Nature and Identification of *Exophiala werneckii*

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The morphological and physiological characteristics of 44 isolates of *Exophiala werneckii* recovered from human and environmental sources were indistinguishable from 2 isolates that caused tinea nigra. Casein hydrolysis and inability to decompose tyrosine differentiate *E. werneckii* from *Exophiala jeanselmei*, *Exophiala spinifera*, and *Wangiella dermatitidis*.

*Exophiala werneckii* (Horta) Arx 1970 is the etiological agent of the superficial mycosis tinea nigra (1, 4, 8, 10). Its distinguishing morphological feature is a yeast form which is typically two celled. These yeast cells are annelid-like that form one- and two-celled conidia. The conidiation and dimorphism of this fungus have been examined extensively (6, 7, 9, 13, 14). *E. werneckii* is differentiated from similar fungi solely by morphology. The physiological properties of this fungus have not yet been adequately studied.

Recently, we isolated *E. werneckii* from several unusual human and environmental sources. One isolate was recovered from an inflammatory scalp lesion in a girl, and another came from a macerated interdigital lesion in a man’s foot. Two isolates were associated with plaque-like *Candida* infections in the crural areas of patients’ bodies, and another was associated with a case of tinea capitis already under treatment. In none of these five cases did we notice pigmented macules at the infection sites or fungal elements in epidermal scrapings. Additional isolates were obtained from asymptomatic superficial human dermal areas and from superficial moldy plaques in spoiled salted freshwater fish (16). The objectives of our study were to confirm the identity of the *E. werneckii* isolates and to evaluate commonly employed physiological tests as possible species-specific diagnostic criteria to supplement the morphological identification of *E. werneckii*.

We studied 46 isolates of *E. werneckii*: 5 were recovered from superficial dermal lesions in humans, 34 from asymptomatic human scalp as well as the interdigital areas of hands and feet, 5 from moldy plaques on air-dried salted Amazonian fish (*Osteoglossum bicirrhosum*), and 2 controls (B2803a, B2804a) from human tinea nigra. The 44 test isolates were maintained in the fungal culture collection at the Instituto Nacional de Pesquisas da Amazônia (INPA).

All isolates were maintained on Sabouraud dextrose (SAB) agar. Colonial morphology and thermotolerance were studied by inoculating each isolate onto duplicate tubes of SAB agar and Czapek-Dox agar and incubating the cultures at 25, 28, 38, 42, and 45°C for 1 month. Weekly observations on colonial morphology were made.

Each isolate was inoculated onto duplicate tubes of SAB agar containing NaCl at 10, 20, and 30% (wt/vol) at 25°C for 1 month. Weekly observations were made. The biochemical properties examined were hydrolysis of casein, tyrosine, xanthine, hypoxanthine, and starch; gelatin liquefaction; paraffin utilization; sodium nitrate utilization in Czapek-Dox agar; proteolytic activity in Loeffler coagulated serum; and fermentation of glucose, maltose, sucrose, galactose, and lactose. The tests were performed by the methods described by Beneke and Rogers (2), Berd (3), Conti-Diaz et al. (5), McClung (12), Silva (21), and Silva-Hutner and Cooper (22).

For comparison purposes, temperature and biochemical studies were also carried out with the following fungi: *Exophiala jeanselmei* (Instituto de Higiene de Montevideo [IHM] isolates 1860, 1783), *Exophiala spinifera* (IHM isolates 1610, 1611, 1740, 1781), and *Wangiella dermatitidis* (IHM isolates 1763, 1765, 1766 and INPA isolates 105, 109, 119, 123, S11, S76, S93). The IHM cultures had been examined and were furnished by I. A. Conti-Diaz of the IHM, Montevideo, Uruguay (5). The INPA isolates were recovered from bats (20) and soil (15) in Manaus, Brazil.

Colonies of the *E. werneckii* isolates on SAB agar and Czapek-Dox agar were initially yeast-like and shiny black. With age, they produced submerged peripheral hyphae and abundant aerial mycelia and turned olive in color. Colonial development was similar at 25, 28, and 38°C. There was diminished metallic tonality in the colonies of cultures kept at the higher temperatures. At 42°C, all isolates showed slow but steady growth. At 45°C, there was no growth. Microscopically, all isolates showed early hy-
TABLE 1. Physiological characteristics of some *Exophiala* and *Wangiella* species

<table>
<thead>
<tr>
<th>Fungus</th>
<th>No. of isolates tested</th>
<th>Casein</th>
<th>Tyrosine</th>
<th>Xanthine</th>
<th>Hypoxanthine</th>
<th>Starch</th>
<th>Protein</th>
<th>Gelatin liquefaction</th>
<th>Paraffin utilization</th>
<th>Na$_3$NO$_3$ utilization</th>
<th>Maximum growth temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. werneckii</em></td>
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</tr>
<tr>
<td>Non-tinea nigra isolates</td>
<td>44</td>
<td>+</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>42</td>
</tr>
<tr>
<td>Tinea nigra isolates</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>42</td>
</tr>
<tr>
<td><em>E. jeanselmei</em></td>
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<tr>
<td>a</td>
<td>2</td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>38</td>
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<td><em>E. spinifera</em></td>
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<td>b</td>
<td>4</td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>38$^b$</td>
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<tr>
<td><em>W. dermatitidis</em></td>
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<tr>
<td>IHM isolates</td>
<td>3</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>42</td>
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<tr>
<td>INPA isolates</td>
<td>8</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>42</td>
</tr>
</tbody>
</table>

$^a$ Classified as *Phialophora gougerotii* by the original authors (5).

$^b$ Reported as 41°C by the original authors (5).

phal development and characteristic annellides and annelloconidia, some being two celled with prominent darkly pigmented septa. Chlamydoconidia and spiral hyphae were also present. All structures were discernible as early as 10 days after inoculation. There was no difference in morphological development among the cultures of the 44 test isolates and those of the 2 control isolates on SAB agar, potato dextrose agar, and cornmeal agar. However, growth was more rapid and abundant on cornmeal agar.

All *E. werneckii* isolates were resistant to high salinity (30%) in the culture medium. The colonial development of isolates on SAB agar saturated with NaCl was indistinguishable from those grown on SAB agar without NaCl. The test and control isolates consistently decomposed casein but did not hydrolyze tyrosine, xanthine, hypoxanthine, or starch. All isolates utilized sodium nitrate in Czapé-Dox agar. However, none liquefied gelatin or utilized paraffin, except for two INPA isolates which showed a very slight degree of paraffin utilization. The isolates also did not hydrolyze protein in Loeffler coagulated serum. None of the isolates fermented glucose, maltose, sucrose, galactose, or lactose.

Table 1 presents data on the physiological characteristics and maximum growth temperatures of the *E. werneckii*, *E. jeanselmei*, *E. spinifera*, and *W. dermatitidis* isolates. The following characteristics are being reported for the first time: inability of *E. spinifera* to hydrolyze xanthine, lack of paraffin utilization by *E. spinifera* and *W. dermatitidis*, and utilization of sodium nitrate by *E. spinifera*. Results on the other biochemical reactions confirm those reported earlier by various investigators (5, 11, 17-20). Except for the maximum growth temperature of *E. spinifera*, which we found to be lower than that reported by Conti-Díaz et al. (5) (38 as against 41°C), the temperature results were consistent with the published data (5, 11, 17-19). The ability to hydrolyze casein and the inability to decompose tyrosine are biochemical characteristics that differentiate *E. werneckii* from *E. jeanselmei*, *E. spinifera*, and *W. dermatitidis*. They are additional supportive evidence of differences among these species.

Results of the morphological and biochemical studies show that the isolates of *E. werneckii* evaluated in this study, from human and environmental origins, are identical to typical isolates recovered from patients with tinea nigra. The diverse origins and halophilic nature of our isolates indicate the ubiquitous nature of *E. werneckii*.

I thank Libero Ajello for furnishing the tinea nigra isolates and Ismael A. Conti-Díaz for the IHM isolates, and appreciate the technical assistance of M. S. Barreto da Silva and R. C. C. Luizão.

LITERATURE CITED


ERRATUM

Detection of Bacteremia in Patients Receiving Antimicrobial Therapy: an Evaluation of the Antimicrobial Removal Device and 16B Medium

GARY V. DOERN AND NELSON M. GANTZ

Department of Clinical Microbiology and Division of Infectious Disease, University of Massachusetts Medical Center, Worcester, Massachusetts 01605

Volume 18, no. 1, p. 45, column 1, lines 25–26: "Streptococcal salivarius" should read "Streptococcus salivarius."
Page 45, Table 1, column 1, line 3: "Staphylococcus fecalis" should read "Streptococcus faecalis."
Page 45, Table 1, column 1, line 4: "Staphylococcus bovis" should read "Streptococcus bovis."
Page 46, column 2, line 17: "Streptococcus fecalis" should read "Streptococcus faecalis."

Author’s Correction

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Volume 16, no. 5, p. 976–978: It has come to the attention of the author that the utilization of sodium nitrate by Exophiala spinifera was reported previously by I. Conti-Diaz et al. (in The Black and White Yeasts, IV Int. Conf. Mycoses—1977, Pan American Health Organization scientific publication no. 356, p. 109–114, 1978). She and the editor regret the omission of this citation.