Phototaxis in the Marine Fungus *Rhizophydium littoreum*

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Phototaxis appears to be a factor that influences the dispersal of zoospores of the marine fungus *Rhizophydium littoreum*. By using a quantitative method to study phototaxis, zoospores were found to be positively phototactic toward blue wavelengths of light. White light in the range of 20 to 6,000 microeinsteins m\(^{-2}\) s\(^{-1}\) and blue light in the range of 1 to 300 microeinsteins m\(^{-2}\) s\(^{-1}\) gave positive responses in the laboratory. Results of field studies confirmed the ability of zoospores to respond to light under natural conditions. Phototaxis may be an important adaptive mechanism which influences vertical migration of zoospores in the photic zone where plant products are available as nutrients.

The relationship of wavelengths and intensities of light in the marine environment is important in studies of phototaxis (20, 23). Various wavelengths of light are absorbed differentially by clear seawater, with red light being absorbed first and blue light penetrating to the greatest depths. In the more turbid coastal waters, yellow substances in the water absorb mostly UV and blue wavelengths, shifting the wavelength of primary transmittance from blue to blue-green (9, 10).

Many organisms, both unicellular and multicellular, exhibit phototaxis. Phototaxis is an orientation of and movement of the entire organism with reference to light (7, 19, 20) and may imply the ability to differentiate among light stimuli of differing intensities and wavelengths (25). Phototaxis may include movement either toward or away from a particular light source, depending on the nature of the light. Positive phototaxis has been observed in some aquatic fungi (3, 5, 11, 22). Action spectra of the photoresponses in a variety of organisms, including fungi, consistently have pronounced peaks in the range of 435 to 475 nm (8, 18).

Phototaxis may be important to organisms in avoiding unsuitable light conditions and guiding them into zones of favorable conditions for survival (7). The photoreponse may aid microorganisms in seeking food sources and available mates (25). Carlile (4) suggests that photoresponses in some fungi are particularly significant in relation to spor dispersal. Blue light receptors may be advantageous to organisms in open ocean marine ecosystems because the blue wavelengths of light have the ability to penetrate to the greatest depths (10, 21). In this study we examine the effects of light intensity and quality on the migration of *Rhizophydiurn littoreum*, a marine chytrid that has been described as having a photoreceptive organelle.

MATERIALS AND METHODS

The marine fungus *Rhizophydiurn littoreum* (1) used in this study was derived from the original *Phlyctochyrium* sp. isolate 71-1-E (ATCC 36100) described by Kazama (13). *Rhizophydiurn littoreum* was maintained, as described by Amon and Arthur (2), on 1 g of yeast extract–1 g of peptone–10 g of glucose–12 g of Bacto-Agar (Difco Laboratories, Detroit, Mich.) (YPD) in 1 liter of Instant Ocean (Aquarium Systems, Mentor, Ohio) seawater at 20% salinity. Zoospores were spread on YPD plates and incubated at 24°C under continuous fluorescent light at an intensity of 50 microeinsteins m\(^{-2}\) s\(^{-1}\). The suspension of zoospores used in the phototaxis experiments was obtained by flooding 46-h-old (± 2 h) zoospore-producing plates with sterile seawater. The zoospore concentration was adjusted to 10\(^7\)/ml, and motility was checked microscopically. All experiments were done with zoospore suspensions that had greater than 75% motility.

A study was also done to compare the blue light phototactic response of *Rhizophydiurn littoreum*. Amon (1) and another isolate of *Rhizophydiurn littoreum* designated P.C. (1) which was isolated from the green alga *Codium* sp. *Rhizophydiurn littoreum* was maintained in the same manner as *Rhizophydiurn littoreum*. Except that the medium contained only 3 g of glucose per liter, and zoospores were harvested at 72 h. Laboratory phototaxis experiments were similar to that described by Kazama (13), except that a heat barrier was found to be unnecessary. Experiments were carried out at room temperature in the dark with a carousel slide projector (125 V, 300-W lamp; Eastman Kodak Co., Rochester, N.Y.) as the light source. Light intensity was measured with a quantum meter (model LI-185A; Li-Cor). Light was projected to a front surface mirror and reflected to a piece of black cardboard with an X pattern (2 by 10 mm) cut out to allow the passage of light. A petri dish (diameter, 60 mm) containing a 6.5-ml zoospore suspension in seawater was placed on top of the cardboard pattern (16).

The wavelengths of light were varied by using either broad-spectrum filters (Roscolux; Rosco, Port Chester, N.Y.) or narrow-spectrum (10-nm bandwidth) interference filters (Pomfret Research Optics, Stamford, Conn.) placed in the light path. The broad-spectrum filters had wavelengths ranging from 600 to over 740 nm; and the narrow-band filters had maximum transmissions at 400, 420, 440, 460, 480, 500, 520, 540, 550, 577, 589, 600, and 620 nm. The rate at which zoospores responded was determined by measuring the time required for the appearance of a sharply defined X pattern of zoospores in the dish (16). Exposure times of 15 to 600 s were used. A negative response resulted in an accumulation of zoospores outside of the X pattern formed by light.

Inhibition of phototaxis by chemoattractants was tested by carrying out the phototaxis assay in dishes as described above, except that they contained approximately 0.1 to 0.001 M concentrations of the various chemicals.

In field studies a method that was different from the one described above was used. Clear tubes of acrylic plastic (2.5
cm in diameter by 6.5 cm in length) were painted black over half their length and fitted with rubber septa in each half. Vent ports allowed sampling without creating internal negative pressure. Duplicate tubes were completely filled with zoospore suspensions (2.5 × 10⁶ ml⁻¹) and attached to a piling at 2, 4, and 6 m below the mean sea level by scuba divers. The tubes were leveled and oriented with the septa facing down. By using 1-ml syringes and 2.5-cm 23G needles, divers withdrew 0.3-ml samples from both the light and the dark sides after 20 min of exposure to ambient light levels. Zoospore numbers in the light and dark sides were compared by using a counting cell (Petroff-Hauser), and field light measurements were made with a submersible quantum sensor (Li-Cor). These field studies were accomplished at the Pivers Island Pier, Duke University Marine Laboratory, Beaufort, N.C.

Preliminary experiments carried out under laboratory conditions were used to prove the design of the plastic vessels. Counts of zoospores always accurately reflected the number of zoospores placed in the tubes.

RESULTS

*R. littoreum* was strongly attracted to unfiltered white light at intensities of 20 to over 6,000 microeinsinns m⁻² s⁻¹. At low intensities of light the response time was proportional to intensity (Fig. 1). At low intensities only blue light stimulated phototaxis, and the most rapid response was at a wavelength of 400 nm (Table 1). When broad-spectrum blue filters (450 ± 30 nm) were used, zoospores responded to intensities of over 300 microeinsinns m⁻² s⁻¹. No response was observed at wavelengths of 500 nm or greater (up to 700 nm), unless the intensity of light was increased, and then no response was seen at wavelengths greater than 600 nm. The pattern of the response indicated an action spectrum with a major peak at 400 nm (or less) and a minor peak at 440 to 460 nm (Table 1). Known chemoattractants (17) do not block the response to blue or white light, but some attractants such as glucose and maltose stimulate the cells to a quicker response.

In nature zoospores responded well to ambient light at intensities as low as 2 microeinsinns m⁻² s⁻¹ in 6 m of turbid water (Table 2). Zoospores that were held for more than a few minutes at approximately 0 to 10 cm under the surface in bright (c.a. 12,000 microeinsinns m⁻² s⁻¹) sunlight died.

In one study two other marine chytrids were compared with *R. littoreum*. *R. littoreum* P. C. exhibited a negative response to blue (340 to 500 nm, peak 400 nm, broad-band filter) light at an intensity of 6 microeinsinns m⁻² s⁻¹, while *R. littoreum* 71-1-E was strongly attracted and *R. aestuarii* was weakly attracted to the same wavelength and intensity.

**DISCUSSION**

*R. littoreum* 71-1-E has a strong positive response to blue light, so it is reasonable to conclude that this species has a specific blue light receptor (6, 18, 23). Kazama and Schornstein (14) have studied the presumptive photoreceptor organelle and have demonstrated the membrane particles which respond to white light, but attempts in our laboratory to characterize extracted pigment have been unsuccessful. The action spectrum shows some similarity to flavins (18), but more research is needed to substantiate that conclusion.

In the laboratory the highest intensity of white light provided by the experimental apparatus stimulated a positive phototactic response. That level of light is comparable to that which we measured on a cloudy day at the summer solstice. Because direct sunlight in 1 cm of water appears to kill the zoospores, it must be concluded that the photoreponse is useful in directing the motile cells toward the photic zone but not to the surface.

Results of our studies show that very low intensities of light also stimulate a positive response. Wavelengths which penetrate best in oceanic waters evoke a positive response at intensities lower than 2 microeinsinns m⁻² s⁻¹. If 15,000 microeinsinns m⁻² s⁻¹ is used as a surface sunlight value and 10% extinction per m is assumed in clear ocean water (24), zoospores could respond to light at depths of greater than 70 m, much beyond the zone of abundant plant life. In turbid coastal areas a 40% extinction is expected (24), providing the possibility of phototactic responses at a depth of up to 13 m. The results of our studies done in turbid water

**TABLE 1. Time required for phototactic response**

<table>
<thead>
<tr>
<th>Intensity (microeinsinns m⁻² s⁻¹)</th>
<th>400</th>
<th>420</th>
<th>440</th>
<th>460</th>
<th>480</th>
<th>500</th>
<th>520</th>
<th>540</th>
<th>550</th>
<th>577</th>
<th>589</th>
<th>600</th>
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<td>A</td>
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<tr>
<td>1.5</td>
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<td>60</td>
<td>60</td>
<td>30</td>
<td>30</td>
<td>60</td>
<td>90</td>
<td>B</td>
<td>B</td>
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<tr>
<td>3.5</td>
<td>C</td>
<td>30</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>180</td>
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<td>B</td>
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<td>15</td>
<td>15</td>
<td>15</td>
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<td>B</td>
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</table>

Abbreviations: A, only weak responses (illumination for 3 to 10 min did not improve response); B, no response at any time tested; C, data unavailable illuminating system unable to produce this intensity and wavelength. All data are based on five replicates. By using 15-s sampling intervals, all recorded response times were the same in each replicate.
TABLE 2. Photoresponses in nature by using a submerged sampling device

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Light intensity (microwatts m⁻² s⁻¹)</th>
<th>No. of zoospores ± SD²</th>
<th>Initial Light side</th>
<th>Dark side</th>
<th>After 20 min on: Light side</th>
<th>Dark side</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>100</td>
<td>48 ± 2.0</td>
<td>61 ± 11.0</td>
<td>34 ± 5.8</td>
<td>38 ± 3.3</td>
<td>38 ± 3.3</td>
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<tr>
<td>4</td>
<td>21</td>
<td>50 ± 2.5</td>
<td>59 ± 5.8</td>
<td>39 ± 3.4</td>
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<td></td>
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<tr>
<td>6</td>
<td>2</td>
<td>45 ± 2.0</td>
<td>55 ± 4.5</td>
<td>38 ± 3.3</td>
<td></td>
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</tr>
</tbody>
</table>

² The light meter measured only in the 400- to 700-nm range. This experiment was carried out at noon.

Initial value was for both light and dark sides.

at the Duke University port area and marshland showed positive phototaxis at a 6- to 7-m depth in nature.

The ecological advantage of positive phototaxis to blue light may be multifold. In many estuaries, dissolved and small particulate nutrients are homogeneously suspended in the water column, so migration toward that type of nutrient is unlikely. Conversely, sessile algae are often rigorously stratified in the photic zone and are well known for zonation related to light quality (21). Migration toward light would bring motile propagules toward the zones where they could contact host algae such as *Bryopsis* spp. (12) and *Codium* spp. (1). In addition, phototactic responses toward blue light allow these fungi to find their way to the surface more effectively than a response to less penetrating, longer wavelengths. A photoresponse may also serve to prevent migration away from the photic zone or it may help to extricate the zoospore found in sediment. Phototaxis may even tend to prevent competition. If one strain responds positively and another negatively, as we have shown, they might settle on the top and bottom of an algal frond, respectively. Once in the immediate vicinity of potential food sources, phototaxis may cease to become an important factor. Although our results indicate that photoresponses appear to dominate over chemotropes, contact or close proximity to foods with a highly chemotactic exudate may, in fact, control behavior during the final approach of the zoospores.

The data suggest that this fungus, like many other motile aquatic microbes (18), has a blue light receptor pigment. Such a conclusion is also supported by the yellow pigmentation of the zoospore mass in reflected light (complementary colors). All three strains tested had both phototropes and yellow pigments, but it is curious that *R. aestuarii*, with the most intense pigmentation, had the weakest response. If the rumposome is a necessary part of the light-gathering system (14), the observation (15) that this complex is reduced in *R. aestuarii* takes on added importance. We could then suggest that the structure of the light-gathering organelle is at least as important as the pigment which may also be involved.

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LITERATURE CITED