The cytochrome systems of two classes of aquatic fungi, the Oomycetes and Chytridiomycetes, were studied by means of reduced-minus-oxidized difference spectra at room and at low temperature. At room temperature, all of these fungi have a c-type cytochrome with an absorption maximum at 551 μm and a b-type cytochrome at 564 μm. The Oomycetes have a-type cytochromes at 605 μm, and the Chytridiomycetes have a-type cytochromes at 606 μm (Blastocladiiales) or at 609 μm (Monoblepharidales). Additional b-type cytochromes are found at 557 μm in the Oomycetes and at approximately 560 μm in the Chytridiomycetes. The data obtained from spectra at low temperature are consistent with these conclusions. Thus, the difference spectra reveal variation between the cytochrome systems of these two classes of aquatic fungi.

Boulter and Derbyshire (2) examined the visible absorption spectra of a large number of fungi (including Alomyces, Saprolegnia, and Pythium) with a Zeiss hand spectroscope and a Hartridge reversion spectroscope. They concluded that the cytochrome systems in these fungi are similar to yeast. With this technique, however, it is difficult to resolve absorption peaks of cytochromes which are close together. Further data obtained with improved spectrophotometric techniques led Lindenmayer (7) to suggest that, in general, the cytochrome system of the fungi is remarkably similar to that of mammalian and avian cells and unlike the systems found in bacteria and in the mitochondrial of green plants. However, more recent work has shown that the cytochrome systems of biflagellate aquatic fungi belonging to the class Oomycetes differ substantially from that of yeast (3, 4, 10). The purpose of the present study was to compare the cytochrome systems found in the posteriorly uniflagellate fungi (Chytridiomy- cetes) with those in the biflagellate fungi (Oomy- cetes).

**Materials and Methods**

A culture of Pythium ultimum (RDC4) was obtained from Joseph G. Hancock, Jr., Department of Plant Pathology; cultures of Alomyces catenoides, Blastocladia alabamensis, Monoblepharis sp. (CR-33) were kindly provided by Ralph Emerson, Department of Botany, University of California, Berkeley. The sources of Aphanomyces astaci J1 and Monoblepharis sp. O are discussed by Unestam (8, 9). A. astaci J1 was grown in a shake culture on a medium containing 0.6% glucose, 0.3% peptone (Difco), and salts (8). Monoblepharella and Monoblepharis were grown in a shake culture on a medium containing 0.5% glucose, 0.5% Tryptone (Difco), 0.66 mM phosphate, and 25% of the concentration of salts used for Aphanomyces. The other fungi were also grown in a shake culture but on a medium containing 0.3% glucose, 0.125% peptone (Difco), 0.125% yeast extract (Difco), and salts (4).

The mycelia of these fungi were fragmented, by a Virtis homogenizer, in a 0.25 M sucrose solution maintained near 0°C in an ice bath; the suspension was concentrated by sedimentation at 12,000 x g. The difference spectra were observed according to the method described by Gleason and Unestam (4), with the following modification. An unsilvered dewar flask was built into the scattering transmission accessory of a Cary 14 split-beam spectrophotometer for low temperature spectra. Plastic absorption cells were used in place of glass cells, and, after reduction or oxidation of the mycelium, they were immersed in liquid nitrogen for several minutes. The absorption cells were raised above the surface of the liquid nitrogen in the dewar flask while the spectrum was being recorded. Oxygen was used instead of hydrogen peroxide for oxidation of the homogenates, because none of these Chytridiomycetes had sufficient catalase activity for release of oxygen bubbles from hydrogen peroxide. All of the Oomycetes tested had vigorous catalase activity.

To study the effect of antimycin A on the cytochrome system, several drops of 5 X 10^-4 M antimycin A (in ethyl alcohol) were added to 3 ml of the suspension. After about 10 min, the suspension was bubbled with oxygen, placed in the absorption cell, and then frozen in liquid nitrogen. Antimycin A inhibits electron transport between cytochrome b and cytochrome c in higher plants, yeasts, and mammalian cells (1, 5). It appears that antimycin A also inhibits electron transport between cytochrome b and cytochrome c in aquatic fungi. In (reduced by succinate or antimycin A plus oxygen) minus (oxidized) difference spectra, the absorption maxima are due to
reduced b-type cytochromes, since the c- and a-type cytochromes become oxidized.

RESULTS

Chytridiomycetes. Figure 1D shows the difference spectrum observed with suspensions of Allomyces at room temperature between 500 and 650 m\(\mu\). The a-type cytochromes have absorption maxima at 606 (\(\alpha\)) and 446 m\(\mu\) (\(\gamma\); not shown). The b-type cytochromes have broad peaks at 560 (\(\alpha\)), 530 (\(\beta\)), and 426 m\(\mu\) (\(\gamma\); not shown), and the c-type cytochromes have broad peaks at 553 (\(\alpha\)) and 522 m\(\mu\) (\(\beta\)). The broad peak at 426 m\(\mu\) in the Soret region was resolved into two components by use of the respiratory electron transport inhibitor antimycin A. In Allomyces, the \(\gamma\) peak of the c-type cytochromes was at 425 m\(\mu\) and the \(\beta\) peak of the b-type cytochromes was at 430 m\(\mu\). The broad peaks at 560 and 553 m\(\mu\) in the \(\alpha\) region could not be resolved further at room temperature.

The spectrum of Allomyces was then compared with the spectra of the other Chytridiomycetes. A major difference between the absorption maxima of a-type cytochromes in the Monoblepharidales and those in the Blastocladiales was evident in room temperature spectra: the a-type cytochromes in Monoblepharella and Monoblepharis had an absorption maximum at 609 m\(\mu\), whereas those of Allomyces and Blastocladiella had an absorption maximum at 606 m\(\mu\).

In low-temperature spectra of Monoblepharella, the absorption maximum of a-type cytochrome was at 608 m\(\mu\), and a shoulder appeared at 598 m\(\mu\); in Allomyces and Blastocladiella, a single absorption maximum was observed at 603 m\(\mu\) (Fig. 2). Spectra at low temperature revealed that Allomyces, Blastocladiella, Monoblepharis, and Monoblepharella probably have two c-type cytochromes and two or three b-type cytochromes. The absorption maxima of c-type cytochromes consisted of a shoulder usually at 545 m\(\mu\) and

**Fig. 1.** Cytochrome system of Allomyces catenoides. Difference spectra: D. (reduced by hydrosulfite) minus (oxidized) at room temperature; E. (reduced by hydrosulfite) minus (oxidized) at low temperature.

**Fig. 2.** Cytochrome systems of Allomyces, Blastocladiella, and Monoblepharella: reduced hydrosulfite-minus-oxidized difference spectra at low temperature.
two peaks at approximately 552 and 548 µm (Fig. 2). The data suggest that two c-type cytochromes occur, one with an absorption maximum at 548 µm and the other with an absorption maximum at 552 µm. It is possible that these cytochromes are equivalent to cytochromes c and c₁ characterized in mammalian cells. Figure 3 illustrates that only the b-type cytochromes remain reduced in the presence of antimycin A, but some differences in the wavelengths of the three absorption maxima are evident. The c-type cytochromes became oxidized with the addition of antimycin A.

The room and low temperature absorption peaks of all of the b- and c-type cytochromes were estimated after examination of a large number of spectra. The wavelengths of maximum absorption of all cytochromes were shifted downward about 2 µm after the homogenized mycelia were frozen in liquid nitrogen. These data are summarized in Table 1.

Oomycetes. The difference spectra obtained from Aphanomyces at low temperature are shown in Fig. 4, and the cytochromes are listed in Table 2. There are two b-type cytochromes, one with an absorption maximum at 557 µm and a second with an absorption maximum at 564 µm, cytochrome c (551 µm), and cytochrome a-a₃ (605 µm) in this fungus at room temperature. The reduced-minus-oxidized difference spectrum of Pythium revealed the same cytochrome system as that in Aphanomyces.

**DISCUSSION**

The wavelengths of absorption maxima of the cytochromes found in Aphanomyces and in Pythium at room temperature were identical to those found in other Oomycetes (Saprolegnia, Apodachlya, Leptomitus, Sapromyces, and Mindellia) by Gleason and Unestam (4) and Unestam and Gleason (10). Thus, all of the Oomycetes from the three orders studied have two b-type cytochromes, a c-type cytochrome, and cytochrome a-a₃. The Chytridiomycetes, on the other hand, probably have three b-type cytochromes and two c-type cytochromes, one of which may be c₁. The Monoblepharidales (Monoblepharis and Monoblepharella) differ from the Blastocladiales (Allomyces and Blastocladiella) in that they have a-type cytochromes which absorb at 609 µm instead of at 606 µm at room temperature. The b-type cytochrome which has an absorption maximum at 557 µm is found in the Oomycetes, but not in the Chytridiomycetes. An absorption maximum at 564 µm, due to b-type cytochrome, was found in both groups of fungi.

The colorless algae in the Chlorophyta possess two b-type cytochromes and one c-type cytochrome as far as is known (D. A. Webster, Ph.D.

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**FIG. 3.** The b-type cytochromes of Blastocladiella, Monoblepharella, and Allomyces: (reduced plus succinate plus antimycin A plus oxygen) minus (oxidized) difference spectra at low temperature.

**TABLE 1.** Cytochrome systems of Chytridiomycetes*

<table>
<thead>
<tr>
<th>Order</th>
<th>Temp</th>
<th>a-type cytochrome</th>
<th>b-type cytochrome</th>
<th>c-type cytochrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastocladiæs</td>
<td>Low</td>
<td>603</td>
<td>563</td>
<td>552, 548</td>
</tr>
<tr>
<td></td>
<td>Room</td>
<td>606</td>
<td>564</td>
<td>553</td>
</tr>
<tr>
<td>Monoblepharidæs</td>
<td>Low</td>
<td>608</td>
<td>563</td>
<td>552, 548</td>
</tr>
<tr>
<td></td>
<td>Room</td>
<td>609</td>
<td>564</td>
<td>553</td>
</tr>
</tbody>
</table>

*Absorption maxima detected in reduced-minus-oxidized difference spectra at room temperature and at low temperature in the visible (α) region.
Fig. 4. Cytochrome system of Aphanomyces astaci. Difference spectra at low temperature: A. (reduced by succinate) minus (oxidized); B. (reduced by succinate plus antimycin A plus oxygen) minus (oxidized); C. (reduced by succinate) minus (reduced by succinate plus antimycin A plus oxygen).

Table 2. Cytochrome system of Oomycetes

<table>
<thead>
<tr>
<th>Temp</th>
<th>a-type cytochrome</th>
<th>b-type cytochrome</th>
<th>c-type cytochrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
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<td>Room</td>
<td>605</td>
<td>564</td>
<td>557</td>
</tr>
</tbody>
</table>

*Absorption maxima detected in reduced-minus-oxidized difference spectra at room and at low temperature in the visible (α) region.

The Oomycetes and the Chytridiomycetes also differ in the pathway of lysine synthesis (11) and in the patterns of association of enzymes for tryptophan synthesis (6). The cytochrome studies provide further evidence that the Oomycetes and the Chytridiomycetes are not closely related.

Acknowledgments

The authors are deeply indebted to Allan C. Wilson, Michael Doudoroff, and Ralph Emerson for providing the equipment and cultures used in this investigation and for criticism of this manuscript.

This investigation was supported by Public Health Service fellowship 1-F2-GM-20,026-01 and by a grant from the Swedish Natural Science Research Council, 1156-1, A3.

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