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Sexual and Asexual Reproduction of *Aspergillus nidulans* in vivo

Sexuelle und asexuelle Reproduktion von *Aspergillus nidulans* in vivo

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Key words: Sexual fruiting bodies of *A. nidulans* in vivo

Schlüsselwörter: Sexuelle Sporen von *A. nidulans* in vivo

Summary: Both the sexual and asexual fruiting bodies of *Aspergillus nidulans* were discovered in the lung tissue of a captive rhinoceros, in a biopsy specimen from a human with bronchial stump aspergillosis, and in the guttural pouch of a horse. In addition, a zygomycete with unusual morphology was found in the lungs of the rhinoceros.

Zusammenfassung: Es wurden sowohl sexuelle als auch asexuelle Sporen von *Aspergillus nidulans* im menschlichen Biopsiematerial eines Patienten mit Bronchialstumpfmykose sowie im Luftsack eines Pferdes und im Lungengewebe eines Nashorns gefunden. Beim Nashorn wurde zusätzlich zur *Aspergillus*-Invasion auch eine Zygomyceteninvasion mit ungewöhnlichen Strukturen beobachtet.

Introduction

Aspergillosis and zygomycosis are well known diseases in humans and in a large variety of nonhuman species (1, 7, 9, 16).

Asexual fructification in vivo of etiologic agents of both diseases is well documented in the literature (1, 2, 7, 16). The findings of both the sexual and asexual reproductive states in a host are very rare. Only a few workers have reported the sexual reproduction of aspergilli in vivo (3, 6, 11, 12, 17) and apparently none have reported the sexual reproduction of the zygomycetes in vivo.



Fig. 1: Hyphae of a zygomycete in rhinoceros lung. Grocott methenamine-silver nitrate stain. Orig. Mag. 160 $\times$.



Fig. 2: Bizarre "zygospore-like" structure of the zygomycete and sporangiospores (arrows) in rhinoceros lung. Grocott methenamine-silver nitrate stain. Orig. Mag. 160 $\times$.

Fig. 3: Unusual sac-like structures of the zygomycete in rhinoceros lung. Grocott methenamine-silver nitrate stain. Orig. Mag. 160 $\times$.

Fig. 4: Conidial head, resembling those seen in *A. fumigatus*, with uniseriate sterigmata with narrow ends in rhinoceros lung cavity. Short chains of conidia and detached conidia are also present. Grocott methenamine-silver nitrate stain. Orig. Mag. 400 $\times$.

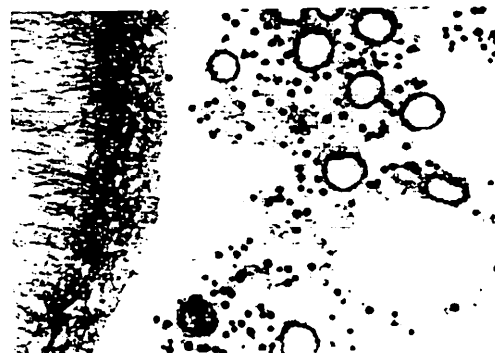


Fig. 5: Numerous cleistothecia and hülle cells (black) in rhinoceros lung. The fungal elements are surrounded by organized fibrin, inflammatory cells, and necrotic debris. A mat of *A. nidulans* mycelium is present in the cavity wall; hyphae have also penetrated the lung parenchyma. Grocott methenamine-silver nitrate stain. Orig. Mag. 90 $\times$.

The concomitant occurrence of aspergillosis and zygomycosis has been observed in birds and in mammals (10, 12).

The purpose of this paper is to report both sexual and asexual reproduction of *Aspergillus nidulans* in a human, a rhinoceros, and a horse, and concomitant aspergillosis and zygomycosis in the rhinoceros.

Material and Methods

Histological sections of rhinoceros and horse tissue were prepared by routine procedures and stained with hematoxylin and eosin (H & E), with the Grocott-modified Gomori methenamine-silver nitrate (GMS), and with the two above combined.

Sputa and material obtained by bronchoscopy from a human were cultured at 37°C and 27°C on Sabouraud's dextrose agar and Czapek's solution agar. The specimens were also examined directly after they were treated with 10% KOH or examined histologically after they were stained with H & E.



Fig. 6: Numerous thick-walled hülle cells associated with a cleistothecium in the rhinoceros lung cavity. The fungus elements are surrounded by polymorphonuclear leucocytes and necrotic debris. H & E stain. Orig. Mag. 200x.



Fig. 7: A cleistothecium with intact asci and a hülle cell (black) in the cavity of rhinoceros lung. Grocott methenamine silver nitrate stain. Orig. Mag. 40x.

Case Studies

Rhinoceros: An African rhinoceros (*Diceros bicornis*), that died at the Schönbrunn Tiergarten, Wien, Austria, was taken to the Institut für Pathologie und Gerichtliche Tierheilkunde der Veterinärmedizinischen Universität Wien on April 20, 1965, for pathological investigations. Histological examination of the lungs revealed severe chronic bronchitis, pneumonia, and cavities. In a few foci in the parenchyma and in the cavities there were many freely irregularly branching hyphae (3.2–13.2 µ wide) with walls of irregular thickness and occasional swellings or septa (Fig. 1). The morphology of these hyphae was compatible with that of a zygomycete. Sporangiohores with fragments of sporangia and many sporangiospores, most of which were elongated and measured 2.2–3.2 µ × 3.2–3.7 µ, were also observed. Peculiar structures (15–80 µ in diameter) resembling zygosporangia or azygosporangia were also found (Figs. 2 and 3). Some of them had dark, thick walls 3.5–4.7 µ in width, which were visible both in unstained and H & E stained sections.

Some foci of infection had been invaded by septate hyphae (3–4 µ wide) with dichotomous branches at approximately a 45° angle. On the surface of some cavities, compact masses of these hyphae or "fungus balls" were seen as were a few aspergillus heads resembling those seen in *A. fumigatus* borne on smooth conidiophores, 3.2–8 µ wide (Fig. 4). The vesicles were globose to subglobose, 12.2–22.8 µ in diameter and had uniseriate, narrow-ended sterigmata

(2.4–3.2 µ × 4.8–7.6 µ). Many globose, delicately spinulose, olive-yellow (14) conidia, most of them 2.4–3.2 µ in diameter and either free or borne on sterigmata, were seen in unstained and in H & E stained sections as were occasional laterally borne conidia (3.2 µ).

In one cavity, numerous cleistothecia (≤ 160 µ in diameter) in various stages of development (Fig. 5) were present. They were surrounded by hülle cells (11.2–15.2 µ in diameter) (Fig. 6) and were embedded in organized inflammatory cells, fibrin, and necrotic debris. The cleistothecial walls consisted of one to three layers of regularly flattened cells. Some intact eight-spored asci (5.8–7.0 µ × 9.4 µ) were present in a few cleistothecia (Fig. 7); in others many free, coral-red (15) lenticular, smooth-walled ascospores with two equatorial crests (5.6 µ long, including the crests which were 0.7 µ) were seen in unstained and in H & E-stained sections (Fig. 8). Some ascospores were also seen outside of the cleistothecia. Near the cleistothecia some brownish hyphae with occasional peculiar structures were found (Fig. 9). In some foci mixed growth of an aspergillus and a zygomycete was observed (Fig. 10).

Horse: Histological sections from the guttural pouch of a horse were sent in 1966 to one of the authors (H. S.) by Prof. Dr. J. Nicolet, Vet.-Bakt. Institut, Bern, Schweiz. (The sections had originally been sent to Prof. Nicolet by Prof. Dr. T. Teuscher of the Veterinärpathologisches Institut der Universität Zürich, Schweiz). Branched, septate hyphae, some of which were ochre-yellow and had occasional swellings, had extensively invaded the tissue. Many round hülle cells, some cleistothecia, and red lenticular smooth-walled ascospores with two equatorial crests were also seen.

Human: Between July and November 1967, four sputa, material obtained by bronchoscopy, and material from two lung biopsies on a patient suffering from tuberculosis of the lungs, were sent to one of the authors (H. S.) by Dr. C. Virchow, Hochgebirgsklinik Davos-Wolfgang, Schweiz, who later reported on the clinical aspects of the disease (19).

In wet mounts of the sputa, the material obtained by bronchoscopy, and in histological sections of material from one biopsy, many branched, septate hyphae, hülle cells, and yellowish conidial heads of an *Aspergillus* sp. were seen. Most of the heads did not have sterigmata, although a few had atypical swollen sterigmata (Fig. 11).

In material from one of the biopsies, branched hyphae, a vesicle with biserial sterigmata (Fig. 12), round hülle cells, and cleistothecia (Fig. 13) some of which with asci and red, lenticular, smooth-walled ascospores with two equatorial crests were seen. A fungus, whose colonial morphology was compatible with that of *A. nidulans*, was recovered in cultures from the sputa and from material collected by bronchoscopy. The microscopic features of this fungus were similar to those of *A. nidulans*, with the exceptions that only nontypical or atypical swollen sterigmata were noted. Cleistothecia with asci and red ascospores subsequently developed.

Discussion

Several *Aspergillus* species including those in the *A. nidulans* group produce an ascomycetous state in culture. Unlike other aspergilli, members of the *A. nidulans* group produce red ascospores in cleistothecia that are surrounded by, or associated with, thick-walled hülle cells (13). These distinguishing structures were observed in the tissues reported on here, indicating that the *Aspergillus* sp. involved was indeed a member of the *A. nidulans* group. This belief was further substantiated by the recovery of an *A. nidulans* group fungus from the human clinical material. It is noteworthy that the sexual form of the *Aspergillus* sp. in the tissues from the three sources was comparable to those of the *A. nidulans* group. The conidial heads, which were also found in the rhinoceros, and human specimens, were not always typical in that they only had uniseriate or swollen sterigmata or lacked them entirely. However, it cannot be excluded that the rhinoceros had more than one *Aspergillus* in the lung cavities, e.g. *A. nidulans* and *A. fumigatus*.



Fig. 8: A cleistothecium with free lenticular ascospores that bear two equatorial crests and hülle cells (black) in the rhinoceros lung. Grocott methenamine-silver nitrate stain. Orig. Mag. 400 $\times$.



Fig. 9: A peculiar structure of the aspergillus and hülle cells (black) in rhinoceros lung cavity. Grocott methenamine-silver nitrate stain. Orig. Mag. 400 $\times$.



Fig. 10: Concomitant growth of an aspergillus and a zygomycete in rhinoceros lung. Grocott methenamine-silver nitrate stain. Orig. Mag. 160 $\times$.



Fig. 11: *A. nidulans* conidial heads without sterigmata or with atypical swollen sterigmata in a human biopsy specimen. H & E stain. 1550 $\times$ approx.

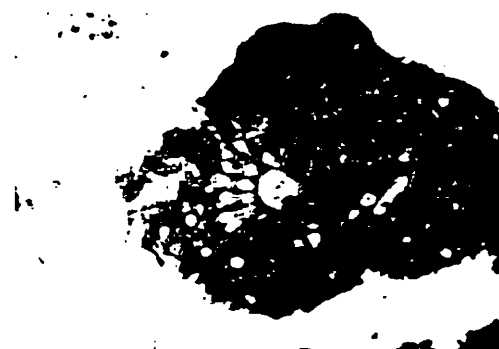


Fig. 12: *A. nidulans* conidial heads with typical biserial sterigmata in human tissue. H & E stain. 750 $\times$ approx.

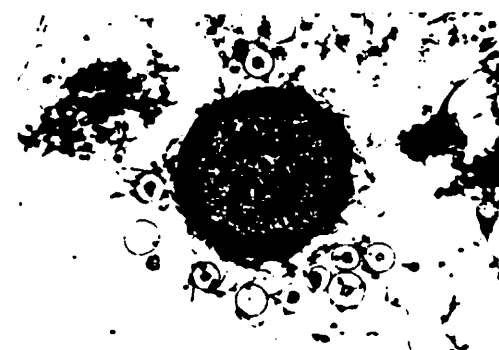


Fig. 13: A cleistothecium associated with hülle cells in human tissue. H & E stain. 600 $\times$ approx.

The fact that the ascigerous state of *A. nidulans* was found in necropsy tissue specimens from the rhinoceros and horse raises the question of whether this form developed after the hosts died. However, the fact that the sexual forms were embedded in an organized mass of inflammatory cells, fibrin and necrotic debris indicates that the ascigerous state developed while these animals were alive.

Because insufficient developmental stages were available it was not possible to identify the genus and species of the zygomycete present in the tissue of the rhinoceros. Abnormal morphology of zygomycetes in vivo resembling our findings have been observed by other workers (4, 8, 18). The significance of these peculiar structures, that resemble zygospores or azygospores, is not yet clear.

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Hemmung extrazellulärer Sproßpilzproteinasen durch Pepstatin A und Leupeptin im Plattentest

Inhibition of Extracellular Proteinases from Yeasts by Pepstatin A and Leupeptin in vitro

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Schlüsselwörter: Sproßpilze - sekretorische Proteinase - Pepstatin A - Leupeptin

Key words: Yeasts - Secretory proteinases - Proteinaseinhibitors - Pepstatin A - Leupeptin

Zusammenfassung: Auf Serumalbuminagar nach Staib wurde eine Proteinaseaktivität bei 23% der *Candida albicans*-, bei 78% der *Candida tropicalis*- und bei 64% der *Candida parapsilosis*-Stämme nachgewiesen. Andere fakultativ-pathogene Sproßpilze wie *Candida guilliermondii*, *Candida krusei*, *Candida pseudotropicalis*, *Candida (Torulopsis) glabrata* und *Trichosporon capitatum* verhielten sich durchweg proteinasenegativ. Allein das Ausmaß der induzierbaren Proteolyse liefert somit im Einzelfall kein eindeutiges Indiz über den Krankheitswert eines Pilzes. Unter dem Einfluß von Pepstatin A wurde die Proteolyse völlig gehemmt, während Leupeptin nur einen geringen Aktivitätsverlust bewirkte.

Summary: Activity of secretory proteinases was detected on serum albumin agar by the method of Staib for 23% of *Candida albicans* strains, 78% of *Candida tropicalis* strains, and 64% of *Candida parapsilosis* strains. Tested strains of *Candida guilliermondii*, *Candida krusei*, *Candida pseudotropicalis*, *Candida (Torulopsis) glabrata*, and *Trichosporon capitatum* were free of any detectable extracellular proteinases. In a particular case the degree of inducible proteolysis cannot be the sole criterion for virulence of any of these potential pathogens. Proteolysis was completely inhibited by Pepstatin A, whereas Leupeptin caused only a slight decrease of enzyme activity.

Einleitung

Es scheint gesichert, daß Metaboliten des Pilzstoffwechsels am Manifestwerden einer Mykose beteiligt sind. Insbesondere den proteolytischen Enzymen wird in dieser Hinsicht eine wichtige Funktion zugeschrieben. Nach Staib steht die stammspezifische Proteolyseaktivität von *Candida (C.) albicans* gegenüber Serumalbuminen in engem Zusammenhang mit dem Pathogenitätsverhalten. Proteolytische Stämme zeigten hohe Mäusepathogenität (21, 22, 23). Nachweislich werden vom Erreger auch in vivo im Verlauf einer Candida-