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First Report of Involvement of *Nodulisporium* Species in Human Disease

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Allergic fungal sinusitis is a common disease that results from a hypersensitivity reaction mounted by the host against fungi living in the paranasal sinuses. We have recently treated a patient with allergic fungal sinusitis due to a *Nodulisporium* species. This is the first description of a *Nodulisporium* species involved in human disease. The genus *Nodulisporium* contains both dematiaceous and nondematiaceous members. These fungi occur worldwide in nature, often as accompanying conidial anamorphs of certain wood decay ascomycetes. Clinical mycology laboratories may encounter this new agent of phaeohyphomycosis.

Allergic fungal sinusitis (AFS) is a common disease process that causes significant discomfort and disability in afflicted patients. The pathophysiology of AFS involves a hypersensitivity reaction mounted by the host against fungi living in the paranasal sinus cavities (34). Fungal conidia are most likely inhaled into the sinuses by the host, and progressive growth can occur if the conditions are favorable. Chronic obstruction of the sinuses, either by nasal polyps or chronically congested nasal mucosa, provides inhaled conidia with a warm, moist environment that is conducive to growth. By definition, there is no tissue invasion by fungi in AFS. The mycology of AFS shows that most cases are due to dematiaceous fungi or *Aspergillus* species (12). We describe here a case of a young woman with AFS caused by a *Nodulisporium* species, which is the first report of a *Nodulisporium* species implicated in human disease. Mycologic details of the fungus are presented.

The patient is a 43-year-old female with diabetes mellitus and a long history of chronic sinusitis. She had been treated for several years for chronic nasal congestion, allergic rhinitis, and nasal polyps, with several treatment courses of decongestants, antibiotics, and steroid nasal sprays. Her condition worsened to the point at which she could no longer breathe through her nose and had lost her sense of smell. She often had thick, purulent nasal discharge and frontal headaches. Because of these symptoms, she was referred for an otolaryngology evaluation. On physical examination, the nasal cavity showed significant mucosal edema without erythema. There was purulent material in the nasal cavity, and the middle meatae were obstructed by polyps. A computed tomography scan showed pansinusitis and almost complete opacification of the paranasal sinuses. The patient underwent endoscopic sinus surgery to relieve obstruction and allow ventilation of the sinuses. There was thick, golden, sticky material resembling peanut butter found in her maxillary and ethmoid sinuses. Bilateral ethmoidectomies, sphenoidotomies, maxillary antrostomies, and frontal sinusotomies were performed. Histologic examination of three biopsies taken from the sinuses during the operation showed chronic sinusitis with extensive necrosis and debris containing eosinophils and septate hyphae (Fig. 1). No tissue invasion by the fungus was seen. Potassium hydroxide preparations showed septate, hyaline hyphae in all three specimens. The remainders of the specimens were streaked directly onto culture media. Culture of all three specimens on inhibitory mould agar (BBL, Cockeysville, Md.) grew a dematiaceous mould that was subsequently identified as a *Nodulisporium* species. Notably, the hyphae observed in the biopsies were colorless; this finding is common for several species of dematiaceous fungi associated with AFS (31). Six months following surgery, the patient had no evidence of recurrent AFS.

Nodulisporium sp. colonies grown for 14 days on potato dextrose agar (Difco Laboratories, Detroit, Mich.) at 21 to

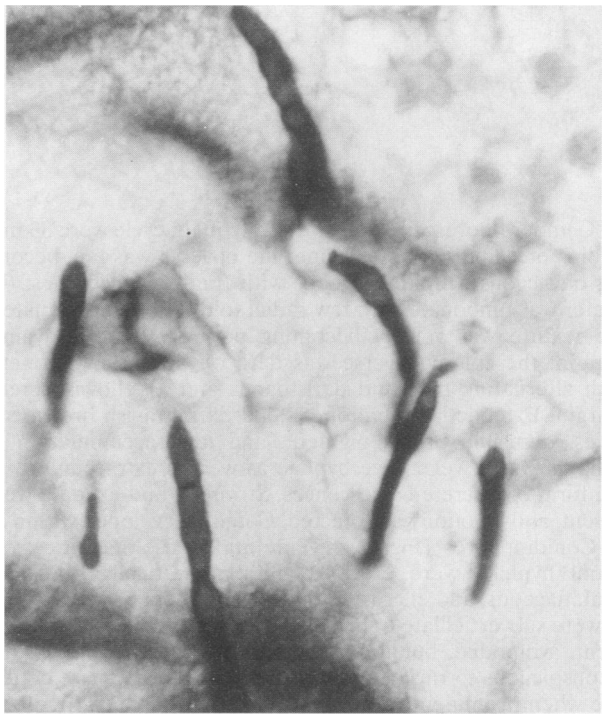

FIG. 1. Gomori methenamine-silver stain of biopsy specimen from the right ethmoid sinus showing septate hyphae and cellular debris. Magnification, ×600.

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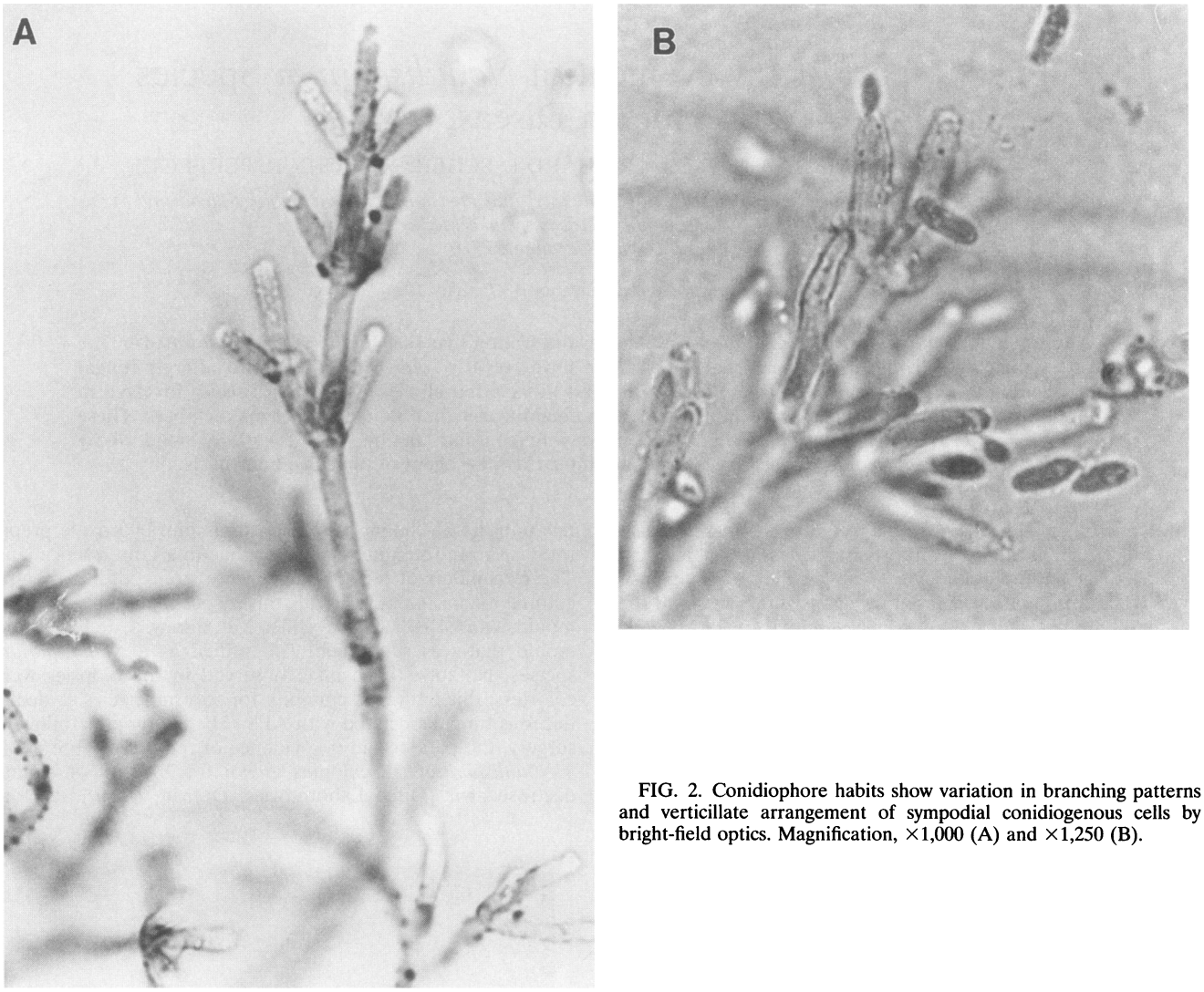

FIG. 2. Conidiophore habits show variation in branching patterns and verticillate arrangement of sympodial conidiogenous cells by bright-field optics. Magnification, ×1,000 (A) and ×1,250 (B).

23°C under fluorescent light with a day-night cycle were 83 mm in diameter and initially hyaline; the obverse was tan, becoming olivaceous to midolivaceous with transient lilac hues; the center was umbonate with few radial folds and faintly striated; the texture was velvety becoming powdery, with a hyaline margin; the colony reverse was dark olivaceous and zonate, with alternating pale and dark bands and a yellowish green margin. Extended incubation yielded dark grayish brown colonies, diffusible brown pigment, and hyperaccumulation of melanin within vegetative hyphae as well as extracellularly in the form of discrete excrescences. Stromata and exudate were absent, and no odor was detected. Conidia developed within 24 h. Conidiophores (Fig. 2), evenly and densely arising from aerial hyphae, were erect, long, asymmetrically branched, dematiaceous, and encrusted in age. Conidiogenous cells (Fig. 3) were subverticillate to predominantly verticillate in arrangement, sympodial, but not prominently swollen at apex, with inconspicuous to raised and prominent scars that were crater-like when prominent. Conidia were variably shaped, pyriform to cylindric, mostly oval, with flat basal scars, unicellular (averaging 4.7 μm long by 2.6 μm wide), distally tapered but not pointed, and subhyaline to pale brown. Susceptibility testing of the isolate was done by a macrodilution tube method as previously described (32), with the exception of yeast nitrogen base as the culture medium. MICs were >25 μg/ml for flucytosine, 12.5 μg/ml for itraconazole, 12.5 μg/ml for fluconazole, and 0.78 μg/ml for amphotericin B.

This paper documents the first reported case of a *Nodulisporium* species implicated in human disease. A literature search of the National Library of Medicine MEDLINE database and the Reviews of Medical and Veterinary Mycology failed to turn up any references of *Nodulisporium* species involved in human disease.

Nodulisporium species occur worldwide in nature, often as accompanying conidial anamorphs to wood decay fungi of the ascomycete genera *Hypoxylon*, *Xylaria*, *Daldinia*, *Entonaema*, and *Biscogniauxia* (4, 14, 15, 20, 21, 24). Stromata of many of these ascomycetes are woody to carbonaceous in texture and may persist for many months. The *Nodulisporium* anamorph grows in conjunction with the developing stromata briefly during favorable conditions and, in many cases, is able to grow independently on various organic matter as well.

The type species for the genus *Nodulisporium* (*N. ochraceum*) reportedly is not dematiaceous, and accordingly, it had

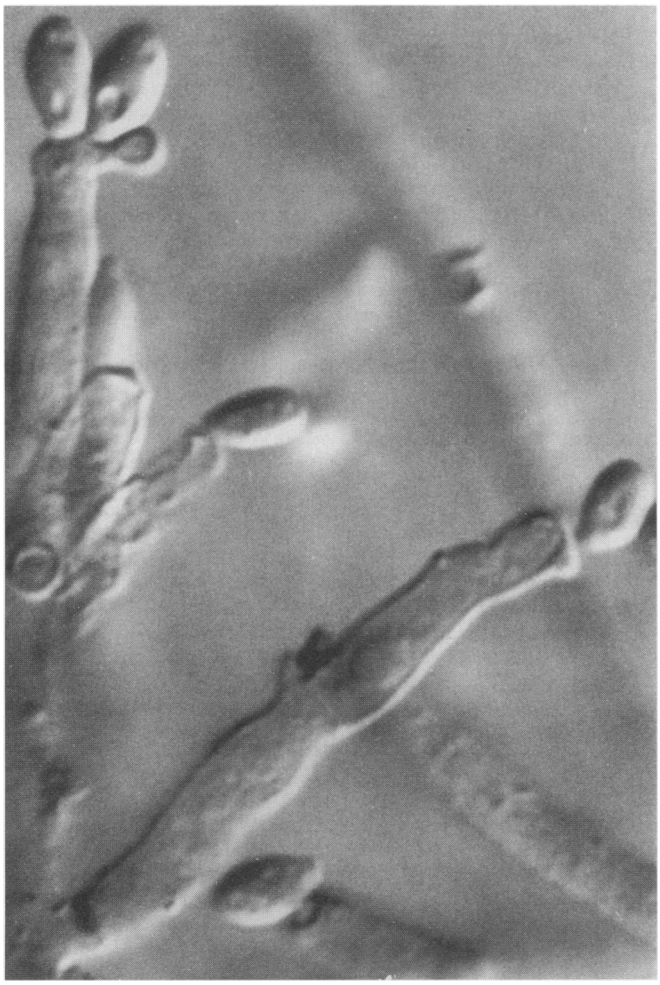

FIG. 3. Conidia develop sympodially and often leave refractile, raised scars (by interference contrast optics). Magnification, ×1,500.

been proposed that dematiaceous fungi of otherwise similar morphology be classified in the genus *Acrostaphylus* (2, 35). This approach has not gained acceptance, and the genus *Nodulisporium* presently accommodates both dematiaceous and nondematiaceous isolates (3, 4, 6, 14, 15, 21). The generic concept of *Nodulisporium* Preuss (1849) has been well summarized and illustrated by Barron (3). Conidial genera that can be confused with *Nodulisporium* include *Calcarisporium*, *Geniculosporium*, *Hansfordia*, *Phaeoisaria*, *Rhinocladiella*, *Sporothrix*, and *Virgaria* (3, 11, 19, 33). Identification of this fungus at the genus level presents no unusual problems, and this level of identification is adequate for the clinical laboratory. Identification of *Nodulisporium* at the form species level, however, is difficult. Discussion of the nomenclatural and taxonomic problems presented by complex fungal life cycles (27) is beyond the scope of this communication. Briefly, because these fungi are pleomorphic and because more attention has historically been devoted to the ascomycetous (teleomorph) element of their life cycles, descriptions of their conidial morphs are more often found under the ascomycete names than under the name *Nodulisporium*. These earlier anamorph descriptions were intended as supplements to the teleomorph descriptions; understandably, separate names were not provided for the accompanying conidial forms. Unfortunately, knowledge of which anamorph (at the form species level) might be invariably connected to a particular teleomorph species is scant. When the conidial morph is isolated in the absence of the ascomycete element (e.g., in the clinical laboratory), one may legitimately question whether it is appropriate to apply a description and a name that is based in part on the ascomycete form, such as the *Nodulisporium* anamorph of *Induratia apiospora*. Whether these anamorphs occurring in the absence of teleomorphs would benefit from a wider application of form species names remains to be determined.

For this group of fungi, there is increasing interest in using anamorph characteristics to facilitate taxonomic decisions regarding the whole fungus (i.e., inclusive of teleomorph and anamorph) (24, 26). A number of the published descriptions, however, are somewhat difficult to employ when only the anamorph is available for study. These studies also varied significantly with regard to the growth conditions of the material being examined, for example, host substrata versus artificial media. In addition, it seems that there is a problem of overlap between anamorph descriptions, which needs further consideration. Study of our isolate included review of more than 120 descriptions of *Nodulisporium* and *Nodulisporium*-like strains (3–5, 7–11, 13–18, 21–25, 28–30, 33, 36, 37). None of the descriptions was entirely in agreement with our isolate; however, it was concluded that it most closely resembles the accounts of *N. hinnuleum* given by Smith (33) and Barron (3). This species has been reported from millet seeds, sorghum, oats, air, and soil.

Nodulisporium species are rarely encountered in the clinical mycology laboratory, and a review of isolates identified in our laboratory over the last 10 years shows that two other cultures besides the one reported here grew a *Nodulisporium* sp. Both were determined to represent contamination rather than infection.

The patient described in this case report was suffering from chronic sinusitis, which, after surgery, was found to be due in large part to the presence of a *Nodulisporium* sp. in her sinuses. The primary treatment for this condition consists of surgical debridement to restore ventilation and drainage of the sinuses (1). Corticosteroids can be given perioperatively to lessen the host hypersensitivity reaction. The role of antifungal drugs is not completely known. It is possible that with the availability of the new potent oral triazole drugs, there is a role for antifungal agents as adjuncts to surgery in the treatment of AFS. In our experience, we have tried short courses of treatment with antifungal agents for patients with recurrent episodes of AFS. However, most patients with recurrent or persistent symptoms of AFS probably require repeated surgical intervention.

Currently, there are no therapeutic implications based on the identification of the particular genus or species of fungal isolates in cases of AFS. However, culture of sinus material does have an important role since it is more sensitive than tissue stains in confirming the presence of fungi. In addition, we believe that documentation of new fungal pathogens is important, given the evolving nature of opportunistic infections. As the population of immunocompromised patients grows, we will likely see an increase in the number of infections due to environmental fungi.

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