Red Light-Blue Light: Alkenone Production in *Isochrysis galbana* is Enhanced by Red Light, while Growth is Optimal under Blue/Violet Light

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BACKGROUND:

Isochrysis galbana is commonly used as a food source in the aquaculture industry because of its nutritional content and the ease with which it can be cultured and harvested. In addition to being a rich source of omega-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid, *Isochrysis galbana* is one of five species of algae that produce alkenones. Alkenones are very long chains fatty acids that contain between 35 and 41 carbon atoms and between 1 and 5 trans double bonds. While the genes and proteins involved in the biosynthesis and metabolism of alkenones remain largely unknown, their ability to absorb UV radiation, and the antifungal and antibacterial properties they exhibit, suggests they may have some valuable commercial applications. For example, alkenones may be useful as an active ingredient in sunscreen, a novel drug delivery system, and/or as a protective coating for food products. Because of these potential commercial applications, the focus of our lab is to determine optimal conditions to maximize alkenone biosynthesis and growth of *I. galbana*. In this experiment, we determined the effects of different colored lights on the growth and alkenone production when *I. galbana* was cultured under different colored lights.

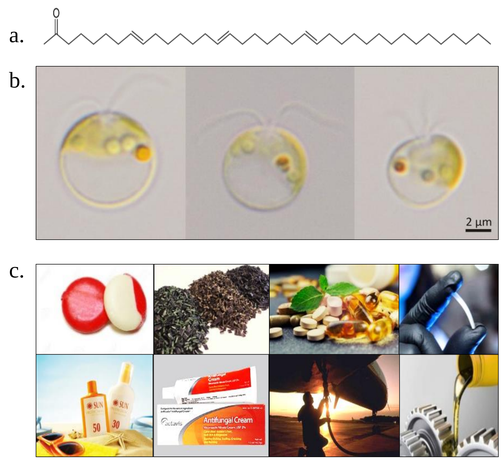


Figure 1. Alkenones are unusual fatty acids that have potential commercial value and are produced by only a select group of haptophytes. Alkenones are long-chain polyunsaturated methyl and ethyl ketones that have between 35 to 41 carbons and 1-5 trans double bonds (a). *Isochrysis galbana* is one of 5 species of marine phytoplankton that produce alkenones (b). The unique properties of these lipids are being exploited for industrial applications (c).

OBJECTIVES:

- To determine the effects of different colored lights on the growth of *I. galbana*
- To examine alkenone production when *I. galbana* is cultured under different colored lights

METHODS:

- **Cultivation**
  - *I. galbana* grown in FSW for 14 days at 19±1° C and under different colored lights at constant 580±50 lux
  - Monitored daily by Nile Red staining and flow cytometry
- **Lipid Extraction**
  - Total lipid extraction using organic solvents
  - Neutral lipid purification by silica chromatography
- **Neutral lipid profiling**
  - GC/MS
  - Identifying and quantifying alkenones

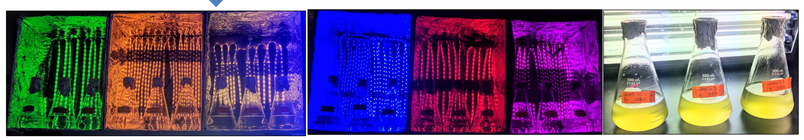
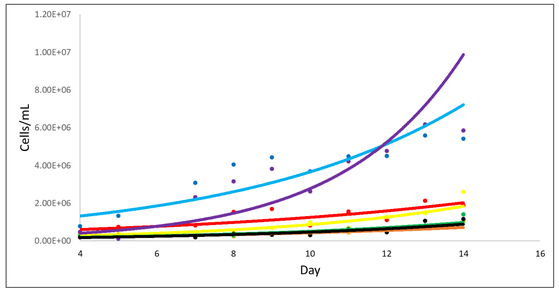


Figure 2. The growth of 250 mL cultures of *I. galbana* in F/2 filtered seawater medium was monitored for 14 days under continuous illumination with green, orange, yellow, blue, red, purple, and white lights.

RESULTS:

- Growth of *I. galbana* is highest under blue and violet lights
- Maximal alkenone production is achieved when cells are grown under red light
- Red, orange, blue, and violet lights trigger the production of C38:5 alkenones
- The production of the C38:4 alkenone was elicited exclusively under blue light
- A total of five different species of alkenones were identified across the different cultures

I. galbana grows faster when cells are illuminated with blue or violet light at 580 lux



Color	Doubling Time (Hour)
Red	26.1
Orange	45.0
Yellow	29.0
Green	30.7
Blue	31.6
Violet	11.1
Control	20.1

Figure 3. Growth of *I. galbana* under blue, purple, red, and yellow was monitored for 14 days. The data points represent the average of triplicate samples under each wavelength.

Nile red staining shows the neutral lipid content of *I. galbana* cells increases up until day 11 at which time it appears to plateau

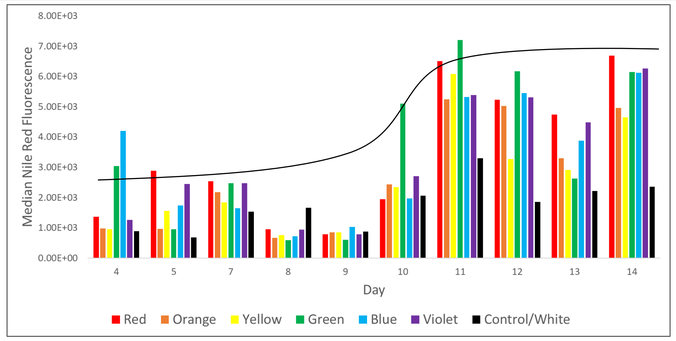


Figure 4. Total lipid concentration is indicated by Nile Red fluorescence and was monitored by Nile Red staining and flow cytometry.

GC-MS can be used to identify and quantitate alkenones in *I. galbana*

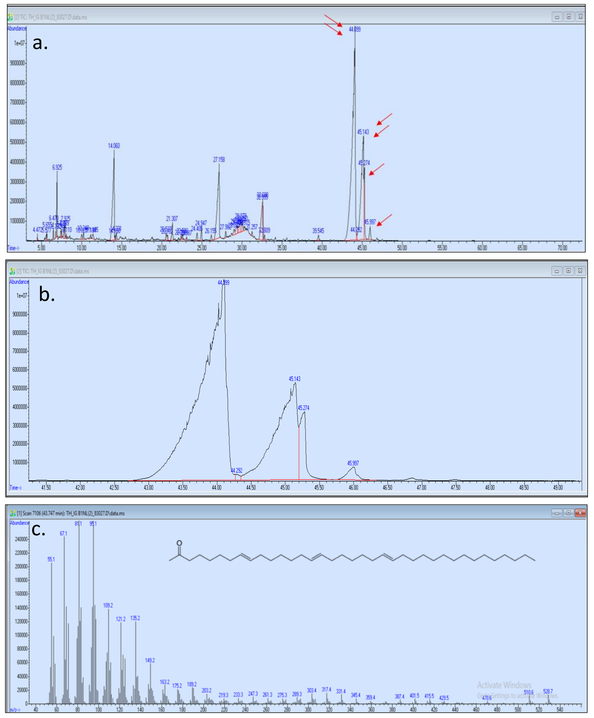


Figure 5. Neutral lipids were extracted and fragmented by gas chromatography (GC). A presentative neutral lipid profile where alkenones are indicated by arrows (a). Major alkenones present in *I.galbana* are Me37:3, C38:5, C38:4, C38:3, and Et38:2 (panel b). MS spectra and fragmentation patterns are indicative of Me37:3 (c).

Alkenones are typically the predominant neutral lipids in *I. galbana*

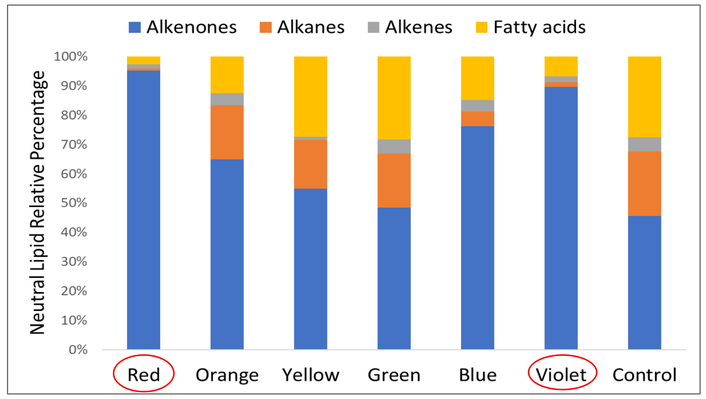


Figure 6. While alkenones are the predominant neutral lipids in *I. galbana*, they become even more prevalent when cultures are grown under red and violet light.

The absolute amount of alkenones produced is greatest when cells were illuminated with red light

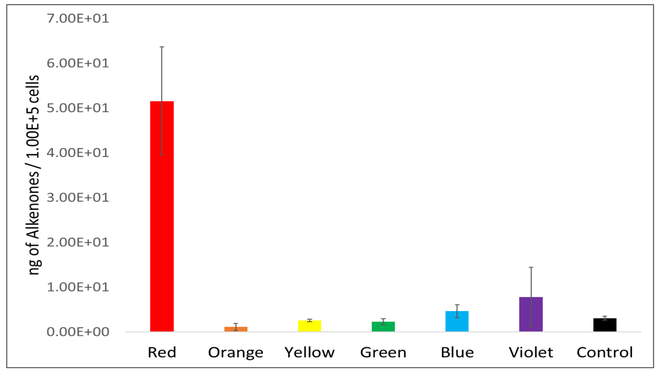


Figure 7. The absolute amount of alkenones produced, determined using internal standards, was 17 times greater when cells were grown under red light compared to white light control.

Alkenone profiles vary under different colored lights

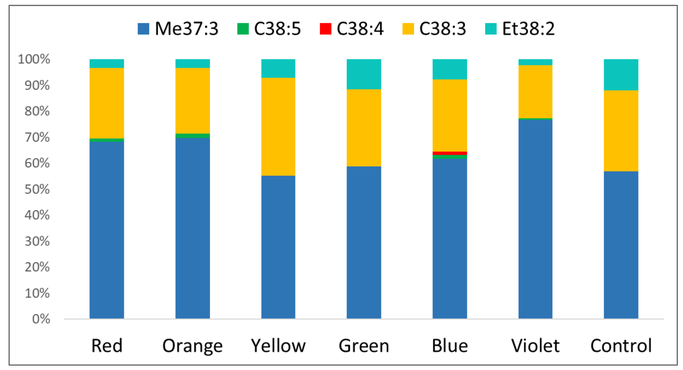


Figure 8. The relative amounts and species of alkenones varies when *I. galbana* produces is cultured under different color lights. Me37:3, C38:3, and Et38:2 alkenones were present in cultures grown under all seven colors of light. C38:5 was detected in cultures grown under red, orange, blue, and violet light, while C38:4 was identified only when *I. galbana* was grown under the blue light.

FUTURE DIRECTIONS:

- Identify genes and promoters involved in alkenone production
- Fine tune wavelengths to modulate alkenone production
- Purify individual species
- Determine the mass balance ratio