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Investigation of Growth Rate and Phycocolloid Content from *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) under Different Light Conditions Using Vibrational Spectroscopy

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Abstract

Kappa-carrageenan (*K*-carrageenan) is an important phycocolloid which is a major constituent of the cell wall of *Kappaphycus alvarezii*. The chemical structure of *K*-carrageenan comprises a linear backbone of D-galactose residues linked with alternating α-(1,3) and β-(1,4) linkages which are substituted by one ester-sulphonic group per di-galactose repeating unit. The spectral qualities of light as well as the ambient carbon

dioxide concentration, both play an important role in the photosynthetic pathway in plants and this investigation set forth to establish the effect of different wavelengths of light and carbon dioxide supplementation on the chemical structure of *K*-carrageenan obtained from *K. alvarezii.* Specimens were cultivated under a range of monochromatic light spectra and assessed for chemical composition using Fourier Transform Infrared (FTIR) spectroscopy.The *K. alvarezii* control was irradiated with full light spectrum, treatments were carried out using blue (492-455 nm), green (577-492 nm) and red (780-622 nm)light. One experiment was carried out by supplementation with carbon dioxide. Samples were collected after 14 days. The effect of different wavelengths of light on the growth rates of experimental samples was determined. Red light had the most significant impact on the growth rate of *K. alvarezii* as compared to those treated with blue light. The FTIR fingerprint of the ground seaweed was found to be identical to that of commercial *K*-carrageenan (Sigma). Special emphasis was given to the 800-1300 cm^{-1} region, which presents several vibrational modes. All the samples produced similar FTIR spectral profiles, suggesting that genes related to the carrageenan biosynthesis are not affected by different wavelengths of light or CO_2. The results obtained from FTIR spectroscopy demonstrated that different wavelengths of light and supplementation with CO_2 have no influence to the chemical structure of *K*-carrageenan in *K. alvarezii*.

Keywords: *Kappaphycus alvarezii*, Growth, Carrageenan, FTIR

1. Introduction

Contemporary *in vitro* plant propagation techniques rely on artificial lighting for the mass production of plants which eventually contributes to the operating costs of a commercial operation. Recent developments in Light Emitting Diode (LED) technology offer a viable alternative to fluorescent and incandescent lighting systems (Singh et al., 2015), however the impact on final product quality and yield has yet to be determined in the case of red algae. Investigations involving the effect of light spectra on red algae have demonstrated that affecting metabolism and growth (Figueroa, 1993; Figueroa et al., 1994), in addition light also influences the relative pigment composition and structure (Talarico et al., 1993; Godinez-Ortega et al., 2007). Carbohydrate metabolism is the most active metabolic process in seaweed and is a key component of the primary life cycle which involve the transformation of energy and the fixation of atmospheric carbon. Light regulates the timing of seaweed growth and reproduction, agar yield and properties, starch content, and others (Ho et al., 2009). Changes in light conditions have been reported to be associated with the regulation of cell wall modification in some plants (Sasidahran *et al.,* 2010). In this study, FTIR spectroscopy was used to analyze the influence of light spectra on the chemical composition of the carrageenan polymer.

FTIR (Fourier Transform Infrared) spectroscopy is widely applied in organic synthesis, polymer science, petrochemical engineering, pharmaceutical industry and food analysis. Several studies have used FTIR spectroscopy to investigate vibrational frequency changes in seaweeds (Figueroa et al., 1999; Sheng et al., 2004; Chen et al., 2006). FTIR provides excellent information on the nature of the bands present on the algae surface. The range used in seaweed surface analysis is the mid-infrared range (4,000-200 cm^{-1}).

2. Materials and methods

Seedlings of *Kappaphycus alvarezii* (var. tambalang 'giant') were obtained from Biotechnology Research Institute (BRI), Universiti Malaysia Sabah. The young seedlings were cultured in under laboratory conditions (Yong et al., 2014). Three replicates consisted of five propagules (2 g) were used for each experimental condition. The seedlings were cultured in the Fernbach culture vessel with 800 mL artificial seawater (Fluval marine salt, 36 g L^{-1}, salinity 31.4 ppt) enriched with 50% Provasoli's enriched seawater (PES) media. For light experiment, the cultures grown under 75 µmol photons m^{-2} s^{-1} white light (WL), blue light (BL) (wavelength = 492-455 nm), green light (GL) (wavelength = 577-492 nm) and red light (RL) (wavelength = 780-622 nm), respectively. For CO_2 enrichment, the cultures were illuminated by white LED, providing an irradiance of 75 µmol photons m^{-2} s^{-1}. CO_2 was provided 500 mL/day and controlled by supplying chambers with air/CO_2 mix. Provision of 500 mL of CO_2 per day was proved to achieve the highest growth rate of *K. alvarezii* (Barat, 2011). To avoid overly acidifying cultures, supplemental CO_2 was only supplied during photoperiod. Both experiments were carried out at 25±1.0 °C with 18 h light and 6 h dark cycle.).

The seaweeds were harvested after 14 days. The weight of experimental samples were recorded for daily growth rate (DGR) determination using the formula DGR = [(Wt/W0)1/t-1] × 100% according to Yong *et al*. (2013) where W0 is the initial fresh weight and Wt is the final fresh weight of the samples after t days of culture. A one way analysis of variance (ANOVA) was used to evaluate significant differences in daily growth rate of cultures and difference was consider significant at $p<0.05$. All statistical analyses were performed using statistical package software SPSS version 16 (SPSS, Chicago, IL).

The cultured seaweeds were harvested from each treatment and washed with tap water. The clean seaweeds were dried at the 60 °C for 48 h. Size reduction was carried out by using Kinematica Polytron PT1200E homogenizer (Fisher Scientific). The dry seaweeds were first grinded with standard aggregate size Ø7 mm followed by Ø5 mm until fine powders were obtained. Assessment of the carrageenan chemical structure was performed by Fourier transform infrared (FTIR).

3. Results and Discussion

3.1 Growth Rate Study

The mean of each replicate was determined. The averages of the replicates were then used to analyze the data and from the calculated means, determine the overall average for each treatment, standard deviations and 95% confidence intervals. The growth rate of *K. alvarezii* was found to be dependent on the wavelengths of light they are exposed to, with longer wavelengths promoting a faster growth rate. After 14 days of cultivation of algal tips under different light qualities and carbon dioxide, RL was determined to be ideal for the growth and direct-regeneration of *K. alvarezii* with daily growth rate of 8.1±1.4% day^{-1} (Figure 1). The algal cultured under WL with CO_2 had the second highest (6.8±1.0% day^{-1}) with the slowest growth rate belonging to the blue treatment (3.5±1.2% day^{-1}). The WL treatment had growth rates (6.6±0.9% day^{-1}) closer to that of the WL+CO_2 followed by the green treatment (5.2±1.1% day^{-1}). There is no significant differences in growth rate were observed between GL and BL ($p>0.05$).

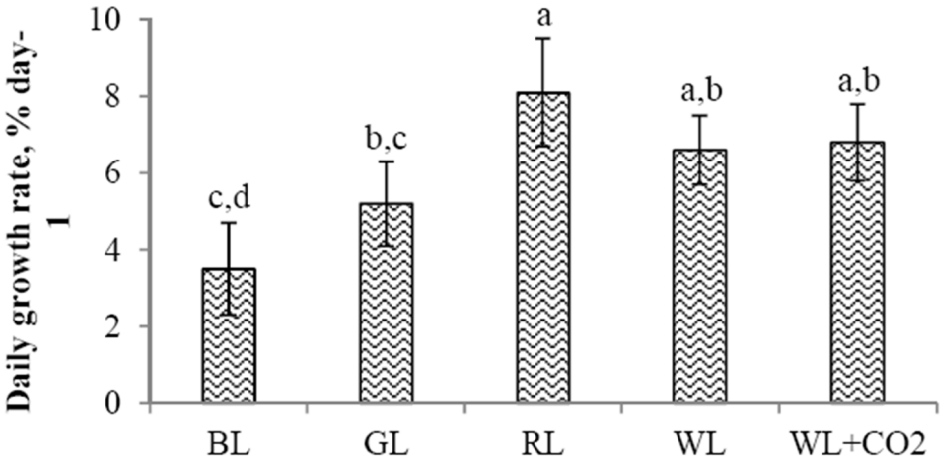

Figure 1. The average growth rate of each treatment over the course of 14 days. The black bars on each treatment represent the standard deviation. Different letters indicate that the values are significantly different ($p<0.05$)

The red wavelengths are known as warm light and they are naturally more prevalent in sunlight. It has been shown that RL favors thallus expansion, cell division and carbon accumulation in red alga *Porphyra leucosticta* (Korbee et al., 2005). Growth rate under blue light, the shortest wavelength, was lower than in green, red and white light in *K. alvarezii*, as it has been previously shown in other red algae, for instance, *Porphyra leucosticta* (Korbee et al., 2005) and *Palmaria palmata* (Parjikolaei et al., 2013). The better growth rate in red light as compared to blue light is probably due to higher photosynthetic efficiency and quantum yield associated with red light.

Lopez-Figueroa (1991) observed that the synthesis of chlorophyll, phycoerythrin, phycocyanin and total protein synthesis in the red algae have been shown to be modulated by different wavelengths of light. Although the chlorophyll a and carotenoids are present in quantities in *K. alvarezii* comparable to the green algae, their function is apparently not that of a primary light absorber, this role is taken over by the phycobilins. Phycoerythrin synthesis in red algae was mainly stimulated by green light and phycocyanin synthesis was induced by red light. One possible explanation for changes in growth rate in blue light is an alteration in the photosynthetic performance. In *K. alvarezii*, an increase in the amount of phycocyanin and reduction in that of phycoerythrin under RL, the action spectra reflects this, with increasing activity in the orange-red region (600-640 nm) where phycocyanin absorbs. In the view of the photosynthetic effectiveness of phycocyanin and chlorophyll *a* in *K. alvarezii*, this would contribute to the high photosynthetic rates under RL. According to Haxo & Blinks (1950), the photosynthetic rates are high in the spectral regions absorbed by phycobilins. They also reported that in red algae, photosynthesis is almost minimal at 435 nm and 675 nm, where chlorophyll shows maximum absorption.

One of the possible explanation for the higher observed growth rate of *K. alvarezii* under RL and GL as compared to BL may be attributed to the fact that RL and GL favor excitation of photosystem II (PSII) rather than Photosystem I (PSI). The spectrum of BL is more likely to favor excitation of PSI, thus limiting the turnover of PSII. The light absorption around the

maximal transmission of the BL (440-480 nm) is 1.2-1.5 times smaller than that around the maximal transmission of RL (630-670 nm) (Figueroa et al., 1994). As suggested by Cunnigham *et al*. (1990), different wavelengths of light can induce changes in photosynthetic efficiency of red algae due to an imbalance of the photosystems or differences in their turnover rates via alterations in the electron flow between PSII and PSI and/or the carbon fixation rate. Our findings corroborate the results obtained by investigators in related species of red algae.

The growth of *K. alvarezii* was influenced positively by CO_2 enrichment. The addition of CO_2 into cultures slightly increased the growth compared to control. This is accordance with the results obtained for the red alga *Hypnea spinella* (Suarez-Alvarez et al., 2012) and *Gracilaria lemaneiformis* (Xu *et al.,* 2010). High CO_2 levels generally enhance photosynthesis due to the diffusion of more CO_2 to the active site RuBisCO (Giordano et al., 2005). Full light spectrum consists of all visible colors, not just blue, green and red wavelengths, and so are perceived as white by visual scene. Full light spectrum emitting light in every spectral range. So it is no surprise that cultures grown under WL have similar growth rate to RL.

3.2 FTIR Analysis of Carrageenan

Carrageenan is the polysaccharide composed of the monosaccharide galactose in compounds containing varying quantities of sulphate. Carrageenan is long, flexible chains with about 25,000 galactose components. Their gelation properties are dependent on the variety and their behaviour is affected by the surrounding conditions of pH, ion content and temperature (Mouritsen, 2013).

The chemical structure of *K*-carrageenan obtained from *K. alvarezii* specimens subjected to different photosynthetic spectra was assessed using FTIR spectroscopy in order to determine the effect of light on chemical composition of *K*-carrageenan. Special emphasis was given to the 800-1300 cm^{-1} region (Figure 2), also known as fingerprint region, which presents several vibrational modes. All the samples produced similar FTIR spectral profiles, suggesting that genes related to the carrageenan biosynthesis are not affected by different wavelengths of light or CO_2. The spectra of the ground seaweed were found to be identical to that of commercial *K*-carrageenan (Sigma). The study of carrageenan by FTIR shows the presence of very strong absorption bands in the 1220 cm^{-1} region (S=O of sulfate esters), being correlated to the sulphate content of the sample (van de Velde, 2002) and in 1010-1080 cm^{-1} region (glycosidic linkage) of all carrageenan types (Tuvikene et al., 2006). The other chemical groups are characteristics of *K*-carrageenan type: 3,6-anhydro-D-galactose at 925 cm^{-1}, and D-galactose-4-sulfate at 840 cm^{-1} (Sekkal & Legrand, 1993; Pereira et al., 2009; Dewi et al., 2012).

The morphological plasticity of *Kappaphycus alvarezii* and *Eucheuma denticulatum* causes confusion between two species. These two species produced different type of carrageenans. *K. alvarezii* is predominantly *kappa*-carrageenan and *E. denticulatum* is largely *iota*-carrageenan. In comparative studies of carrageenan types, the FTIR spectra provide enough information. The main difference of *kappa*- and *iota*-carrageenan was differentiated from wide spectra appeared, the bands 840-850 cm^{-1} identified for galactose-4-sulphate for *kappa* (Freile-Pelegrin & Robledo, 2006a and 2006b) and the spectra bands of 800-805 cm^{-1} which is

iota with linked bound of 3,6 anhydrogalactose-2 sulphate (Freile-Pelegrin & Robledo, 2007). In this study, the characteristic and distinctive of iota-carrageenan band is absent and allowed us to prove that seaweeds derived by *in vitro* culture methods are phenotypically identical to their wild types.

Several intense characteristic bands in the IP spectra can be attributed to the functional groups present in seaweed proteins and polysaccharides (Fourest & Volesky, 1996). For instance, bands at approximately 1740, 1640, 1420 and 1240 cm^{-1} can be attributed to various carboxyl stretches (free C=O, chelate/asymmetric C=O, symmetric C=O and C=O respectively) while polysaccharide ether and hydroxyl groups exhibit stretches of around 1160 and 1030 cm^{-1} respectively. On the other hand, seaweed proteins exhibit –NH stretches at approximately 1540 cm^{-1}.

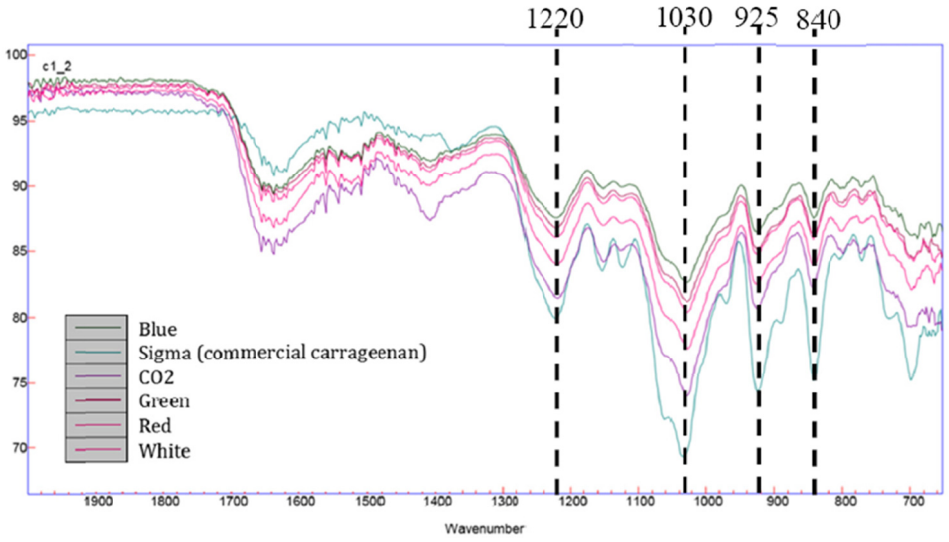

Figure 2. FTIR spectra of commercial *K*-carrageenan (Sigma) and FTIR spectra of *K. alvarezii* extracted carrageenan from different wavelengths of light and CO_2 enrichment

4. Conclusion

From the study conducted, it can be concluded carrageenan obtained from *in vitro K. alvarezii* belonged to *Kappa*-carrageenan. FTIR spectroscopy shows that different wavelengths of light and CO_2 enhancement have no influence to the chemical structure of *K*-carrageenan in *K. alvarezii*. Red light demonstrated the most significant effect as compared to blue light had the least effect on growth rate of *K. alvarezii*. In addition, this study supports the claim that seaweeds derived by *in vitro* culture methods are phenotypically identical to their wild types.

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