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ASPERGILLUS PENICILLOIDES SPEGAZZINI, A XEROPHILIC FUNGUS ISOLATED FROM LENSES

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Abstract

In tropical or subtropical regions with humid periods, glass elements of optical instruments are sometimes quite moldy. Since *Aspergillus penicilloides* Spegazzini is isolated from various optical materials, this fungus is primarily responsible for the damage to optical equipment in humid weather. The growth of the fungus both in nature and in culture has been investigated in order to carry on further studies on control measures. In nature, except for spore germination, *A. penicilloides* grows usually in concentrated and dry habitats. In culture, its growth is negligible on Czapek's agar, poor on Czapek's with 20% sucrose, and luxuriant only on Czapek's with 40% sucrose and enriched with supplements of malt and yeast extract. Spore germination takes place when large amount of gaseous water is available surroundingly.

Introduction

The aspergilli affect our life in a variety of ways. They are common contaminants in laboratories and cause decay of exposed foodstuffs. Particularly, in tropical and subtropical areas, they grow on leather and cloth fabrics. People have to keep the storage as dry as possible to prevent molding. In addition to that it is a big nuisance to the optical instruments because a specific aspergillus which has been isolated and identified as *Aspergillus penicilloides* Spegazzini (Ohtsuki, 1943). Lenses, prisms, and films are its natural habitats.

Aspergillus penicilloides has at least three synonyms, i.e. *A. glaucus* var. *tonophilus* Ohtsuki, Suda, and Sai, *A. vitricolae* Ohtsuki, and *A. pertardus* Biourge (Raper, Fennell, and Austwick, 1965). Its variation is believed to be at the variety or strain level. It was first described in 1896 by Spegazzini in Argentina (Blochwitz, 1929). Smith in 1931 recognized the intermediate forms between the *A. glaucus* and the *A. fumigatus* groups as constituting the *A. penicilloides* group. Strains have been isolated from cane products in Louisiana and from mildewed textiles in the United Kingdom (Smith, 1931). Although it has been isolated from cases of Keloidal blastomycosis in Brazil, it was non-pathogenic for laboratory animals and embryonated eggs. Strain isolated by Borelli of

Venezuela, probably representing a casual isolated in clinical material from a case of *tinea pedis,* is assignable to the species but differs in producing somewhat more broadly spreading and deeper colonies on enriched Czapek's agar (Fonseca and Lacaz, 1971). It is the purpose of this study to describe the fungus isolated mainly from lenses in Hong Kong so that further studies could be based on.

Materials and Methods

Fungi isolated from lenses of microscopes and from cellophanes and related materials such as photographic slides were used in this study. Since the fungus grows firmly on the surface of substrata, the molded area was inverted onto artificial media so that the surfaces of both molding material and culture media were contacted each other. Cutting of cross groove on media was also made to provide partial aeration for growth. In order to make a successful transferring of the fungus mycelium, this treatment should be kept for at least three days. The micro-climate within the quadrant type of Petri dish was kept at 100% relative humidity by adding sterilized water in one of the compartments at bottom.

Pure culture upon known substrates is essential to the identification of fungi. Although Czapek's solution agar is an optimum substrate for most aspergilli, it is not suitable for this specifically xeroilic one. However, upon Czapek's and malt agars with additional sugar or salt to boost the osmotic tension of these substrates seems good in solving this problem. Four media were used in this study, e.g. Czapek's agar ($NaNO_3$ 3g, K_2HPO_4 1g, $MgSO_4$ 0.5g, KCl 0.5g, $FeSO_4$ 0.01g, sucrose 30g, agar 15g, and distilled water 1000 ml), C20 agar (Czapek's agar with 20% sucross), and C40 agar (Czapek's agar with 40% sucrose and enriched with supplements of 25g of malt and 10g of yeast extract).

Results

Cultural Details

Aspergillus penicilloides is very osmophilic, failing to grow or growing very poorly on Czapek's solution agar at room temperature (20–22°C), never attaining diameters exceeding 2–3mm even after four weeks.

Colonies on malt extract agar grew somewhat more rapidly than on standard Czapek's agar, becoming 5–10mm in diameter at room temperature in four weeks, nonsporulating or producing only a limited number of aberrant conidial heads, rich dark green with paler edge, turning darker and duller, and finally becoming dirty greenish-grey; reverse brown, greenish-brown, and dark green in patches; surface much wrinkled and folded.

Colonies on Czapek's agar with 20% sucrose reached diameters of 10–15mm in four weeks at room temperature but remained thin and nonsporulating; sporulation but not

rate of growth was enhanced by incubation at 30°C; and no growth occurred at 15°C.

Colonies on enriched Czapek's agar with 40% sucrose grew quite rapidly, attaining diameters of 50–60mm in four weeks at room temperature, radially furrowed, most commonly forming a thin tough felt; heavily sporulating in dark yellow-green shades; reverse from uncolored to dirty greenish brown or very dark green, with color most pronounced at colony centers; odor slight, not distinctive.

The perfect stage of this species has not been observed in both natural habitat and artificial cultures.

Structural details

The vegetative mass consists of both submerged mycelium and aerial felt of branching and interlacing hyphae. They are septate. The mycelium produced an abundance of conidiophores arising either from substratum or from aerial hyphae. Conidial heads arised primarily from the substrate but produced in limited numbers from a thin weft of aerial mycelium, globose when young and remaining so for a considerable time, reaching an overall diameter of 80–90 μm; in age or at drying margins becoming loosely and irregularly columnar, somewhat ragged and up to 200–300 μm long; heads from aerial mycelium smaller and more quickly columnar. The structure of conidial head somewhat resembles that of a *penicillium,* hence the specific epithet of *penicilloides.* Conidiophores were smooth, comparatively thin walled, somewhat flexuous, showing no marked taper from just below to vesicle to the point of origin, ranging from 150 to 450 μm in length and from 4 to 10 μm in diameter but most commonly 200–300 μm by 5–7 μm. Vesicles are pear shaped to subglobose, rather sharply marked off from conidiophores, mostly 10–20 μm in diameter but ranging from 6 to 25 μm, uncoloured or with very slight greenish tinge, bearing sterigmata over the upper half to two-thirds; sterigmata uniseriate, radiately arranged but with those at the periphery lending to be upcurved, numerous, and comparatively crowded, mostly 7–10 μm by 2.5–3.5 μm, conidial tubes short and blunt with walls somewhat thickened; conidia ovate to truncate-elliptical when first form, 3–3.5 μm by 4–4.5μm, quickly roughened and at maturity becoming barrel shaped to globose, usually showing connective, with very dark coloured walls, conspicuously echinulate with short coarse spines, mostly 3.5–5 μm by 3–4 μm but up to 6 μm in diameter.

Discussion

In rainy season characterized by the high humidity and high temperature of the tropics and subtropics, a more or less heavy clouding of the polished glass surfaces of optical elements due to the growth of *Aspergillus penicilloides* may be commonly observed. Nakamura (1918), Nakazawa et al. (1932), Teshima (1934) and Ohtsuki et al. (Ohtsuki; 1943, 1950, 1954 and 1962) have previously studied this problem. The last author gained several important results and proposed new scientific names for the responsible fungus. The studies were since then suspended for some time. The

spores of this fungus are ubiquitous and the fungus itself is obligatory tonophily for establishment and has its mycelium with enormously high osmotic pressure. Only extraordinarily hypertonic media such as Czapek's with 40% sucrose and enriched with supplements of 25g of malt and 10g of yeast extract are suitable for the culture of this fungus. Though its habitats should be highly concentrated with large amount of osmoactive substances such as sucrose or table salts, the humidity and temperature of its surroundings are the other limiting factors for growth. Wherever the fungi find suitable environments, they begin to germinate and develop without requiring noticeable amounts of nutritive substances. In natural conditions such as on lenses, the foreign nutrition of tiny amounts of organic matter could well be from traces of grease, enamel, or even dust and impurities that are always present in instruments. Under severe cases, the fungus may even penetrate into the glass by forming etched replica of the mycelium due to its metabolic activities. Optical instruments with the growth of this fungus are of course not workable.

To control the damage to optical instruments due to the growth of this fungus, an initiated studies of its general habitat both under natural and artificial conditions would be of primary interest. This report provides the studies of artificial culture of the fungus. The most feasible mean of control is to reduce the relative humidity of the air inside and around glass objects to about 70%. This is to prevent the germination of spore but not the growth of mycelium. To control mycelium growth, relative humidity should be further reduced to much less than 70%. After establishment of this fungus on the optical elements, however, it is extremely resistant to dessication and dry conditions. And an ideal fungicide has not been developed for the case. At the present time, a good

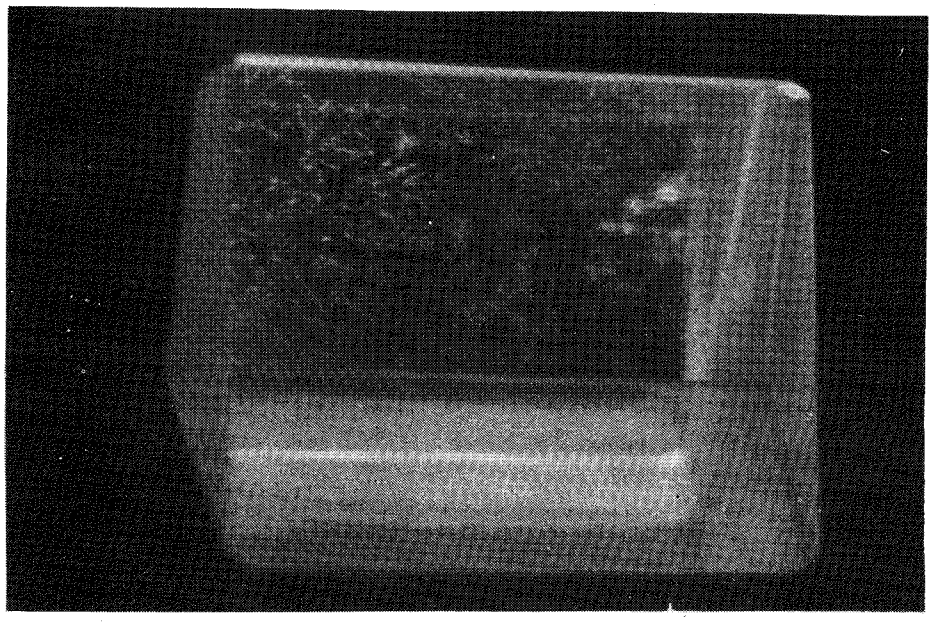

Fig. 1. The growth habit of *Aspergillus penicilloides* on a prism. (x 8)

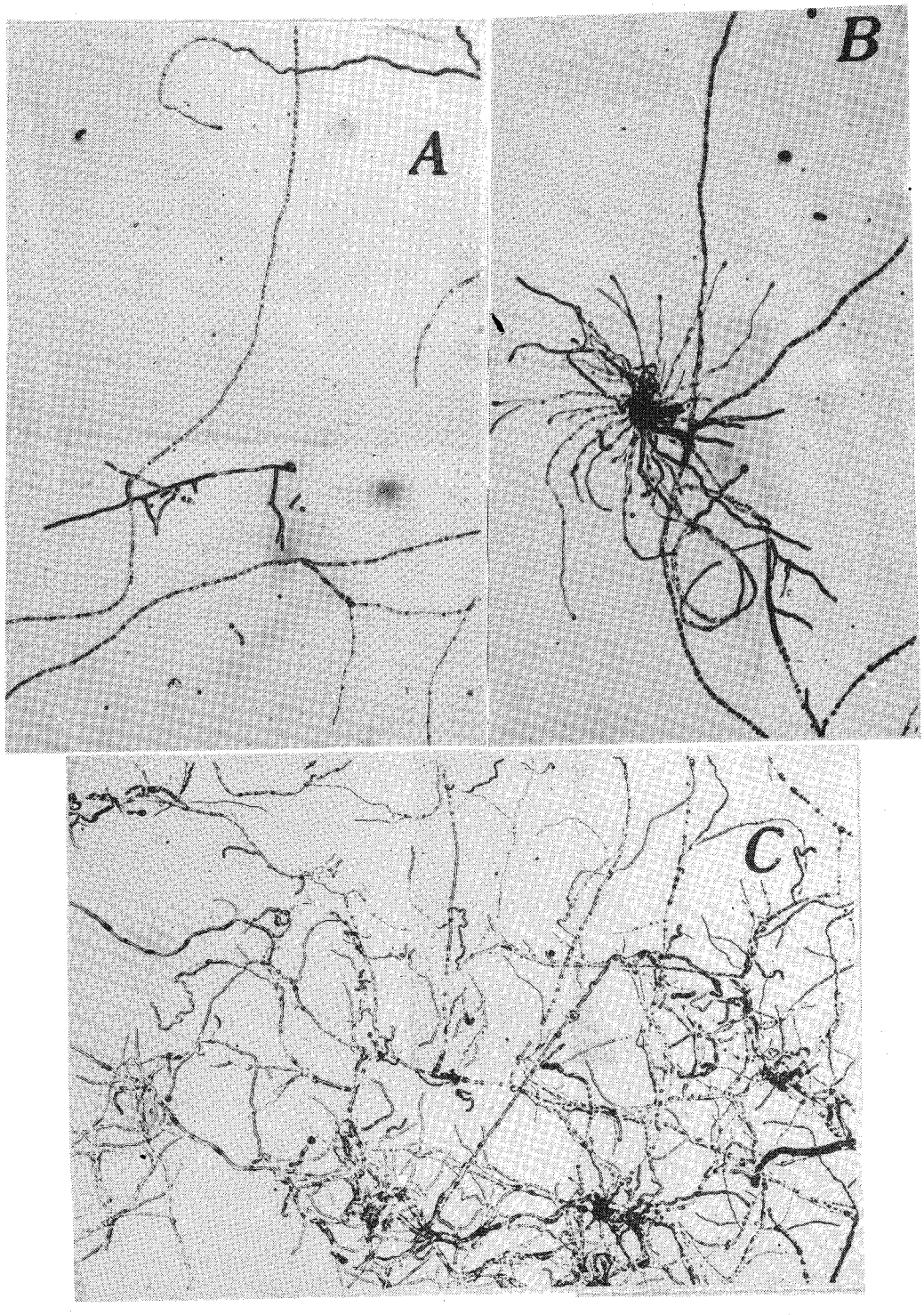

Fig. 2. Growth habit of *Aspergillus penecilloides* in culture. A. Germinating stage. (x 400). B. Established stage. (x 350). C. Advanced stage. (x 300).

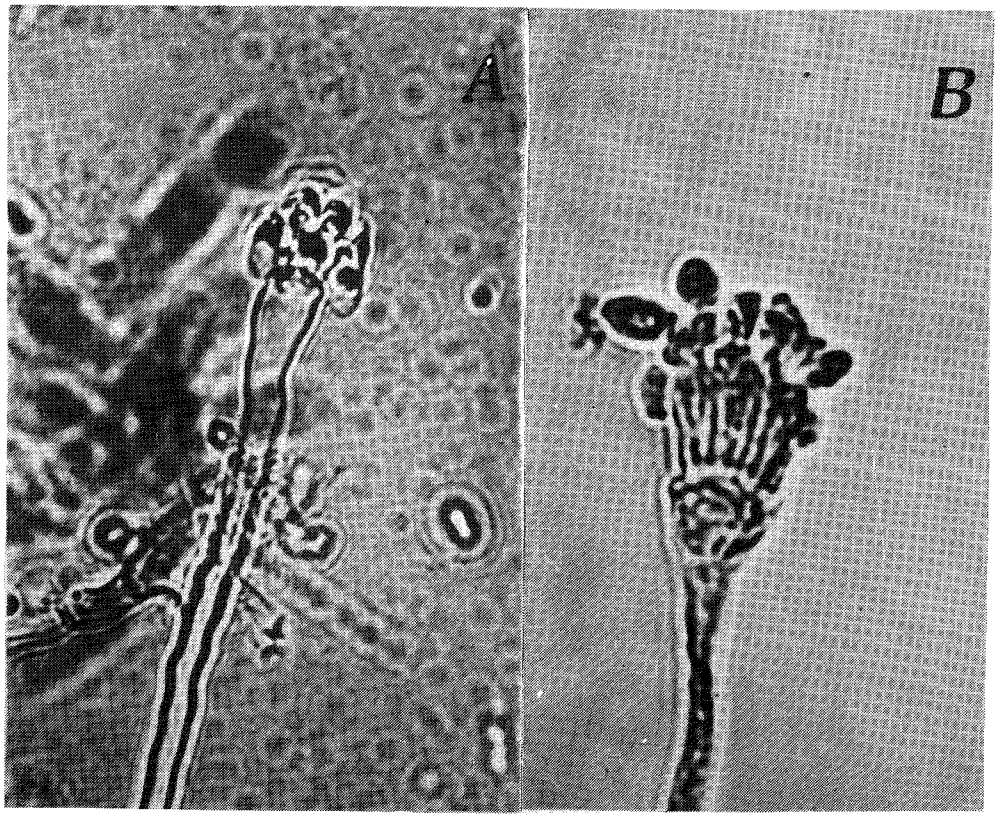

Fig. 3 Apex of a conidiophore of *Asperigillus peniculloides*. A. Initial stage. B. Late stage. (x 800).

suggestion to protect optical elements against fungus growth is to store the optical elements or instruments in a dry and ventilated place free from dust and impurities.

It is generally experienced that lenses of some camera are less moldable in wet season. This is probably due to the difference of quick-freezing and natural-freezing techniques adopted in lens making. Another possibility is the different types of lens coating. Unfortunately, many new glasses required for particular high-quality optical systems are especially susceptible to the growth of this fungus.

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從光學顯微鏡片分離之一種好旱性眞菌
Aspergillus penicilloides Spegazzini

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熱帶或亞熱帶地區當濕季來臨時，空氣中含水量大增，光學儀器放置其間易使其內之玻璃組件長霉。從許多類型之光學儀器分離菌種的結果得知，潮濕的環境導致儀器的損害，主要是由 *Aspergillus penicilloides* Spegazzini 所造成。爲求有效控制，乃對該菌之生長，分別從自然狀態與人工培養中加以研究以謀對策。於自然狀態下，*A. penicilloides* 除在胞子萌芽時，皆密集於乾燥的角落。當用 Czapek's agar 人工培養時，其生長則視培養基調配之不同而異。在純agar中幾乎不長，添加 20% 蔗糖時亦僅有少許生長，但在含40% 蔗糖並補以 malt 及 yeast 抽出液之培養基中始長得茂盛。至於胞子萌芽則必需在水液含氣量多的環境中才能進行。