5110 and WB 5052 (Raper and Fennell, **1965**). These two strains were obtained from Northern Regional Research Laboratory, Peoria, Illinois. Careful comparison revealed that the strain ATCC 26606 was similar to WB 5110 and WB 5052 and that these three human isolates are sufficiently distinct from typical strains of *A. fumigatus* to warrant their description as a new species.

In this paper a new species based on these three strains is described and the major differences distinguishing the species from *A. fumigatus* are presented.

MATERIALS AND METHODS

The cultural characteristics of the three clinical isolates of an *Aspergillus* with septate phialides (ATCC 26606, WB 5110, and WB 5052) were compared with those of three strains of *A. fumigatus,* two NIH clinical isolates, NIH 14495 and NIH 26823, and one soil isolate, NIH KF5, and one strain of *A. fumigatus* var. *ellipticus* Raper & Fennell, WB 5109 (Raper and Fennell, **1965**). The media used were

Czapek's solution (3% and 20% sucrose), malt extract and $M_{40}Y$ agars (Raper and Fennell, **1965**). Incubation was at 25 and 37 C. Color references are based upon plates in Ridgeway (**1912**).

The pathogenicity of strains ATCC 26606 and WB 5110 was compared in mice with that of *A. fumigatus,* NIH 14495 and NIH 26823. Conidia from 2-wk-old cultures grown on malt extract agar at 25 C were suspended in 0.025% Tween 80 solution and stirred on a Vortex Jr. mixer for 5 min. The suspensions were filtered through sterile cotton tampons to remove spore chains and clumps, and centrifuged. The spore pellets were suspended in saline and serial 10-fold dilutions were prepared. For each strain, three dilutions containing 1×10^5, 1×10^6, and 1×10^7 spores/ml, as determined by both haemocytometer and plate count, were used as inocula. Female general purpose (NIH) white mice weighing 19–21 g were infected by injecting 0.5 ml of inocula into a lateral tail vein. Eight mice were infected with each inoculum and were observed daily for survival. On the 30th day post infection the surviving mice were sacrificed and the organs of the animals were used to recover the organism and to prepare tissue sections as described elsewhere (Kwon-Chung, **1968**).

DESCRIPTION

Aspergillus phialiseptus Kwon-Chung, sp. nov. FIGS. 1–2, 6–9

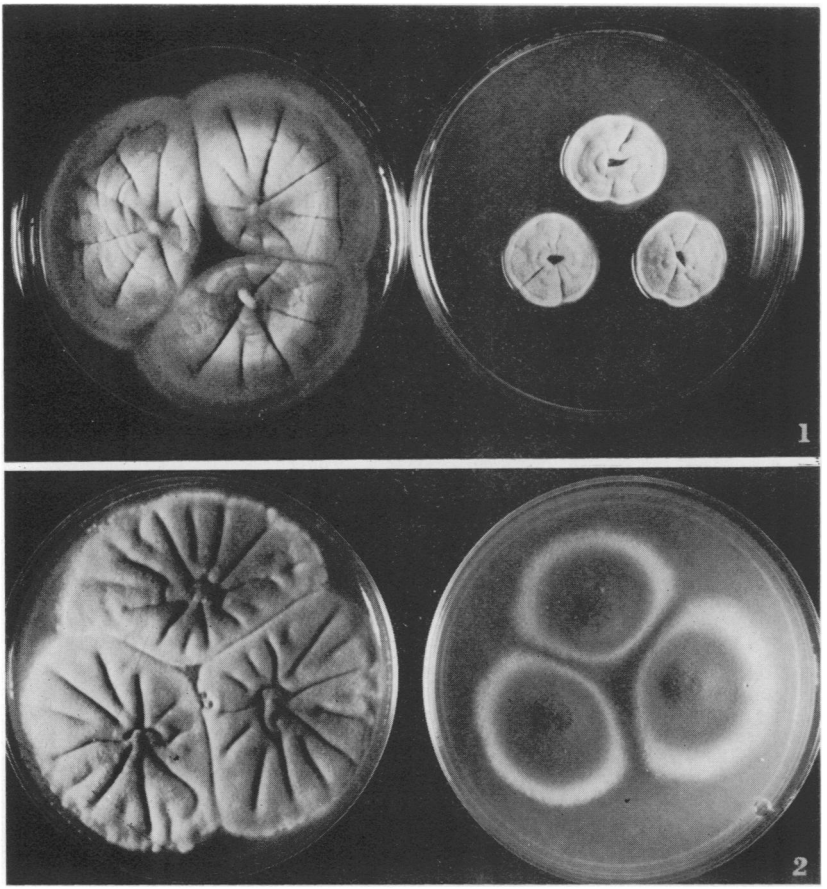
Coloniae in agaro Czapeki (3% sucroso) restrictae ruguseque, intra hebdomades duas ad temperaturam 25 C 2–3 cm. diam.; capita conidica numerosa, parva, griseocaerulea, 30–50 × 40–60 μm; conidiophora 10–14 × 130–250 μm, glabra et tenuitunicata; vesiculae 7–30 μm, latae, 20–25 μm longae aut definite lageniformes aut ex apicibus leniter expansis conidiophororum constitutae; phialides uniseriatae, quaque phialide saepe septo transversali simplici praedita, 9–30 × 3–5 μm, saepissime 14–16 × 3–4 μm; conidia piriformis vel ovalia 4–6 × 5–8 μm, vel subglobosa 3–5 μm diam., glabra usque ad subasperata.

Holotypus BPI 71850 e materia sinus sphenoid aegri isolata.

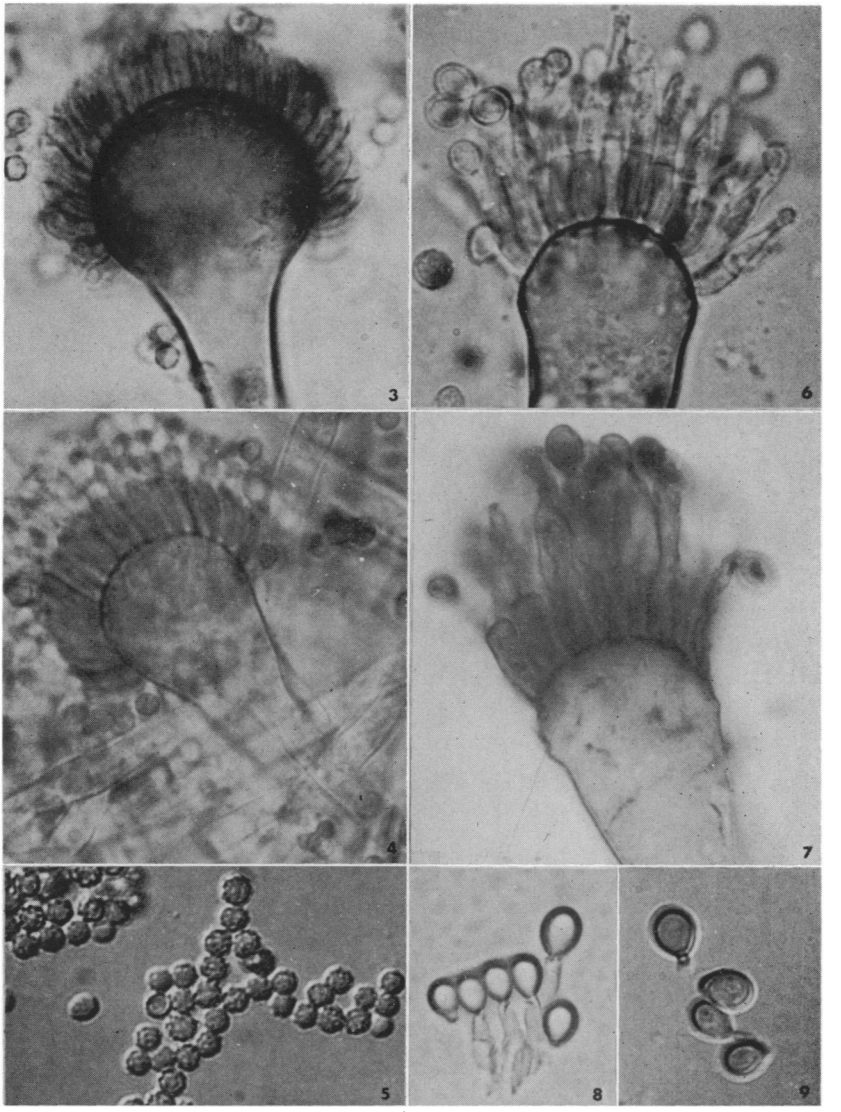
Colonies on Czapek's solution agar (3% sucrose) restricted, raised and wrinkled with tendency to split at the center (FIG. 1), diameter ranging from 2 to 3 cm in 2 wk at 25 C, Pale Smoke Gray (R., Pl. XLVI) or Deep Bluish Gray-Green (R. Pl. XLII) with the production of abundant bluish-green dimunitive and inconspicuous conidial heads; reverse wrinkled, yellowish brown. *Conidial heads* with short conidial chains, rarely becoming loosely columnar, 30–50 × 40–60 μm; *conidiophores* thin walled, smooth, straight or slightly sinuous, occasionally septate, without well-defined foot cells, 10–14 × 130–250 μm, *vesicles* (FIGS. 6, 7), 7–30 μm wide and 20–25 μm long, flask-shaped or consisting of slightly expanded apices of conidiophores, slightly greenish in the upper part, infrequently set at a slight angle on the conidiophore;

phialides uniseriate, frequently septate (FIG. 6), slightly greenish, variable in size, 9–30 × 3–5 μm; most commonly 14–16 × 3–4 μm, upper halves of phialides collapse readily in age (FIG. 7); conidia (FIGS. 8, 9) usually fewer than five per chain, pyriform to oval, 4–6 × 5–8 μm or subglobose, 3–5 μm in diam, smooth or slightly rough. The last conidium in each chain is not easily detached from the phialide and when liberated, carries a small to an appreciable part of the phialide with it (FIGS. 8, 9).

Colonies on malt extract agar (FIG. 2) growing well, 4–5 cm in diam in 2 wk at 25 C, plane and velvety, near American Green (R. Pl. XLI)



FIGS. 1–2. *Aspergillus phialiseptus* ATCC 26606. Two-wk-old colonies. 1. On Czapek's solution agar (3% sucrose) at 37 C (left) and 25 C (right). 2. On malt extract agar at 37 C (left) and 25 C (right).



FIGS. 3-5. *Aspergillus fumigatus.* Conidial structures. 3-4. Vesicles and phialides of NIH 14495 and NIH 23823 respectively. 5. Globose, echinulate conidia of NIH 14495. FIGS. 6-9. *Aspergillus phialiseptus* ATCC 26606. Conidial structures. 6. Long, septate phialides. 7. Collapsed upper portion of phialides. 8-9. Oblong or pyriform conidia bearing the fragments of phialides. (All × 1,200).

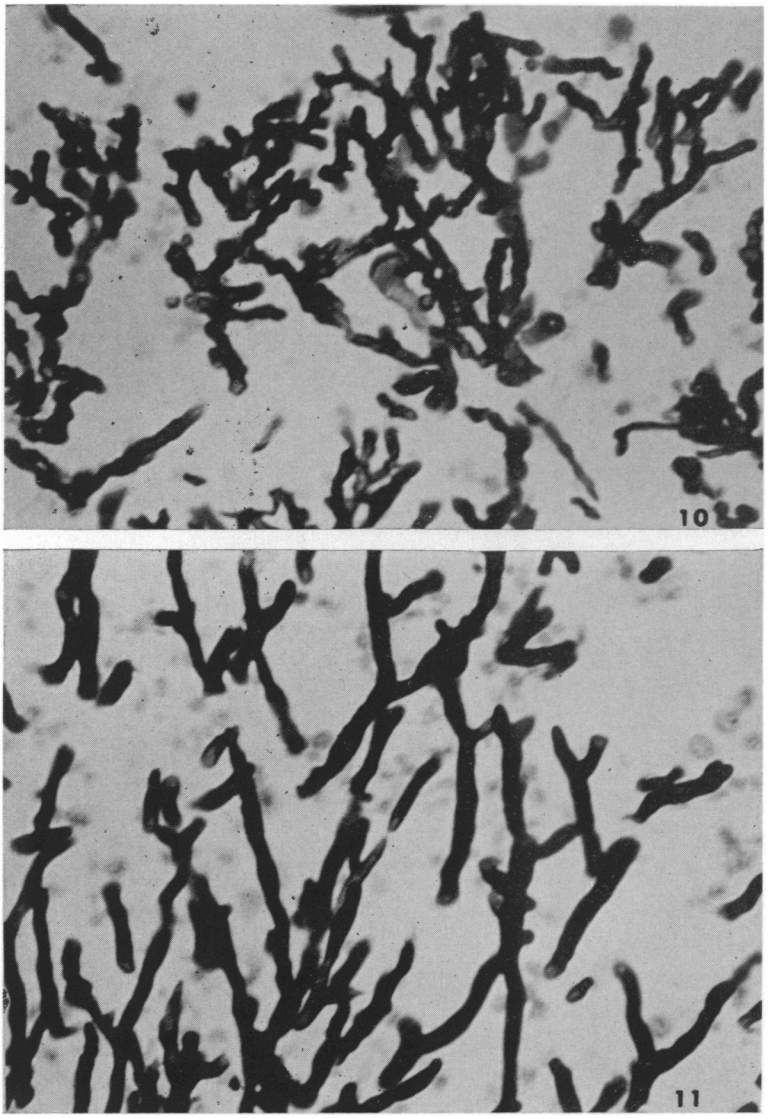
from abundant conidial heads except in a narrow white margin (FIG. 2); reverse cream to light yellowish green. Conidial structures as on Czapek's agar. The species grows faster at 37 C (FIGS. 1, 2) than at room temperature (25 C).

The type (BPI 71850; ATCC 26606), was isolated in 1973 at San Francisco Medical Center, San Francisco, California, from biopsy material of a patient suffering from sinusitis. The binomial *Aspergillus phialiseptus* was selected to denote the phialides with septa.

Aspergillus fumigatus var. *ellipticus,* WB 5109, differed from the strains of *A. fumigatus* only by size and shape of conidia as described by Raper and Fennell (**1965**). Colony characteristics and conidial structures, other than conidia, were the same between the species and the variety. Detailed comparison, therefore, was made only between strains of *A. fumigatus* and *A. phialiseptus.*

Differences between the conidial structures of *A. fumigatus* and *A. phialiseptus,* shown in FIGS. 3–9, are the major criteria for separation of the two species. *Aspergillus phialiseptus* produces septate phialides (FIGS. 6, 7) twice as long as those of *A. fumigatus* (FIGS. 3, 4). Phialides are more uniformly septate in strains WB 5110 and ATCC 26606 than in WB 5052. Conidia of *A. fumigatus* are definitely rough, globose to subglobose, and are readily deciduous, while those of *A. phialiseptus* are smooth to finely roughened, mostly oblong or pyriform, and the last conidium of each chain fails to separate from the phialide. When these conidia are forcibly liberated, they carry with them small or appreciable parts of the tips of their phialides (FIGS. 9, 10) and, in this respect, resemble aleuriospores (Ainsworth, **1961**). Strains WB 5110 and ATCC 26606 produce mostly smooth and large conidia, while WB 5052 tends to produce smaller, finely roughened conidia.

Colony characteristics of *A. phialiseptus* on various agar media at 25 or 37 C are distinct from those of *A. fumigatus.* Strains of *A. fumigatus* produce plane, felted or floccose, rapdly growing greenish colonies on malt and Czapek's agar at both 25 and 37 C. On Czapek's agar at 25 C, strains WB 5110 and ATCC 26606 of *A. phialiseptus* produce restricted, raised, wrinkled, greenish colonies with a tendency to split at the center, but are rapidly growing and cream colored on this medium at 37 C. Sporulation occurs only in marginal areas at 37 C and does not influence colony color. On Czapek's agar at both 25 and 37 C, strain WB 5052 produces floccose white colonies with a tinge of green due to light sporulation. More conspicuous differences in the growth characteristics of the two species, summarized in TABLE I, were observed on high-sugar agar media such as Czapek's solution with 20%



FIGS. 10–11. Hyphal growth as seen in sections of kidney from mice infected with *Aspergillus phialiseptus* ATCC 26606 (above) and *A. fumigatus* NIH 14495 (below) (Gomori Methenamine-Silver). × 480.

sucrose and $M_{40}Y$. On both media at 25 and 37 C, strains of *A. phialiseptus* either failed to grow or formed colonies of less than 1.5 cm in diam with no sporulation in 5 wk, while strains of *A. fumigatus* grew

TABLE I

GROWTH CHARACTERISTICS OF *Aspergillus phialiseptus* AND OF *A. fumigatus* ON DIFFERENT SUBSTRATES AND AT DIFFERENT TEMPERATURES

Strains	Color of colony on Cz[a] at 37 C	Growth at 25 C	
		Cz20[a]	$M_{40}Y$
A. fumigatus			
NIH KF 5	Bluish green	4.5; +++[b]	5.5; +
NIH 14495	Olivish green	4; ++	3; +++
NIH 26823	Bluish green	6; +++	6; +++
A. phialiseptus			
ATCC 26606	Yellowish cream	0.7; −	0; −
WB 5110	Yellowish cream	1.2; −	1.3; −
WB 5052	White with greenish tinge	1; −	0.7; −

[a] Cz—Czapek's solution (3% sucrose) agar; Cz20—Czapek's solution (20% sucrose) agar.

[b] Diameter of colony in centimeters on 35th da; +++ Heavy sporulation, ++ moderate sporulation, + poor sporulation, − no sporulation.

moderately (3–6 cm in diam) and sporulated poorly (NIH KF 5, soil isolate) to heavily (NIH 14495, NIH 26823, clinical isolates) on both media.

PATHOGENICITY

Mouse pathogenicity of isolates (TABLE II) varied within both of the species. All mice injected with 5×10^6 conidia of *A. fumigatus* or *A. phialiseptus* died within 3 da. All mice injected with 5×10^5 conidia of *A. fumigatus* strain NIH 26823 and *A. phialiseptus* strain WB 5110 died during the study period. Seven of 8 mice (88%) injected with 5×10^5 conidia of *A. fumigatus* NIH 14495 and one of 8 mice (13%) injected with the similar inocula of *A. phialiseptus* ATCC 26606 survived. With 5×10^4 conidia of *A. fumigatus* strain NIH 14495 and *A. phialiseptus* ATCC 26606 all mice survived and showed no evidence of disease; whereas, only 50% of the mice injected with *A. fumigatus* strain NIH 26823 and 88% of the mice injected with *A. phialiseptus* WB 5110 survived. Kidneys from all surviving mice were positive for the same *Aspergillus* cultures they received.

Lesions were observed in kidneys of surviving mice infected with 5×10^5 conidia of strains NIH 14495 and ATCC 26606. Sections of the kidneys showed extensive hyphal growth (FIGS. 10 and 11). Although the overall diameter of *A. phialiseptus* hyphae seen in the sections stained with Gomori methenamine silver was about the same as

TABLE II

NUMBER OF SURVIVORS ON EACH DAY AMONG MICE INFECTED WITH CONIDIA OF *Aspergillus fumigatus* STRAINS NIH 14495 AND NIH 26823 AND WITH *A. phialiseptus* STRAIN ATCC 26606 AND WB 5110

Inoculum	Strain	No. of days post infection																						% survival at 30 days
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22–30	
5×10^6	NIH 14495	8	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	NIH 26823	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ATCC 26606	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	WB 5110	8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5×10^5	NIH 14495	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7	88
	NIH 26823	8	8	6	5	4	3	3	3	3	3	3	3	2	2	1	1	1	1	1	1	0	0	
	ATCC 26606	8	8	7	7	7	7	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	1	13
	WB 5110	8	8	8	7	7	7	7	7	6	4	4	4	4	4	1	1	0	0	0	0	0	0	0
5×10^4	NIH 14495	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100
	NIH 26823	8	8	8	8	8	8	8	8	8	8	8	7	7	7	6	6	6	6	6	5	5	4	50
	ATCC 26606	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100
	WB 5110	8	8	8	8	8	8	8	8	8	8	7	7	7	7	7	7	7	7	7	7	7	7	88

those of *A. fumigatus,* hyphae of *A. phialiseptus* tended to be more sinuous and compact than those of *A. fumigatus.*

DISCUSSION

The species concept of *A. fumigatus* formulated by Thom and Raper (**1945**) and adopted by Raper and Fennell (**1965**) and de Vries and Cormane (**1969**) was broad enough to include certain clinical isolates with recognized differences in colony characteristics, morphology, and physiology. Raper and Fennell described the septation of the phialides in strains WB 5052 and WB 5110 but considered both to be aberrant representatives of *A. fumigatus.* Similarly, de Vries and Cormane observed the same phenomenon in their clinical isolate 61 but treated it as an atypical strain of *A. fumigatus.*

In 1943, Sartory and Sartory observed septate phialides in an *Aspergillus* isolated from the sputum of a patient with respiratory disease. They described the fungus as *A. septatus* to denote the septation of the uniseriate phialides but failed to provide a Latin diagnosis and, therefore, the epithet is invalid according to the *International Code of Botanical Nomenclature* (Stafleu, **1972**). The Sartorys noted the resemblance in morphology and mouse pathogenicity between *A. septatus* and *A. fumigatus,* although their fungus produced rose-colored rather than green conidia.

Unable to obtain a culture of *A. septatus* and aware of the frequent occurrence of spontaneous conidial color mutations in *A. fumigatus,* Raper and Fennell (**1965**) listed *A. septatus* as a possible synonym of *A. fumigatus.* A statement was made of the similarity between the phialides of WB 5052 and WB 5110 and those described by Sartory and Sartory for *A. septatus.*

After the extensive comparisons of ATCC 26606, WB 5052, and WB 5110 with typical strains of *A. fumigatus* reported here, I have concluded that the three strains isolated from clinical material represent a distinctive species. The species is easily separable from *A. fumigatus* and *A. fumigatus* var. *ellipticus* by 1) its long septate phialides, 2) nondeciduous nature of the last conidium in each chain, 3) its failure to sporulate on high-sugar media and restricted growth on standard Czapek's agar medium, and 4) its yellowish cream to white colony on Czapek's agar at 37 C.

I have selected the new specific epithet, *phialiseptus,* rather than validating *A. septatus* in order to reserve the Sartorys' name for future use if another isolate with both septate phialides and rose-colored conidia is discovered.

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