Preliminary Studies of Some Physiological Properties of *Torula jeanselmei*

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The morphological characteristics of pathogenic and saprophytic strains of *Torula jeanselmei* (Fungi Imperfecti) are indistinguishable. Some basic physiological properties of *T. jeanselmei* were investigated in the hope of separating the human pathogenic strain from common saprophytes in a clinical laboratory. Nitrogen and carbon assimilation tests were not useful for distinguishing the pathogen, since all strains, pathogenic and saprophytic, were able to assimilate nitrogen from ammonium, nitrate, and nitrite sources, and all were able to utilize glucose, fructose, galactose, cellobiose, lactose, glycerol, and starch. It appeared that temperature range for growth might be useful in the identification of the pathogen. All pathogenic strains grew well at 37°C but poorly at 10°C; in contrast, all saprophytes could grow at 10°C but were unable to grow at 37°C (with two exceptions).

*Torula jeanselmei* Langeron or *Phialophora jeanselmei* (Langeron) Emmons (Fungi Imperfecti) is one of the etiological agents of mycetoma in man (2, 5). This fungus is also a common saprophyte isolated from decayed wood (6), polluted water and sewage (1), and from pulp suspensions collected in paper mills (7). Morphologically, pathogenic and saprophytic strains are similar (8); however, their physiological properties may or may not agree. The purpose of this preliminary investigation was to determine some basic physiological properties of several strains of *T. jeanselmei*. It was hoped that these results would reveal physiological differences valuable in distinguishing pathogenic from saprophytic strains in a clinical laboratory.

**Materials and Methods**

The cultures of *T. jeanselmei* used in this study were as follows: Emmons 7850, isolated by W. B. Cooke from polluted water in the U.S. (1); Emmons 8725, isolated by D. Symmers from mycetoma of the hand in the U.S. (2); Emmons 8728, Langeron's original strain from mycetoma in Canada (5); ENSAN 3412, F. Mangenot's isolate from wood in France (6); Wang 53 and 297, isolated from pulp suspensions collected from the headbox of a paper mill in New York, May and July, 1959 (7); and Wang 318, isolated from pulp suspension collected from the cylinder vat of a board mill in New York, July 1959 (7).

A preliminary study was carried out to determine which synthetic medium to use. Yeast Morphology Agar (Difco) was chosen over Czapek Dox Agar, because it was convenient to obtain the corresponding Yeast Carbon Base (Difco) for the nitrogen requirements study, as well as the Yeast Nitrogen Base (Difco) for the carbon nutrition study. Preliminary tests also showed that the unadjusted pH 4.5 of the Yeast Morphology Agar produced very glabrous growth. The normal woolly appearance of the fungus was restored when the pH of the medium was adjusted with 1 N NaOH to about pH 6.5. Consequently, all media were adjusted after autoclaving to pH 6.1 to 6.7.

**Temperature studies.** Each of the seven strains of *T. jeanselmei*, inoculated in the centers of petri plates containing Yeast Morphology Agar (pH 6.4), was placed in one of seven incubators that were variously set at 10 to 45°C at 5°C increments (with 37°C replacing 35°C). All cultures were examined, and colonies were measured weekly for 3 weeks. Results obtained from the study above indicated that there was a definite difference between the growth of the pathogenic and saprophytic strains at 10 and 37°C. Therefore, further studies were made at these temperatures. The following strains of *Torula*, in addition to those mentioned above, were used: Emmons 8727, from a case treated by Philip Beerman, April 1945, in the U.S.; Emmons 8733, from sputum, December 1957, in the U.S.; and Wang 102, 310, 316, 325, 414, 553, 597, 932, and 936, all isolated from pulp suspensions collected in various paper mills in New York from May to December, 1959 (7, 8).

The 18 strains were each inoculated on Sabouraud Dextrose Agar and blood-agar plates. Each inoculum measured approximately 2 mm² and was taken from 1-week-old stock culture on Malt Agar. Those on Sabouraud Dextrose Agar were incubated at 10 and at
37 C. All of the cultures on blood-agar were incubated at 37 C.

Nitrogen requirement studies. Attention was devoted to three inorganic forms of nitrogen: an ammonium salt, a nitrite, and a nitrate. The media were prepared by use of Yeast Carbon Base (Difco) with 2% purified agar plus one of the following nitrogen sources (milligrams per 100 ml): ammonium sulfate, 51.8; sodium nitrite, 54.1; and potassium nitrate, 79.9 to provide 0.011% of nitrogen. The inoculum was taken from 3-week-old stock cultures of each of the seven strains and was planted in the center of each plate. The inoculum was standardized with a square instrument similar to a cork borer. The stock culture mat was cut into squares measuring 5 mm². Agar on the bottom of the square was scraped off before the square was placed on the new medium. All cultures in this experiment and in the following carbon studies were incubated at 25 C (optimal growth temperature for all strains), and the diameter of each colony was measured weekly for 3 weeks.

Carbon requirement studies. To study the carbon requirements of T. jeanselmi, two sets of media were prepared by use of Yeast Nitrogen Base with 2% purified agar plus 2% concentration of each of the following carbon sources: glucose, fructose, galactose, cellulose, lactose, glycerol, and starch. To determine whether or not autoclaved sugars might give different results, the first set of media was autoclaved for 15 min at 15 psi. For the second set, the nitrogen base with the carbon source was filter-sterilized by use of a Seitz filter according to the Difco Manual (p. 250-254). Strips of filter paper were used as the source of cellulose, and they were autoclaved with the nitrogen base in test tubes. Each inoculum of approximately 2 mm² was placed in the center of each plate. The inocula for the second set, i.e., filter-sterilized media, were taken from the first set of cultures after 1 week of incubation. Each stock culture was on the Yeast Nitrogen Base containing the same carbon source to which it was transferred.

Results

Results from preliminary temperature studies on seven strains of T. jeanselmi indicated that the optimal temperature for all strains was 25 C. For all strains, no growth occurred at either 40 or 45 C. At 10 C, two of the pathogenic strains, Emmons 8725 and 8728, gave only a trace of growth at the end of 3 weeks of incubation, whereas all saprophytic strains produced colonies measuring from 6 to 13 mm in diameter. At 37 C, the pathogenic strains measured 10 to 14 mm in diameter, but the saprophytes, except 318, gave no visible growth. Additional tests were made by use of all available pathogenic and saprophytic strains on hand. It was hoped that the results could be used to separate the pathogens from the saprophytes. Table 1 shows the differences in ability of 18 strains of T. jeanselmi to grow at 10 C and at human body temperature, 37 C. For all pathogens, growth occurred at 37 C on either Sabouraud Dextrose Agar or blood-agar. With the exception of Emmons 8727, pathogen strains grew poorly at 10 C at the end of 3 weeks. All saprophytes grew well at 10 C, but no growth was visible at 37 C, except with strains 318 and 936.

Growth was supported by all of the nitrogen sources (Table 2). No difference appeared between the nitrogen requirements of the two pathogens, Emmons 8725 and 8728, and those of the saprophytes. Table 2 also indicated that good growth was supported by all the carbon sources except cellulose.

As stated previously, the first set of media for the carbon study was autoclaved to compare with the second set which was filter-sterilized. The growth was nearly the same for each strain on both sets of media.

Discussion

The ability of a fungus to grow at 37 C is a useful criterion in separating a pathogenic species from nonpathogens. Kao and Schwarz (4) used the characteristic of good growth of Cryptococcus neoformans (Sanfelice) Vuill. at 37 C as one of the tests for positive identification of the pathogen, since nonpathogens were unable to grow at that temperature. Recently, Greer and Friedman (3) reported that Basidiobolus ranarum Eidam isolated from subcutaneous phycmycosis grew well at 37 C, but that Basidiobolus strains isolated from natural habitats were unable to

Table 1. Difference in the growth rate of pathogenic and saprophytic strains of Torula jeanselmi at 37 and 10 C

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Colony diam (mm) at 3 weeks</th>
<th>37 C</th>
<th>10 C, SD</th>
<th>Blood-agar²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E8725</td>
<td>Human</td>
<td>14</td>
<td>10</td>
<td>T²</td>
<td></td>
</tr>
<tr>
<td>E8727</td>
<td></td>
<td>5</td>
<td>7</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>E8728</td>
<td></td>
<td>12</td>
<td>7</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>E8733</td>
<td></td>
<td>4</td>
<td>5</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>318</td>
<td>Pulp</td>
<td>9</td>
<td>7</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>936</td>
<td>Polluted water</td>
<td>8</td>
<td>7</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>E7890</td>
<td>Wood</td>
<td>0</td>
<td>T</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>ENSAN 3412</td>
<td>Polluted water</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>53, 102, 297, 310, 316, 325, 414, 553, 597, and 932</td>
<td>Pulp</td>
<td>0</td>
<td>0</td>
<td>6-11</td>
<td></td>
</tr>
</tbody>
</table>

* Sabouraud Dextrose Agar.
1 Measured at the end of 2 weeks.
² Trace of growth.
Interesting Results in However, saprophytes slowly strains at well further study with carbon from pathogenic distinguishing in this tests the from media; therefore, strains from our grew from our which were able to investigate, especially two sources. It was able to differentiate saprophytic of all strains of Cryptococcus neoformans. Cryptococcus strain source was unable to assimilate potassium nitrate as the sole source of nitrogen, whereas the pathogenic Cryptococcus strain was unable to do so. Results from our nitrogen assimilation study showed that strains from human as well as from saprophytic sources grew equally well on all three nitrogen media; therefore, they are not useful for distinguishing pathogenic T. jeanselmei from the saprophytes. The same conclusion is also drawn from the carbon requirement tests. Since results from the simple nitrogen and carbon assimilation tests in this study are inconclusive, further studies on the amino acid and vitamin requirements of these strains as well as pathogenicity and serological tests are planned in the hope of devising a few dependable techniques to identify the pathogen in clinical laboratories.

Acknowledgments

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Literature Cited