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 dermatomyces species and related arthrodermaticous anamorphs showed species-specific growth responses to increasing sodium chloride concentrations. In addition, some dermatomyces species showed characteristic morphological responses to low levels of added salt, including induction of macroconidia (7, 17) and inhibition of sterile albimism (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 dermatomyces, 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).

Damiaticaceous 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 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 test.

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 carrioni*, *Exophiala spinifera*, and *Phialophora verrucosa*. A second group, showing inhibition at 9% sodium chloride, contains *Exophiala jeansenii* (including isolates retained in the Ontario Ministry of Health collection under the ambiguous name "Phialophora gaugetii"—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 hawaiensis*, *B. spicifera*, and *Exserohilum rostratum* were included. Finally, the fifth group of isolates, containing *Phaeoannellomyces werneckii*, *Phaeothea 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 conidiation 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 verrucosa*, at 5% salt in *Fonsecaea pedrosoi*, and at 7 to 9% salt in *Exophiala jeansenii*.

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

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Aureobasidium alternata (Fr.) Keissler
Aureobasidium pullulans (de Bary) Arn.
Bipolaris hawaiensis (M. B. Ellis) Tsun. et Uey.
Bipolaris spicifera (Bain.) Subram.
Cladosporium carrionii Trejos
Cladosporium cladosporioides (Fres.) de Vries
Cladosporium sphaerospermum Penz.
Cladosporium spp.
Exophiala sp.
Fonsecaea compacta (Carrion) Carrion
Fonsecaea jeanselmei (Langer.) McGinnis et Padhye
Fonsecaea spinifera (Nells. & Con.) McGinnis
Exserohilum rostratum (Drechsler) Leon. et Suggs
Fonsecaea compacta (Carrion) Carrion
Fonsecaea pedrosoi (Brumpt) Negroni
Phaeoannellomyces werneckii (Horta) McGinnis et Schell
Phaeoophora fassurella Sigler, Tsun. et Carm.
Phialophora verrucosa Medlar
Wangiella dermatitidis (Kano) McGinnis
Xylohypha bantiana (Sacc.) McGinnis, Borelli, Padhye, et Ajello

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<th>Species</th>
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<th>7</th>
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* 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.

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.

We thank M. R. McGinnis for supplying cultures and S. Albreish for technical assistance.

R. C. S. was partially supported by a Medical Research Council of Canada postdoctoral fellowship.

**LITERATURE CITED**

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