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ENVIRONMENTAL CONDITIONS FAVORING GAMETOPHYTE DEVELOPMENT FROM THE CHANTRANSIA STAGE OF BATRACHOSPERMUM (RHODOPHYTA)

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ABSTRACT

(1) A red alga resembling *Audouinella* was introduced into culture after collection from a spring-fed stream. (2) Variation in temperature, light intensity and light-dark cycles resulted in the production of juvenile gametophytes from the *Audouinella*-like filaments. (3) Based on the appearance of the gametophytes, *Audouinella*-like filaments were determined to be a chantransia stage of *Batrachospermum*. (4) Analysis of data from culture experiments indicated that conditions in the spring-fed stream were unsuitable for development of stages other than the chantransia stage. (5) Juvenile gametophyte development was influenced by a low nitrate and high phosphate concentration in combination with a temperature of 11 C, a light intensity of either 46.4 or 92.9 $\mu E \cdot m^{-2} \cdot s^{-1}$ and a light-dark regime of 8:$\overline{16}$. (6) At 15 C, a temperature higher than that recorded in the stream, gametophyte development occurred at a light intensity of 46.4 or 92.9 $\mu E \cdot m^{-2} \cdot s^{-1}$ under all light-dark regimes used (8:$\overline{16}$, 12:$\overline{12}$, 16:$\overline{8}$). (7) It is suggested that maturation of gametophytes was not observed because of an apparent lack of free carbon dioxide in the stationary cultures. (8) Hair formation on gametophyte pleuridia occurred only in cultures kept in the same medium for 85 days at 11 C under a light intensity of 46.4 $\mu E \cdot m^{-2} \cdot s^{-1}$ with a light-dark cycle of 8:$\overline{16}$. (9) Production of swollen tips on chantransia fila-

ments (club filaments) was controlled by complex interactions involving temperature, light intensity and light-dark photoregimes. (10) These club filaments were most abundant in flasks kept at 15 C and exposed to 16:$\overline{8}$ under any one of three light intensities (46.4, 92.9 or 185. 8 $\mu E \cdot m^{-2} \cdot s^{-1}$) used, or those at 4, 8, 11 or 15 C under 46.4 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 8:$\overline{16}$.

INTRODUCTION

Collection of material from a fast-flowing stream in West-Central Illinois revealed a red alga that morphologically resembled *Audouinella violacea* (Kütz.) Hamel. This alga remained constant in its appearance on all sampling dates during a two-year period and has maintained this form in collections made over the last 13 years. Identification of some species of freshwater red algae, including *Batrachospermum*, is complicated by the presence of "chantransia" stages in their life histories. These stages resemble species of the *Audouinella (Rhodochorton-Acrochaetium)* complex (Fritsch, 1959; Prescott, 1978; Woelkerling, 1983). The objectives of this study were to introduce this alga into culture and examine its life history.

MATERIALS AND METHODS

The alga was collected from a spring-fed stream located in Wild Cat Springs Park, Hamilton, Illinois. It grew attached to rocks, sticks and roots in water flowing from a north-facing limestone cliff. An oak-hickory community formed a dense canopy over the stream during most of the year. Material was collected and transported in stream water to Western Illinois University at Macomb. Water temperature and light intensity were measured at the same site on each sampling trip to the stream. Light intensity was recorded in microEinsteins$\cdot m^{-2} \cdot s^{-1}$ ($\mu E \cdot m^{-2} \cdot s^{-1}$) with a quantum sensor (LI-190S, Lambda Instruments) and light meter (LI-170, Lambda), and temperature with a mercury thermometer. Chemical analyses of stream water were done on samples taken at one-week intervals from 20 June to 25 July 1979 using standard methods (APHA, 1976) as modified by the Hach Company.

Of the many culture media tested, best growth and condition of filaments were obtained in an alteration (Torres and O'Flaherty, 1976) (Table 1) of Bold's modified Bristol's medium (Starr, 1978). This altered Torres-O'Flaherty medium, and modifications of it, contained 10 mg of GeO_2/L to inhibit diatom growth. All experiments were conducted using 20 mL of autoclaved medium contained in 50-mL erlenmeyer flasks that had been washed in detergent, rinsed seven times with tap water, once with 3N HCl and then seven times with glass distilled water. Flasks were labeled and placed in an environmental chamber (I-36L, Percival) and illuminated with fluorescent lighting (20-watt, cool white, Westinghouse).

In one set of experiments, temperatures of 4, 8, 11 and 15 C were combined with light intensities of 46.4, 92.9 and 185.8 $\mu E \cdot m^{-2} \cdot s^{-1}$ and light-dark photoregimes of 8:$\overline{16}$, 12:$\overline{12}$, 16:$\overline{8}$ and 24:$\overline{0}$. A second series repeated a portion of these experiments with temperatures of 11 and 15 C, light intensities of 46.4 and 92.9 $\mu E \cdot m^{-2} \cdot s^{-1}$ and light-dark cycles of 8:$\overline{16}$, 12:$\overline{12}$ and and 16:$\overline{8}$. Observations of the alga from each flask were made 30 and 40 days after inoculation. Some flasks

were returned to the environmental chamber and algal material examined again 85 and 130 days after inoculation. A final set of experiments combined three concentrations (1.1×10^{-2}, 1.1×10^{-1} and 1.1 mg/L) of nitrate (as $NaNO_3$) with three concentrations (6.0×10^{-3}, 6.0×10^{-2} and 6.0×10^{-1} mg/L) of phosphate as K_2HPO_4, two light intensities (46.4 and 92.9 $\mu E \cdot m^{-2} \cdot s^{-1}$) and three light-dark cycles (8:16, 12:12 and 16:8).

Unsuccessful attempts were made to obtain a pure culture by placing tips from *Audouinella*-like filaments in media containing streptomycin (25 mg/L) or actidione (cycloheximide) (50 mg/L) or both of these antibiotics (Stein, 1979). As a result of these unsuccessful efforts, tips, two to three cells in length and free of debris, were cut from filaments with a sterilized, stainless steel scalpel and placed in test medium at 11 C under a light intensity of 92.9 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 24 hours. One mL of a test medium was then withdrawn and used as an inoculum for each flask. Dark periods were initiated by covering each flask with a 250-mL beaker painted with two coats of black enamel. Combinations of factors were each replicated three times for each experiment.

Samples were prepared for scanning electron microscopy (SEM) by fixation in 2% glutaraldehyde in 0.2 M sodium cacodylate buffer. Dehydration was done with a graded ethanol series. Specimens were critical-point dried, mounted on stubs with double-stick tape and sputter-coated with 20 nM of gold-palladium. Filaments were examined with a Jeol JMS-25s II SEM.

RESULTS AND DISCUSSION

Audouinella-like filaments grew from filament tips used as inocula in all experiments. These filaments formed monosporangia and each cell had a spiral, ribbon-like plastid (Fig. 1) typical for *A. violacea* (Smith, 1950). In SEM photomicrographs, cells exhibited striations in their walls (Fig. 2) reported by Garbary (1978) as distinctive for species of *Audouinella*.

Juvenile gametophytes (Fig. 3) arose from *Audouinella*-like filaments cultured under a number of combinations of conditions (Table 2). These gametophytes were structurally those of a species of *Batrachospermum* (Fritsch, 1959; Yoshida, 1959). The *Audouinella*-like filaments were the chantransia stage of *Batrachospermum*. Each gametophyte produced rhizoid-like branches from a basal, axial cell above the point of attachment to the chantransia (Fig. 3). Bead-like pleuridia (Aghajanian and Hommersand, 1980) branched from the main central axis of each gametophyte (Fig. 3). Steps in gametophyte development were not observed, but based on the appearance of the juveniles, they are assumed to be those described by von Stosch and Theil (1979).

Gametophytes were most abundant in flasks at light intensities of 46.4 and 92.9 $\mu E \cdot m^{-2} \cdot s^{-1}$ kept at 15 C under all light-dark photoregimes (Table 2). They did not develop in cultures grown at 4 or 8 C, or in those under a light intensity of 185.8 $\mu E \cdot m^{-2} \cdot s^{-1}$. At 11 C, under 46.4 $\mu E \cdot m^{-2} \cdot s^{-1}$, they were abundant in a light-dark cycle of 8:16 and less abundant at 16:8 (Table 2). Gametophytes were produced at 11 C, under 8:16, 46.4 $\mu E \cdot m^{-2} \cdot s^{-1}$, a phosphate concentration of 6.0×10^{-2} mg/L and nitrate at either 1.1×10^{-2} or 1.1 mg/L or with phosphate at 6.0×10^{-3} mg/L and nitrate at 1.1×10^{-1} mg/L. At an intensity of 92.9 $\mu E \cdot m^{-2} \cdot s^{-1}$ and 11 C, gametophytes formed in a nitrate concentration of 1.1

mg/L with phosphate at 6.0×10^{-3} mg/L and a light-dark regime of 16:$\overline{8}$, or in a phosphate concentration of 6.0×10^{-2} mg/L with 12:$\overline{12}$. Gametophytes were not as numerous under conditions involving nitrate and phosphate variations as they were in the medium containing the original concentrations of these two factors (1.1×10^{-2} mg nitrate/L; 40.9 mg phosphate/L). Nitrate concentrations were not less than 11.0 mg/L and phosphate was not more than 8.0×10^{-1} mg/L in samples from the stream (Table 3). A temperature of 15 C was not observed in the stream water (Table 4). It is unlikely that conditions in the stream remained constant long enough to allow development of gametophytes (Tables 3 and 4). As Minckley and Tindall (1963) noted, *Batrachospermum* has complex relationships with its environment and no single factor can be discussed without assessing the changes and constancies of other factors.

Maturation of gametophytes was not observed in cultures maintained for up to 130 days after inoculation. Since we had a stationary culture situation, a lack of free carbon dioxide probably inhibited this maturation (Rider and Wagner, 1972; Raven and Beardall, 1981).

Hair formation at the ends of pleuridia (Fig. 4) was seen on gametophytes maintained for 85 days at 11 C, under a light intensity of 46.4 $\mu E \bullet m^{-2} \bullet s^{-1}$ and with 8:$\overline{16}$. This formation occurred under a lower light intensity but higher temperature than conditions reported for other red algae. Stimulation of hair formation occurred at high light intensities in low nitrogen concentrations in *Ceramium* (DeBoer and Whoriskey, 1981) and *Acrochaetium* (West, 1971), and at high light intensities and low temperatures (2 and 8 C) in *Chromastrum* (Stegenga and Mulder, 1979). West (1971) indicated that hairs might be sites of nutrient uptake which was supported by studies done on *A. hermannii* (Roth) Duby (Hymes and Cole, 1983) and other red algae (DeBoer and Whoriskey, 1981).

Chantransia filaments grown under various conditions produced enlarged tips (>12.5 μM in width) that resembled clubs (Fig. 5). Gametophytes and these club filaments were not seen in the same flasks but were often in different flasks subjected to the same set of conditions. At 15 C, club filaments were produced at all light intensities and under all light-dark regimes except 185.8 $\mu E \bullet m^{-2} \bullet s^{-1}$ and 24:$\overline{0}$ (Table 5). They were most abundant in flasks kept at 15 C and exposed to 16:$\overline{8}$ under any one of the three light intensities, or those at 4, 8, 11 or 15 C under 46.4 $\mu E \bullet m^{-2} \bullet s^{-1}$ and 8:$\overline{16}$. At 8 C, a light-dark cycle of 16:$\overline{8}$ resulted in an abundant production of these filaments under all light intensities while one of 8:$\overline{16}$ produced them in abundance under 46.4 or 185.8 $\mu E \bullet m^{-2} \bullet s^{-1}$. Under 185.8 $\mu E \bullet m^{-2} \bullet s^{-1}$ and 8 C, these filaments were produced under all light-dark cycles and were in abundance in all but 24:$\overline{0}$. At 11 C, club filaments were abundant only under the two lower light intensities in combination with 8:$\overline{16}$ or 16:$\overline{8}$ (Table 5). Based on these results, the most common temperature observed in the stream (11 C) (Table 4) would allow club filament development only under the most restrictive range of light intensity and, in nature, photoperiod. Club filaments were not observed on material taken from the stream. Conditions favorable for their development would not exist long enough for their formation. In culture, formation required at least 30 days. Structures similar to club filaments were reported for *A. floridula* (Dillwn.) Woelkering (Knaggs, 1967) and Yoshida (1959) gave a vague reference to inflated cells present in *Batrachospermum*. Their function is not known but it

is possible that these filaments were some developmental stage in the life history of *Batrachospermum*. They may have been filaments that were inhibited from forming gametophytes. A swelling at the tips of branches that eventually produced gametophytes was reported by von Stosch and Theil (1979).

After the growth periods necessary for the development of this alga, some contaminants were found. These contaminants were species of *Chlamydomonas*, *Chlorella*, *Chlorotylium*, rarely *Cladophora* (Chlorophyta), *Schizothrix* (Cyanophyta) and *Trachelomonas* (Euglenophyta).

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Table 1. Concentrations of components of a modification of Bristol's medium used in culturing *Batrachospermum*.

Component[1]	Concentration (mg/L)
$NaNO_2$	1.5×10^{-2}
$CaCl_3 \cdot 2H_2O$	25.0
$MgSO_4 \cdot 7H_2O$	75.0
K_2HPO_4	75.0
NaCl	25.0
$FeCl_3 \cdot 6H_2O$	5.4×10^{-1}
H_3BO_3	6.2×10^{-1}
$MnCl_2 \cdot 4H_2O$	1.4
$ZnCl_2$	1.0×10^{-1}
$CoCl_2 \cdot 6H_2O$	5.0×10^{-3}
$CuCl_2 \cdot 2H_2O$	3.4×10^{-5}
Na_2EDTA	3.0
GeO_2	10.0

[1]Forty-eight hours after autoclaving, this medium had a pH of 7.1.

Table 2. Gametophyte production in various combinations of environmental conditions.

Light Intensity ($\mu E \cdot m^{-2} \cdot s^{-1}$)	Temperature (C)	Light-Dark Photoregimes 8:16	12:12	16:8
46.4	11	+ +	None	+
	15	+ +	+ +	+ +
92.9	11	+ +	None	None
	15	+ +	+ +	+ +

(+)indicates that gametophytes were present in this combination but in low numbers.
(+ +)indicates that gametophytes were abundant in this combination.

Table 3. Results of chemical analyses done on stream water samples obtained between 20 June and 25 July 1979.

Factor Analysed	20 June	27 June	4 July	25 July
Alkalinity (ppm $CaCO_3$)	300.0	285.0	270.0	235.0
Carbon Dioxide (ppm)	188.0	102.0	104.0	118.0
Chromium (ppm as Cr^{6+})	2.5×10^{-2}	2.5×10^{-2}	2.5×10^{-2}	4.0×10^{-2}
Copper (ppm)	0	0	2.5×10^{-2}	4.0×10^{-2}
Dissolved Oxygen (ppm)	11.0	11.0	11.0	11.0
Hardness-Total (ppm $CaCo_3$)	280.0	320.0	290.0	260.0
Iron (ppm)	2.0×10^{-2}	2.0×10^{-2}	3.5×10^{-2}	3.5×10^{-2}
Manganese (ppm)	1.5×10^{-1}	2.0×10^{-1}	1.2×10^{-1}	2.0×10^{-1}
pH	6.8	6.8	6.8	7.4
Phosphate-Total (ppm)	8.0×10^{-1}	1.6×10^{-1}	3.5×10^{-1}	4.8×10^{-1}
Nitrate Nitrogen (ppm)	87.5	11.0	10.5	11.75
Silica (ppm)	19.1	25.6	21.9	23.8
Turbidity (JTU)	0	0	7.5	5.0

Table 4. Water temperatures and light intensities at the water surface of the stream during 1979, 1980 and 1981.

Date	Time of Day (CST)	Cloud Conditions	Temperature (C)	Light Intensity ($\mu E \cdot m^{-2} \cdot s^{-1}$)
1979				
16 May	0939	Full Sun	11.0	43.9
20 June	1425	Full Sun	11.0	29.9
27 June	1430	Cloudy	11.0	20.0
4 July	1045	Cloudy	11.0	9.9
25 July	1540	Partly Cloudy	11.5	59.9
1980				
21 April	1210	Full Sun	10.5	190.0 (899.9)[1]
14 May	1308	Full Sun	10.5	46.0 (259.9)
24 July	1045	Full Sun	12.0	17.9
13 September	1130	Full Sun	14.0	43.9
30 December	1115	Cloudy	13.0	160.0
1981				
19 February	1043	Partly Cloudy	11.0	210.0
12 April	1210	Cloudy	11.0	191.3

[1]Numbers in parentheses are values obtained when the canopy allowed full sun to reach the water surface. Other values were obtained when the water surface was in full shade.

Table 5. Club filament production in various combinations of environmental conditions.

Light Intensity ($\mu E \cdot m^{-2} \cdot s^{-1}$)	Temperature (C)	Light-Dark Photoregimes 8:$\overline{16}$	12:$\overline{12}$	16:$\overline{8}$	24:$\overline{0}$
46.4	4	+ +	+	+ +	+
	8	+ +	+	+ +	None
	11	+ +	+	+ +	None
	15	+ +	+ +	+ +	+
92.9	4	+ +	+ +	+ +	+
	8	None	None	+ +	None
	11	+ +	None	+ +	None
	15	+	+	+ +	+
185.8	4	+ +	None	None	None
	8	+ +	+ +	+ +	+
	11	+	None	None	+
	15	+	+	+ +	None

(+)indicates the club filaments were present in this combination but in low numbers.
(+ +)indicates the club filaments were abundant in this combination.

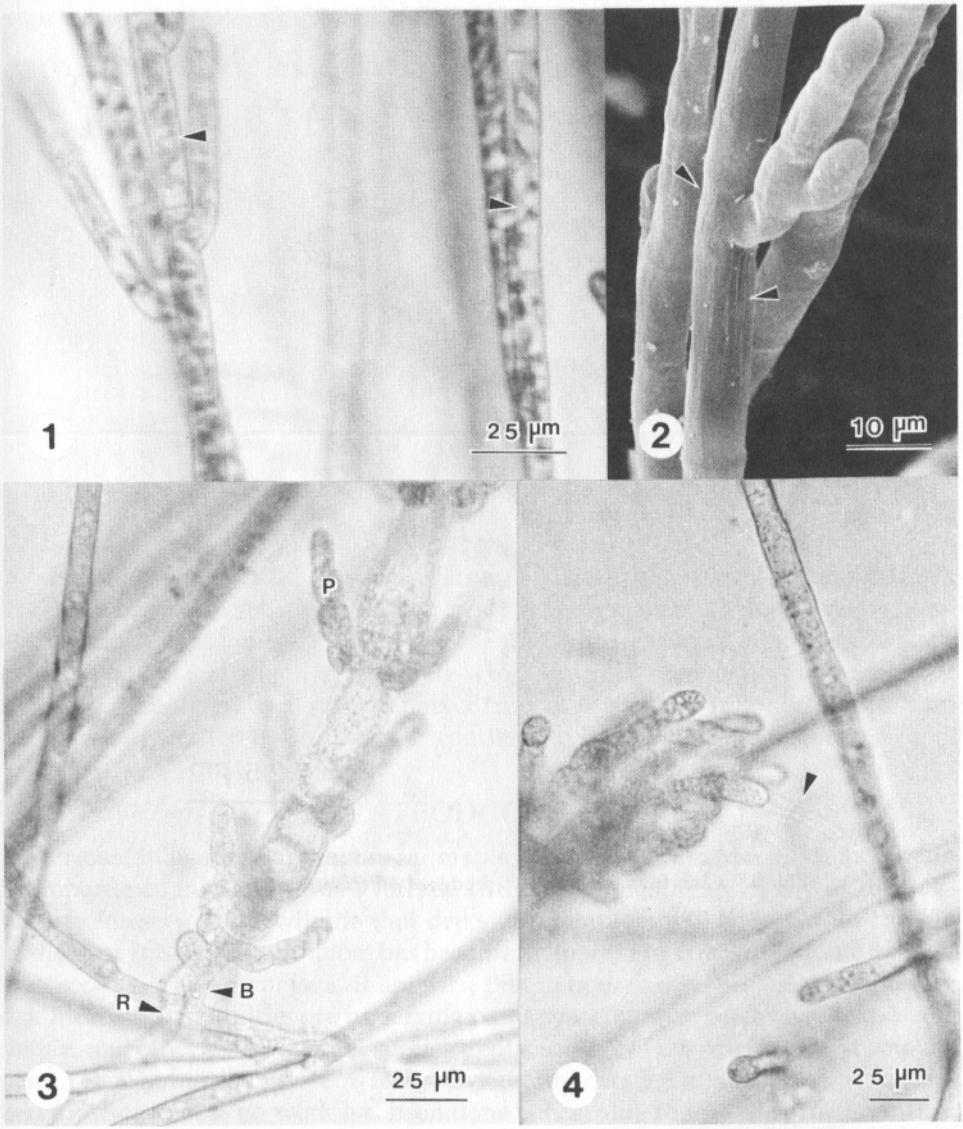

Fig. 1. Chantransia of *Batrachospermum* sp. with cells containing spiral, ribbon-like plastids (arrows).

Fig. 2. Scanning electron micrograph showing longitudinal striations (arrows) on the surface of vegetative cells of the chantransia.

Fig. 3. Gametophyte attachment to the chantransia. Note pleuridia (P), basal attachment cell (B) and rhizoid-like branch (R).

Fig. 4. Hair (arrow) formed at end of a pleuridium.

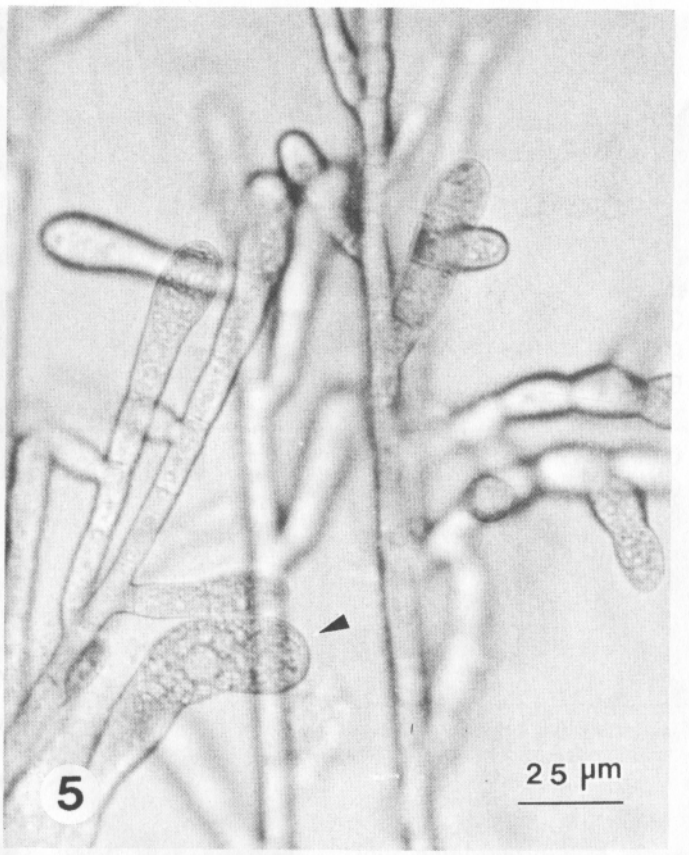

Fig. 5. Club filaments (arrow) produced on chantransia.