ASPERGILLUS LONGIVESICA, A NEW SPECIES FROM NIGERIAN SOIL

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SUMMARY

A new species in the *Aspergillus clavatus* group, *A. longivesica* Huang & Raper, is described. Two isolates of this fungus were obtained from Nigerian soil. It is characterized by blue-green heads, a single series of sterigmata, and conidia with prominent connectives. There are two types of conidiophores: short and long. The short conidiophores bear globose vesicles with uniform elliptical conidia. The long conidiophores are wide and thick-walled, producing elongate, fusoid-clavate vesicles, fusoid-clavate heads in which the conidial chains do not adhere to form divergent columns, and conidia which vary in size and shape. The long conidiophore is positively phototropic and elongates only in the presence of light.

During a microfungal survey of Nigerian soils, a species of *Aspergillus* obviously belonging to the *A. clavatus* group was isolated and is sufficiently different from described species to warrant recognition as a new taxon. It is an interesting fungus from both morphological and photobiological points of view. It produces extremely long conidiophores and vesicles—the longest among species of *Aspergillus*. Furthermore, the long conidiophore of the fungus is strongly phototropic.

MATERIALS AND METHODS

The fungus in question was isolated from one of several soil samples collected in Nigeria by Dr. E. W. Hanson on November 22, 1968. The samples which were air-dried prior to shipment were put into storage at 4 C upon arrival. Isolations were made 6 months later, using a modification of Warcup and Baker's alcohol treatment (7). One half gram of soil sample No. 11 was placed in sterile distilled water in a 15 cm sterile test tube and was heated at 60 C for 30 min in a water bath. The water was then decanted and immediately replaced with 65% ethyl alcohol. After 10 min the alcohol was decanted, bits of the treated soil distributed into petri dishes and the plates then poured with a glucose-ammonium nitrate medium devised by Gochenaur (1). The agar contains streptomycin to inhibit bacteria and rose bengal to reduce spread

of fungal colonies. The plates were rotated gently to disperse the soil particles before the agar solidified. They were incubated in darkness in covered boxes at room temperature. Study in pure culture was carried out by growing the fungus on Dodge's cornmeal (2), Czapek-Dox and malt extract agars (4).

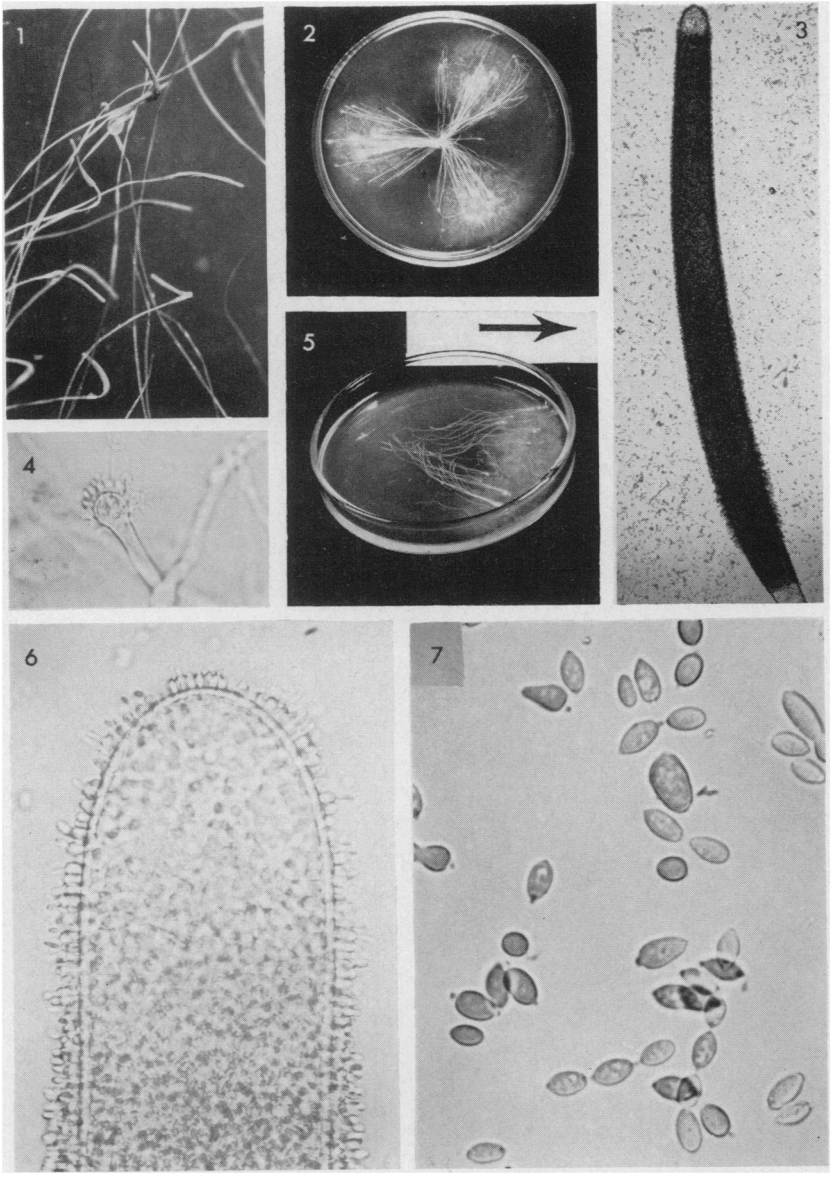
Two methods of inoculation on the cornmeal, Czapek-Dox and malt agars were attempted to demonstrate phototropic response of conidiophores: (1) streaking a conidial suspension on one side of the agar plate, and (2) inoculating the plate at three equidistant points. Inoculated plates were wrapped with black paper. A hole was punched on the edge of the paper cover opposite to the streaked area in case (1) or on the center of the paper cover of the petri dish in case (2) so that light could enter only through the hole. The plates were placed on the table of the laboratory. In another experiment, three point-inoculated plates were wrapped with black paper and placed in black boxes; a second set of plates was placed in an open box and exposed to continuous illumination by a fluorescent lamp with the distance between the light and the cultures being 50 cm, while a third set was incubated in the research laboratory where alternating light and dark periods (13 hr light and 11 hr dark) occurred.

For study of the origin of lateral hyphae on the conidiophore, the lower part of the conidiophore was cut into several pieces about 0.5 cm in length. The pieces were fixed in 3% glutaraldehyde and then post-fixed in 2% OsO_4, followed by dehydration in acetone and embedded in epon-araldite (1:1). Cross-sections were made at 1 μ using a diamond knife on an ultra-microtome. They were stained in haematoxylin and safranin and observed with the light microscope.

Cultures of *Aspergillus giganteus* WB-10 and WB-4763 (from KBR) and N1140 (from Nigerian soil) were grown for comparison.

RESULTS

A new species from Nigeria.—Two isolates of an *Aspergillus* were obtained from a soil sample from Ilesha, Nigeria. Dr. Hanson, who collected the sample, characterized the area as a level rain forest with more than 300 species of large trees. The elevation of the collection site is 600 ft and the average rainfall is 55 in. Cultural and morphological study showed the two isolates to be identical. The fungus appeared to be related to *A. giganteus* Wehmer. However, size and shape of vesicle, size and shape of conidia and other characters were shown to be different from those in the latter fungus. Therefore, the Nigerian isolates are



FIGS. 1–7.

described as a new species. The specific epithet *"longivesica"* was chosen because of the very long vesicles which are produced.

Aspergillus longivesica Huang & Raper, sp. nov. FIGS. 1–7

Coloniae in agaro Czapekii parce celeriter crescentes, ca. 5.0 cm in diam duabus hebdomadibus ca 25 C, albae vel cremeae, reverso luteo-cinnamomeo pallido, mycelio aerio tenui atque flocosso, odore mucido; capitula conidica caeruleo-viridia, compacta et infissurata; conidiophorae continuae, glabrae hyalinesque, duabus formis et duabus magnitudinibus: aliquae breves, aliquantum inconspicuae, tenuitunicatae cum vesiculis subglobosis, aliae grandes, longae et crassitunicatae cum vesiculis fusoideo-clavatis; conidiophorae magnae 60–130 μ in latitudine, 1.5–4.5 cm in longitudine (24 diebus usque et 7.0 cm in longitudine in culturis a latere iluminatis), in parte inferiori per tunicam hypharum delicatularum intertextarum vestitae, cum vesiculis pachydermaticis 130–200 $\mu \times$ 2.2–3.2 mm et capitulis conidicis usque ad 570 μ in latitudine; conidiophorae parvae cum vesicularis tenuitunicatis 18–36 μ in diam; sterigmata uniseriata, 4.2–7.0 $\times$ 3.5–4.2 μ; conidia variabilia ellipsoidea vel globosa vel cylindrica vel piriformia, fere 5.6–12.6 $\times$ 4.2–6.3 μ.

Habitat in solo Africae tropicae.

TYPE LOCALITY: Ilesha, Nigeria.

TYPE: N1129. Holotype, WIS. Isotypes, NY and IMI (3). Living cultures of N1129 have been deposited in the American Type Culture Collection (Rockville, Maryland), the Centraal Bureau voor Schimmelcultures (Baarn, Netherlands) and the USDA, Northern Utilization Research and Development Division (Peoria, Illinois).

Colonies on Czapek's solution agar growing moderately fast at room temperature, attaining a diameter of 5.2 cm in two weeks, very thin floccose, with mycelium mostly submerged, white to cream, reverse pale cinnamon-buff (**5**, XXIX), odor moldy; conidial heads (FIG. 1) grayish blue-green (**5**, XLVIII) to artemisia green (**5**, XLVII), elongate, compact without splitting into divergent columns, gradually decreased in width towards tip and base, 2.1–3.3 mm $\times$ 240–570 μ with a width of 170–360 μ near the tip; conidiophores growing perpendicular to the surface of the agar, the longer ones phototropic, continuing elongation even after two weeks, hyaline to pale yellowish, smooth, falling into two size ranges: (1) longer conidiophores 1.5–4.5 cm $\times$ 60–130 μ, thick-walled (5.6–7 μ), with hyaline exudate and lateral hyphae clothing the

FIGS. 1–7. *Aspergillus longivesica.* 1. Typical elongate conidial heads, ca. $\times$ 4.3. 2. Phototropic response of conidiophores. Light entered through a small aperture through the center of the petri dish. 3. Elongate vesicle on a long conidiophore stained with cotton blue, $\times$ 30. 4. Globose vesicle on a short conidiophore, $\times$ 310. 5. Phototropic response of conidiophores. Light entered from the left as indicated by the arrow. 6. Uniseriate sterigmata on elongate vesicle, $\times$ 240. 7. Conidia showing adherent connectives and variations in size and shape, $\times$ 620.

base, (2) shorter ones 80–420 μ × 7–11.2 μ; vesicles pale yellowish and of two types: (1) elongate, fusoid-clavate, thick-walled, consisting of the swollen terminal region of the long conidiophore, 2.2–3.2 mm × 130–200 μ, with width greatest in the middle part and gradually narrowed towards either end (FIG. 3), (2) globose, rarely oval to flask-shaped, thin-walled, consisting of the swollen tip of the short conidiophore, 18–36 μ in diameter (FIG. 4); sterigmata (FIG. 6) in a single series, with tips gradually reduced to narrow tubes, on long conidiophores ranging from 4.2–7 μ × 3.5–4.2 μ at basal and central parts of the vesicle to 7–11.2 μ × 4.2–8.4 μ at the apex, those on small globose vesicles measuring 4.2–7 μ × 2.4–4.2 μ; conidia pale greenish, appearing blue-green in mass: (1) those on elongated vesicles mostly elliptical but ranging from globose, ovoid, pyriform to cylindrical, varying greatly in dimensions, 4.2–16.8 μ × 2.8–7 μ but mostly 5.6–12.6 μ × 4.2–6.3 μ, with fragments of connectives (FIG. 7), (2) those on globose vesicles elliptical or rarely pyriform, 3.5–5.2 μ × 2.5–3.5 μ, with fragments of connectives evident.

Colonies on malt agar spreading very rapidly, reaching a diameter of 7.2 cm in two weeks, white to cream, mycelium mostly submerged with aerial mycelium thin and floccose; long conidiophores growing through the surface of the agar and dominating the color of the colony; base of the conidiophore strongly hairy; colony reverse cinnamon-buff (**5**, XXIX) to pale brown.

Colonies on cornmeal agar growing rapidly, reaching a diameter of 6 cm in two weeks, colorless, mycelia appressed or submerged; long conidiophores sparse, white to cream, with lateral hyphae clothing the base, projecting through the surface; conidial heads blue green; colony reverse uncolored.

Aspergillus longivesica resembles *A. giganteus* in producing blue-green heads, long conidiophores and sterigmata in a single series. In the former fungus our observations showed that only several out of many conidiophores per colony produced conidial heads. Consequently the colony appeared white to cream which was actually the color of the conidiophores. Colonies of the latter fungus were blue-green due to the presence of abundant conidial heads. Conidial heads of the former do not split into divergent columns whereas those of the latter do. *Aspergillis longivesica* produces longer and wider conidiophores, longer vesicles, wider sterigmata and bigger conidia. The shape of the vesicles is elongate, fusoid-clavate for the long conidiophore and mostly globose for the short conidiophore rather than clavate as is characteristic in *A. giganteus*. Conidia of the new taxon show more variation in size and shape and possess fragments of connectives.

Responses to the light—Conidiophores in three-point inoculations grew towards the center of the plate where a hole had been punched in the paper cover (FIG. 2). In the experiment of one-side streaking on the petri dish, conidiophores grew towards the opposite side where light was allowed to enter (FIG. 5). The length of the conidiophore under the unilateral light 24 days after inoculation reached 7 cm on cornmeal agar. Conidiophores of *A. longivesica* are thus obviously positively phototropic.

The cultures produced more conidial heads in continuous light than in diurnal alternation of darkness and light. In total darkness, growth of conidiophores was inhibited, the largest ones measuring about 2 mm in length. When, after 14 days of dark incubation, the cultures were exposed to the diurnal alternation of darkness and light, the inhibited conidiophores began to grow, reaching a length up to 5 cm on three media used. It appears that light is essential for growth of the conidiophores.

Origin of lateral hyphae on the conidiophore—When cultures were examined with a stereoscope, many thin hyphae were seen climbing up along the base of the long conidiophore. To ascertain the origin of the lateral hyphae, cross-sections made with an ultra-microtome were studied. It was observed from the cross-sections that the thin lateral hyphae were located on the surface of the conidiophore and did not originate from it. The lower part of the conidiophore was cut and laid on the cornmeal agar. After 24 hr hyphal tips of the growing lateral hyphae were cut off, and transferred to sterile agar plates. The transferred tips grew into colonies identical to those of the parent culture. It is concluded that the lateral structures clothing the base of the conidiophore represent vegetative hyphae arising from substrate mycelium.

DISCUSSION

In the present study the Aspergilli isolated following heat-alcohol treatment were typically ascosporic or sclerotial forms. Non-ascosporic *Aspergillus* species were usually obtained from soil without any selective treatment. Although the new taxon was obtained by the heat-alcohol method, it is not clear what structures of the fungus survive the treatment.

Aspergillus longivesica belongs to the *A. clavatus* group, as defined by Raper and Fennell in "The Genus Aspergillus" (**4**). The species most closely allied is *A. giganteus*. Cultural and morphological characters described above clearly distinguish the two taxa. The possession

of evident connectives on conidia of *A. longivesica* is an unique feature among species of the *A. clavatus* group. Other *Aspergillus* groups sometimes show such connective fragments on conidia, e.g., species of the *A. cervinus* group and *A. conjunctus* of the *A. ustus* group. However, the presence or absence of connectives should not be considered a significant character in species separation. At the beginning of the present study we thought that the lateral hyphae on the lower part of the conidiophore might be an important morphological feature characteristic only of the new taxon. However, a comparative study revealed that conidiophores of *A. giganteus* also developed thin lateral hyphae at their bases although not as many or as crowded as those of *A. longivesica.* It is interesting to note that there are many hyaline droplets on the conidiophores, but it is not known what if any effect these may have on the development of the accessory hyphae.

Growth responses of reproductive structures in several Phycomycetes, especially *Phycomyces,* to light have received intensive study in the past two decades. These investigations have contributed in some cases to our understanding of wall synthesis and the synthesis of pigments stimulated by light. In the case of the higher fungi, not until recent years has the quantitative effect of light on conidiophore growth received careful attention. Trinci and Banbury (6) demonstrated that extension of the conidiophore of *Aspergillus giganteus* is restricted to a terminal zone about 200 μ in length. They showed that tips of the conidiophores oscillate about the vertical axis of growth in a left-handed manner. In *A. longivesica,* also, light not only promotes elongation of the conidiophore but also controls the direction of growth of the conidiophore. The conidiophore is positively phototropic and appears to be especially suitable for photobiological studies since it is unicellular, wide and long, and has a fast rate of growth. The fact that *A. longivesica* produces only a limited number of fertile heads suggests other areas for physiological investigation. Whether this deficiency is due to the genetic make-up of the fungus or is something that could be modified by addition of appropriate macro- or micro-elements or vitamins to the medium, or by suitable regulation of environmental factors such as temperature, humidity and quality of light can only be determined by further study.

ACKNOWLEDGMENTS

We wish to thank Dr. E. W. Hanson of the University of Wisconsin for collecting the soil samples used in this investigation. The assistance of Dr. M. P. Backus, who critically read the manuscript and prepared the

Latin diagnosis, and the help provided by Mr. G. E. Coffman in preparing the photographs are likewise acknowledged.

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Accepted for publication July 14, 1970.