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Blue light induces arsenate uptake in the protist *Thraustochytrium*

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The effects of light on arsenic accumulation of *Thraustochytrium* CHN-1 were investigated. *Thraustochytrium* CHN-1, when exposed to blue light from light-emitting diodes (LEDs), accumulated arsenate added to its growth medium to a much greater extent than *Thraustochytrium* cells exposed to fluorescent or red light, or when cultured in the dark. Arsenic compounds in *Thraustochytrium* CHN-1 were analyzed by high-performance liquid chromatography, with an inductively coupled plasma mass spectrometer serving as an arsenic-specific detector. Arsenate, arsenite, monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA) and arsenosugar were identified. The order of arsenic species in *Thraustochytrium* CHN-1 was arsenic(V)> arsenic(III)> MMAA > DMAA at an arsenic concentration of 10 mg dm^{-3} in the medium in blue LED light. As it is known that blue light induces the synthesis of certain metabolites in plants and microorganisms, this indicates that the accumulation of arsenic is an active metabolic process. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: arsenic; light-emitting diode; effect; accumulation; methylation; *Thraustochytrium*; labyrinthulids

INTRODUCTION

The protist *Thraustochytrium* sp. CHN-1 (labyrinthulids) is native to a large range of freshwater and marine environments, and can be cultured in large quantities with relative ease. Labyrinthulids are curious organisms; they have been isolated from a wide variety of marine and freshwater habitats, and are found attached to algae, to vascular plants, and to detrital materials.[1,2] Recently, we isolated a new pigment-containing strain of *Thraustochytrium* sp. CHN-1 from coastal sea water from the Seto Inland Sea (Japan) that contained high levels of docosahexaenoic acid (C22 : 6, DHA).[3] Marine organisms accumulate arsenic to high levels compared with terrestrial organisms.[4] Recently, we reported on aspects of arsenic accumulation in *Thraustochytrium* sp. CHN-1, in particular on the chemical forms of arsenic that are accumulated and the arsenic tolerance of the cells.[5]

Light-emitting diodes (LEDs) have features that make them much better radiation sources than the commonly used fluorescent and metal halide sources. The most attractive features of LEDs are small mass, volume, solid-state construction and long life.[6] Because LEDs emit at specific wavelengths and in narrow bandwidths, they have recently been used for plant culture.[7] We have reported on the effects of blue and red light generated by LEDs on the growth and carotenoid production of *Thraustochytrium* CHN-1 cultured *in vitro*.[8] *Thraustochytrium* sp. CHN-1 responded to blue LED-generated light with the production of carotenoid pigments, including astaxanthin.[8]

This report describes the effects of different types of light on arsenic accumulation and the chemical forms of arsenic in cells of *Thraustochytrium* sp. CHN-1 from the Seto Inland Sea, Japan.

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MATERIALS AND METHODS

Thraustochytrium

Thraustochytrium sp. CHN-1 obtained from sea water of Nagahama in the Seto Inland Sea, Japan, was used throughout the experiments. *Thraustochytrium* sp. CHN-1 was cultured at 23 °C in 1 dm^3 Erlenmeyer flasks containing 250 cm^3 of a medium consisting of 2% glucose and 0.1% KNO_3 in sea water, under low-intensity fluorescent light (60 μmol m^{-2} s^{-1}

intensity) alternated diurnally with darkness, and with rotary shaking at 120 rpm. Cells were harvested at the end of the log-growth phase by centrifugation at 2000 rpm for 20 min. They were washed once with 100 cm^3 of cold reconstituted sea water, freeze-dried and stored at −20 °C prior to extraction.

Arsenic accumulation by *Thraustochytrium* sp. CHN-1

Thraustochytrium sp. CHN-1 cells (1 mg dry weight) were suspended in a 1 dm^3 Erlenmeyer flask containing 250 cm^3 of sea water containing 2% glucose, 0.1% KNO_3 and the experimental concentration of arsenic (10 mg dm^{-3}). Arsenic was added as Na_2HAsO_4. Figure 1 shows the spectral energy distributions of red, blue, and near-infrared LEDs. The arsenic accumulation experiments were carried out in light (60 μmol m^{-2} s^{-1} intensity) from a fluorescent source, from red LEDs (660 nm), and from blue LEDs (470 nm) in sterile air at 23 °C and pH 6.0 with rotary shaking at 120 rpm. At various times, from 4 to 15 days of culture, the arsenic concentrations of each of three randomly selected flasks were determined. After an appropriate time, the cells were collected by centrifugation at 2000 rpm, washed three times with deionized water, and freeze-dried. The freeze-dried cells were stored at −20 °C prior to extraction. Total cell dry weight was determined in each case by a gravimetric procedure.

Analysis of carotenoids in *Thraustochytrium* sp. CHN-1

Carotenoids were extracted from freeze-dried cells samples (50 mg) with acetone. The acetone extract was analyzed by high-performance liquid chromatography (HPLC; Shimazu HPLC LC-8A type, Japan) using a Wakosil 5C18 analytical column (ψ4.6 mm × 150 mm) with a detector wavelength of 450 nm. The carotenoids were eluted isocratically with a mixture of methanol (90%), water (8%), and acetonitrile (2%) at a flow rate of 3.0 ml min^{-1}. The carotenoids were identified by matching the HPLC retention time and the spectrum obtained by using a direct array detector with those of commercially available carotenoid standards. Quantification of each carotenoid was based on calibration curves prepared from the peak areas of the standards.

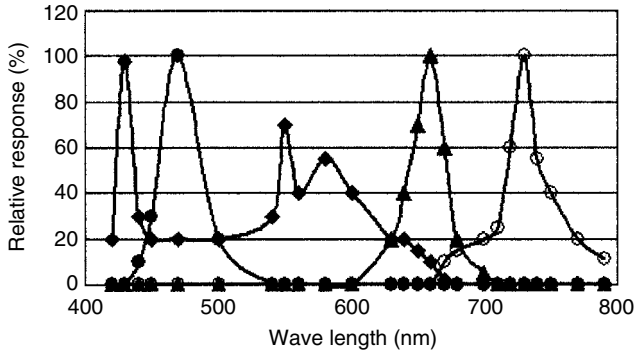

Figure 1. The spectral energy distribution of LEDs (red, blue and near infrared) and fluorescent light: ♦ Fluorescent light; ● blue LEDs; ▲ red LEDs; ○ Near-infrared LEDs.

Analysis of arsenic in *Thraustochytrium* sp. CHN-1[7]

The freeze-dried cells from the arsenic accumulation experiments were digested with a mixture of 3 cm^3 of concentrated nitric acid, 1 cm^3 of concentrated sulfuric acid and 1 cm^3 of 60% perchloric acid.[5] Arsenic was determined by a hydride-generation atomic absorption spectrometer system (Shimazu Model AA-6600G). Wavelength and lamp current for atomic absorption spectrometry were 193.7 nm and 10 mA respectively. All analyses were done in duplicate, and the data are reported as the mean.

Analysis of arsenic species in the *Thraustochytrium* CHN-1 cells[9]

A portion of each of the freeze-dried samples (50 to 100 mg dry weight) was weighed into a centrifuge tube. To each tube was added 5 dm^3 of methanol/water (1 : 1, v/v), and the tube was sonicated for 10 min. After centrifugation (2000 rpm for 10 min), the supernatant solution was removed by Pasteur pipette. The extraction process was repeated five times for each sample; the extracts were combined and evaporated to dryness and dissolved in 2 ml of water. Each solution was filtered through a 0.45 μm disposable filter unit, and an aliquot of the solution was injected into the HPLC–inductively coupled plasma (ICP-MS) system. HPLC–ICP-MS analysis was conducted using an Inertsil ODS column as reported previously.[9] Arsenic compounds were eluted with 10 mM tetraethylammonium hydroxide–4.5 mM malonic acid–0.05% methanol.

Quantification was performed by comparing the peak area of each compound with that of a known concentration of standard arsenic compounds. Interference from chloride ($^{40}Ar^{35}Cl^+$ has the same m/z as $^{75}As^+$) was detected by monitoring ion counts at m/z 77 simultaneously. The concentration of water-soluble arsenic accounted for all arsenic species revealed by HPLC. All analyses were done in duplicate, and the data are reported as the mean. The water-soluble arsenic compounds used as standards were prepared as reported previously.[9]

RESULTS AND DISCUSSION

Effects of arsenic on the growth of *Thraustochytrium* sp. CHN-1

The effect of arsenic on the growth of *Thraustochytrium* sp. CHN-1 was examined by using a medium 2% glucose and 0.1% KNO_3 in sea water containing arsenic as arsenic (V) for 13 days; the results are shown in Fig. 2. The resulting biomass of *Thraustochytrium* sp. CHN-1 obtained under the various light conditions was in the order blue LEDs > dark > red LEDs > fluorescent. The biomass of *Thraustochytrium* sp. CHN-1 decreased with the addition of arsenate. These

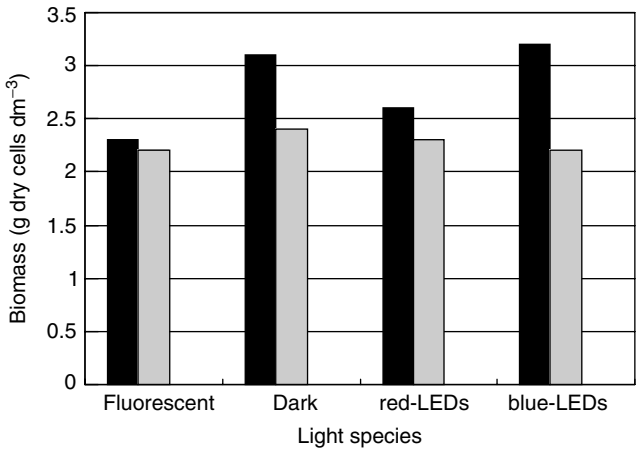

Figure 2. Effects of arsenate on the growth of *Thraustochytrium* sp. CHN-1 under light from various LEDs. Conditions: containing 10 mg dm^{-3} of arsenic(V) (as Na_2HAsO_4); glucose, 2%; KNO_3, 0.1%; in sea water; 23 °C, 60 μmol m^{-2} s^{-1} light intensity, 13 days. ■: Glucose 2%, KNO_3 0.1%; □: Glucose 2%, KNO_3 0.1%, arsenic(III).

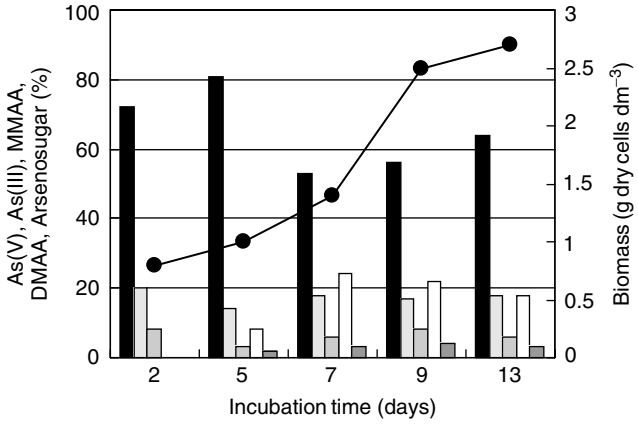

Figure 3. Time course of arsenic accumulation and growth of *Thraustochytrium* sp. CHN-1. Conditions: containing 10 mg dm^{-3} of arsenic(V) (as Na_2HAsO_4); glucose, 3%; KNO_3, 0.1%; in sea water; 23 °C, 60 μmol m^{-2} s^{-1} fluorescent light intensity, 13 days. ■: arsenic(V); □: arsenic; ▨: MMAA; □: DMAA; ▩: arsenosugar; ●: biomass.

results suggest that the growth of *Thraustochytrium* sp. CHN-1 was inhibited by the addition of arsenic in the medium. Maeda *et al*.[10] and Yamaoka *et al*.[11] have recognized similar tendencies for *Chlorella vulgaris* and *Dunaliella* sp. respectively.

Time course of arsenic accumulation and growth of *Thraustochytrium* sp. CHN-1

The major water-soluble arsenic species in *Thraustochytrium* sp. CHN-1 were identified as arsenate, arsenite, monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) by comparison with the standards. Yamaoka *et al*.[5] identified arsenic-containing ribofuransides (so-called arsenosugars) in *Thraustochytrium* sp. CHN-1. Arsenosugars were found to be the major arsenic species in macroalgae.[9] Figure 3 shows the biomass and the accumulation of arsenic species during the growth of *Thraustochytrium* sp. CHN-1. The biomass of *Thraustochytrium* sp. CHN-1 was approximately 0.8–2.5 g dm^{-3} of dry cells in the preliminary logarithmic growth phase (5–9 days), but increased to 2.5–2.8 g dm^{-3} of dry cells in the stationary growth phase (13 days). The arsenic concentration in *Thraustochytrium* sp. CHN-1 was 43 mg g^{-1} dry cells. The arsenic concentration in *Thraustochytrium* sp. CHN-1 was higher than the 10 mg g^{-1} dry weight in *Dunaliella* sp.[11] obtained from the arsenic experiments under the same concentration of arsenic (10 mg dm^{-3}). These results suggest that arsenic was accumulated to a high concentration by *Thraustochytrium* sp. CHN-1 within the logarithmic growth phase. Arsenic in the marine green algae *Dunaliella* sp. is accumulated as arsenic(V) within the logarithmic growth phase.[11] The arsenic species accumulated in *Thraustochytrium* sp. CHN-1 in the preliminary logarithmic growth phase (2 days) was composed of 72% arsenic(V), 20% arsenic(III), 8% MMAA, 0% DMAA and 0% arsenosugar, whereas the arsenic species in *Thraustochytrium* sp. CHN-1 in the stationary growth phase was composed of 64% arsenic(V), 18% arsenic(III), 6% MMAA, 18% DMAA and 3% arsenosugar. Arsenate has been widely found as a major arsenic compound in marine phytoplankton.[9] Matutou *et al*.[12] and Maeda[13] reported that, in a marine micro-alga taking up arsenic(V), the majority of the arsenic(V) was reduced, methylated and released to the surrounding medium. We assume that arsenic in *Thraustochytrium* sp. CHN-1 is metabolized by similar processes to *Dunaliella* sp.

Effects of light generated by LEDs on arsenic species in *Thraustochytrium* sp. CHN-1

The effects of the 60 μmol m^{-2} s^{-1} intensity fluorescent light and light from red and blue LEDs on the growth and on the arsenic species present in *Thraustochytrium* sp. CHN-1 cells are shown in Figs 2 and 4. The biomass yields from 13 days incubation in the medium containing 2% glucose, 0.1% KNO_3, arsenate (10 mg dm^{-3}) with fluorescent light, light from two LEDs and in the dark were 2.1–2.4 g dry cells per liter (see Fig. 2). *Thraustochytrium* sp. CHN-1 was able to grow in the medium without light, and the biomass obtained under dark conditions was about the same as that under fluorescent light conditions. This result suggests that light conditions did not affect the growth of *Thraustochytrium* sp. CHN-1 in the medium containing arsenate. Figure 4 shows that there were differences in the arsenic species accumulated in the dark and under the various light conditions. The quantity of arsenic accumulated by *Thraustochytrium* sp. CHN-1 incubated for 13 days under various light conditions was in the order blue LEDs > fluorescent > dark = red LEDs. The arsenic(V)/arsenic species ratio of *Thraustochytrium* sp. CHN-1 incubated for 13 days in fluorescent

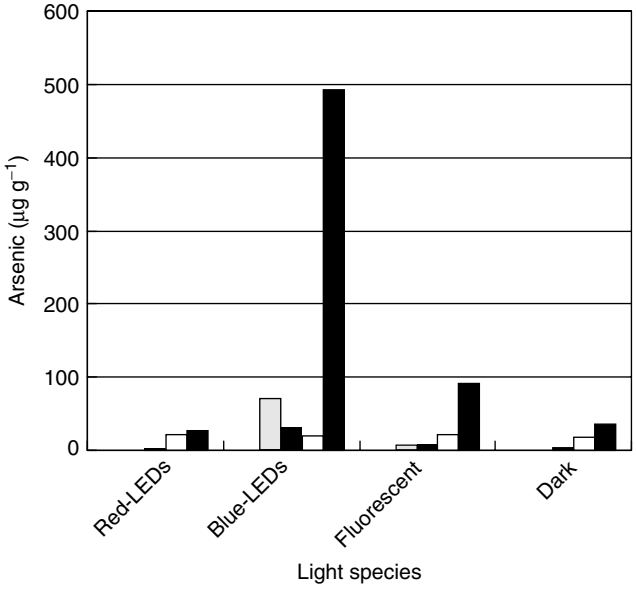

Figure 4. Effect of different light types on arsenic accumulation by *Thraustochytrium* sp. CHN-1. Conditions: containing 10 mg dm^{-3} of arsenic(V) (as Na_2HAsO_4); glucose, 3%; KNO_3, 0.1%; in sea water; 23 °C, 60 μmol m^{-2} s^{-1} light intensity, 13 days. ■: As(V); □: arsenic(III); ■: MMAA; □: DMAA.

light was arsenic(V)/arsenic(III) = 0.7, arsenic(V)/DMAA = 0.7 and arsenic(V)/MMAA = 0.23. The arsenic(V)/arsenic species ratio in the dark was arsenic(V)/DMAA = 0.1, arsenic(V)/MMAA = 0.49. The arsenic(V)/arsenic species ratio in blue LED light was arsenic(V)/arsenic(III) = 0.14, arsenic(V)/MMAA = 0.06 and arsenic(V)/DMAA 0.04. Arsenic accumulation in the light from blue LEDs was 11 times greater than in the dark. Blue LEDs induced the accumulation of more arsenic (arsenic(III) or arsenic(V)) in the cells. These results demonstrate that arsenic uptake and accumulation by *Thraustochytrium* sp. CHN-1 under light from blue LEDs exceeds that under fluorescent light. The color of *Thraustochytrium* sp. CHN-1 grown in the light were orange and red, indicating the accumulation of pigments. Carotenoids in *Thraustochytrium* sp. CHN-1 were separated and shown to be astaxanthin, phenocoxanthin, canthaxanthin, echinenone, and β-carotene.[6] Figure 5 shows the effects of light type on carotenoid production by *Thraustochytrium* sp. CHN-1. The quantities of carotenoids produced by *Thraustochytrium* sp. CHN-1 were in the order blue LEDs > fluorescent > dark. Astaxanthin was the major carotenoid compound in *Thraustochytrium* sp. CHN-1 incubated for 13 days in the light from blue LEDs. Blue light activates the production of various metabolites (carotenoid synthesis, etc.) and behavioral processes both in plants and in prokaryotic and eukaryotic micro-organisms.[14] Also, blue light is a strict requirement for the production of carotenoids in the myxobacterium.[15] Thus, we speculate that there is a possible relationship between increased carotenoid production

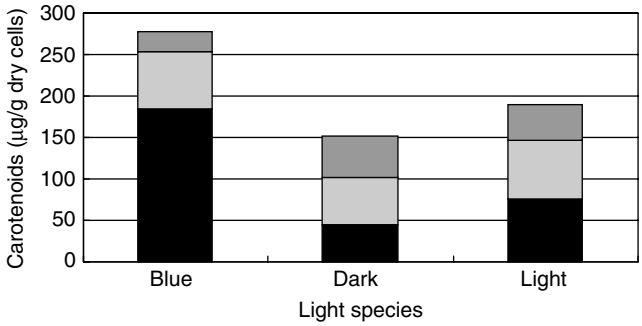

Figure 5. Effect of light type on carotenoid production by *Thraustochytrium* sp. CHN-1. A basal medium of 2% glucose, 0.1% yeast extracts and 0.1% polypeptone in sea water was used; 13 days. ■: astaxanthin; ■: phenocoxanthin; □: canthaxanthin.

and arsenic accumulation under blue LEDs, the causality of which has to be examined in a future study.

CONCLUSIONS

1. Arsenic was accumulated to a high concentration by *Thraustochytrium* sp. CHN-1 within the logarithmic growth phase.
2. The arsenic species accumulated in *Thraustochytrium* sp. CHN-1 in the preliminary logarithmic growth phase (2 days) was composed of 72% arsenic(V), 20% arsenic(III), 8% MMAA, 0% DMAA and 0% arsenosugar, whereas the arsenic species in *Thraustochytrium* sp. CHN-1 in the stationary growth phase was composed of 64% arsenic(V), 18% arsenic(III) 6% MMAA, 18% DMAA and 3% arsenosugar.
3. The quantity of arsenic accumulated by *Thraustochytrium* sp. CHN-1 incubated under various light conditions was in the order blue LEDs > fluorescent > dark ≈ red LEDs.
4. Blue LEDs induced the accumulation of more arsenic (arsenic (III) or arsenic(V)) in the cells.

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