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Sodium Chloride as Aid in Identification of *Phaeoannellomyces werneckii* and Other Medically Important Dematiaceous Fungi

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Seventeen taxa of dematiaceous fungi isolated from humans were tested to determine their responses to various concentrations of sodium chloride in vitro. Five groups of species were recognized on the basis of differing tolerances. *Phaeoannellomyces werneckii* was distinguished by its tolerance of ≥15% NaCl; most dematiaceous pathogens were suppressed at ≤7% NaCl.

Sodium chloride tolerance was first suggested by Kane and Fischer (8) as a useful character in the identification of medically important Hyphomycetes. These authors found that common dermatophyte species and related arthrodermataceous anamorphs showed species-specific growth responses to increasing sodium chloride concentrations. In addition, some dermatophyte species showed characteristic morphological responses to low levels of added salt, including induction of macroconidia (7, 17) and inhibition of sterile albinism (or "pleomorphism"), a differentiated nonsporulating growth form of the aerial mycelium. More recently, Schönborn (20, 21) has confirmed the usefulness of sodium chloride tolerance as a differential character in medical mycology.

In the present study, we investigated the usefulness of sodium chloride in determination of dematiaceous Hyphomycetes isolated from clinical sources. Like the dermatophytes, fungi in this group can be identified by conventional means (3, 11) but are highly variable and present some difficulty. At present, only a small number of physiological features have been described which might serve as confirming characters in the routine identification of dematiaceous Hyphomycetes (4, 6, 10, 13, 22).

Dematiaceous fungal isolates were obtained from the culture collection of the Mycology Section, Laboratory Services Branch, Ontario Ministry of Health, and the collection of M. R. McGinnis, University of North Carolina, Chapel Hill. *Xylohypha bantiana* isolates (OMH 32 = NIH 8579.51 = ATCC 10958 = CBS 173.52 and OMH 33 = NIH 8593.57) were originally received under the name *Cladosporium trichoides* from the collection of the National Institutes of Health, Bethesda, Md. All isolates were maintained on Sabouraud peptone-dextrose agar supplemented with chloramphenicol, cycloheximide, and gentamicin. Cycloheximide-intolerant species were maintained on unamended Sabouraud agar. For testing of sodium chloride tolerance, two series of agar slants were prepared. One series consisted of Sabouraud agar supplemented with 1, 3, 5, 7, 9, 11, 13, or 15% (wt/vol) sodium chloride plus sodium chloride-free controls. The second consisted of Casamino Acids (Difco Laboratories, Detroit, Mich.) agar (5) supplemented with sodium chloride as described above. Each dematiaceous fungus was inoculated onto slants encompassing the entire range of salt concentrations for each type of basal medium. Growth from a point inoculum source was recorded after 14, 21, and 30 days at room temperature. An individual species was considered to be strongly inhibited at a given salt concentration if its colony diameter was less than 2 mm at 21 days. This criterion was applicable both to mycelial species and to species growing primarily in the yeast form. At the end of the growth measurement period, cultures at all salt concentrations on Sabouraud agar were examined microscopically to determine whether sodium chloride had engendered changes in micromorphology.

The responses of 17 fungal species to various sodium chloride concentrations are shown in Table 1. The differences observed among the taxa were not influenced by the nature of the basal medium used in the tests.

On the basis of the information summarized in Table 1, we were able to divide the test isolates into five groups. The first group comprises species strongly inhibited by 5 to 7% sodium chloride: *Xylohypha bantiana*, *Fonsecaea compacta*, *F. pedrosoi*, *Wangiella dermatitidis*, *Cladosporium carrionii*, *Exophiala spinifera*, and *Phialophora verrucosa*. A second group, showing inhibition at 9% sodium chloride, contains *Exophiala jeanselmei* (including isolates retained in the Ontario Ministry of Health collection under the ambiguous name "*Phialophora gougerotii*"—see comments by McGinnis [11] and McGinnis and Ajello [12]). A third distinct group of fungal strains showed a threshold of inhibition at 11% sodium chloride; this group contained two *Aureobasidium pullulans* isolates from humans. The fourth recognized group of isolates showed inhibition at 13% sodium chloride: *Alternaria alternata*, *Bipolaris hawaiiensis*, *B. spicifera*, and *Exserohilum rostratum* were included. Finally, the fifth group of isolates, containing *Phaeoannellomyces werneckii*, *Phaeotheca fissurella*, and cycloheximide-sensitive, nonpathogenic *Cladosporium* spp., were tolerant of 15% sodium chloride.

Certain morphological changes were brought about by salt in various test fungi. Stimulation of conidium production occurred in one isolate of *Alternaria alternata*. Only a few conidia were formed by this freshly isolated culture on plain Sabouraud agar, but greater numbers of conidia were found at 1 to 11% salt, with maximum conidiation at the 9% level. On the other hand, conidiation was suppressed at the 3% salt level in *Fonsecaea compacta* and *Phialophora verruscosa*, at 5% salt in *Fonsecaea pedrosoi*, and at 7 to 9% salt in *Exophiala jeanselmei*.

Other alterations were noted at higher salt concentrations in many of the species examined, including suppression of

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TABLE 1. MICs of NaCl for dematiaceous Hyphomycetes in Sabouraud peptone-dextrose agar

Species	No. of isolates investigated	% Concn of NaCl[a]						
		5	7	9	11	13	15	>15
Alternaria alternata (Fr.) Keissler	3					×		
Aureobasidium pullulans (de Bary) Arn.	2				×			
Bipolaris hawaiiensis (M. B. Ellis) Tsun. et Uey.	2					×		
Bipolaris spicifera (Bain.) Subram.	2					×		
Cladosporium carrionii Trejos	2		×					
Cladosporium cladosporioides (Fres.) de Vries	2							×
Cladosporium sphaerospermum Penz.	2							×
Cladosporium spp.	2							×
Exophiala jeanselmei (Langer.) McGinnis et Padhye	5			×				
Exophiala spinifera (Neils. & Con.) McGinnis	2		×					
Exserohilum rostratum (Drechsler) Leon. et Suggs	1					×		
Fonsecaea compacta (Carrion) Carrion	2	×						
Fonsecaea pedrosoi (Brumpt) Negroni	2	×						
Phaeoannellomyces werneckii (Horta) McGinnis et Schell	6							×
Phaeotheca fissurella Sigler, Tsun. et Carm.	1							×
Phialophora verrucosa Medlar	5	×	×					
Wangiella dermatitidis (Kano) McGinnis	2	×						
Xylohypha bantiana (Sacc.) McGinnis, Borelli, Padhye, et Ajello	2	×						

[a] Suppression of all test isolates of a given species by the same salt concentration is signified by a single cross. For variable species with some isolates more salt tolerant than others, a cross is placed at each concentration causing suppression in a proportion of the isolates.

aerial mycelium, conversion from filamentous to moniliform growth, secretion of oily substances, enhanced roughness of verrucose cell walls, and suppression of cell wall melanization. These characters, however, were too variable to be of potential diagnostic significance.

Testing the sodium chloride tolerance of dematiaceous fungi provides very useful taxonomic information. Particularly remarkable is the case of *Phaeoannellomyces werneckii* (14) which, by growing well at 15% sodium chloride, showed itself to be much more salt tolerant than any other pathogen with which it might be confused. The agents of chromoblastomycosis—*Cladosporium carrionii*, *Fonsecaea compacta*, *F. pedrosoi*, and *Phialophora verrucosa*—showed salt tolerances which are in the low range for filamentous fungi (24). Some agents of phaeohyphomycosis—*Exophiala spinifera*, *Wangiella dermatitidis*, and *Xylohypha bantiana*—also showed low salt tolerance. On the other hand, many dematiaceous potential pathogens, including *Alternaria alternata*, *Aureobasidium pullulans*, *Bipolaris* spp., *Exophiala jeanselmei*, and *Exserohilum rostratum*, showed relatively high tolerance. Several fungi included here which are rarely or never pathogenic—cycloheximide-sensitive *Cladosporium* spp. and *Phaeotheca fissurella*—were also highly tolerant toward sodium chloride, growing well at the 15% salt level.

In contrast to the general usefulness of the salt tolerance test, there appears to be less potential benefit to be derived from morphological examination of dematiaceous fungal cultures grown in the presence of salt. Only stimulation of conidiation in one *A. alternata* isolate gave some preliminary indication that sodium chloride-amended media could give useful morphological information in the study of dematiaceous fungi.

In general, the salt tolerances found in the present study correspond well to existing knowledge about the environmental tolerances of the fungi studied. *P. werneckii*, for example, is known from marine habitats (18) and from dried, salted fish (15). The agents of chromoblastomycosis investigated in this study have seldom or never been isolated from marine or other salty habitats. The only such record we have found is for *F. compacta*, which has been reported once from marine algae in a "tide-washed coastal area" of California (2). On the other hand, occurrence in marine and salt marsh habitats is well documented for *Alternaria* spp. (9, 16, 19), *Aureobasidium pullulans* (9), *Bipolaris hawaiiensis* and *B. spicifera* (16), and nonpathogenic *Cladosporium* spp. (16, 19). The occurrence of *Exserohilum rostratum* in the sea is uncertain because of taxonomic confusion (9), but occurrence in salt marshes has been reported (1). Some of the above-named fungi have been tested previously for salt tolerance (15, 19, 23) and sucrose osmotolerance (16). Particularly interesting to us are the results of Moustafa and Al-Musallam (16), who found sucrose osmotolerance values for *Alternaria*, *Aureobasidium*, *Cladosporium*, and *Bipolaris* species comparable to our own values for salt tolerance. These results suggest that salt tolerance is a highly stable, and hence highly useful, determinative character in dematiaceous fungi.

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