TRANSCRIPTOMIC AND CARRAGEENAN ANALYSES OF *Kappaphycus alvarezii* UNDER DIFFERENT LIGHT WAVELENGTHS WITH CARBON DIOXIDE ENRICHMENT

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations, citations, equations, summaries and references, which have been duly acknowledged.

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ABSTRACT

Kappaphycus alvarezii, a marine red algae belonging to the order Gigartinales (Rhodophyta), is one of the most valuable and cultivated *Kappaphycus* species along with *Kappaphycus striatum* and *Eucheuma denticulatum*. *K. alvarezii* was selected for this study owing to the fact that it has adapted to survive in an ecological niche which is characterised by varying light wavelengths. The process of carbon assimilation in algae is governed by panoply of factors yet much information from the unicellular green alga. Transcriptomes associated with the process of photosynthesis and carbon fixation have offered insights into the mechanism of gene regulation in terrestrial plants; however limited information is available as far as macroalgae/red algae are concerned. The study aims to characterise *K. alvarezii* transcriptome under specific light wavelengths and CO_2 enrichment. The hypothesis was that *K. alvarezii* would respond to different wavelength of light at the molecular level and that it would be possible to detect molecular changes in the treated-cultures and gain some insights into the carbon fixation pathway in this red alga. High-throughput paired-end RNA-sequencing was applied to profile the transcriptome of *K. alvarezii* irradiated with different wavelengths of light (blue 492-455 nm (BL), green 577-492 nm (GL) and red 780-622 nm (RL)) and those exposed to full light spectrum (WL). In addition to this the carbon dioxide (CO_2) induced transcriptome was analysed to identify differentially expressed transcripts. The sequencing reads were subjected to quality filtering and assembly using Trinity. The growth performance of *K. alvarezii* for each treatments was determined and a one way analysis of variance was used to evaluate significant differences in daily growth rate of cultures and difference was consider significant at $p<0.05$. In addition, the chemical structure of *K*-carrageenan obtained from *K. alvarezii* specimens subjected to different photosynthetic spectra was assessed using Fourier Transform Infrared (FTIR) spectroscopy in order to determine the effect of light on chemical composition of *Kappa*-carrageenan. A *de novo* assembly of the transcriptome of *K. alvarezii* generated 76,871 qualified transcripts with a mean length of 979bp and a N50 length of 1,707bp and 55.83% transcripts were annotated on the basis of function. A total of 28,079 annotated transcripts were assigned to 49 functional groups and 3,246 GO terms. KEGG pathway enrichment resulted in 10,460 transcripts being mapped to 273 pathways. The carbon fixation pathway was analysed and key genes encoding enzymes involved in the carbon fixation pathway in *K. alvarezii* such as PPC, PEPC, PRK, PGK, PPDK, provided that unequivocal molecular evidence that most of the C_3 and C_4 pathway genes were actively transcribed in *K. alvarezii*. Setting FDR less than 1e-10 and log2 fold change of 2 as the cutoff, 315 DEGs were obtained. The most DEGs were detected between WL and WL+CO_2. The DEGs detected under different light qualities (BL, GL, RL, WL) and CO_2 accounted for 3.46% of the identified genes. Based on the gene expression patterns among the four lights, the DEGs were designated to four categories: BL-regulated, GL-regulated, RL-regulated or either BL, GL, RL regulated. The numbers of genes encoding light-harvesting proteins transcripts were significantly down-regulated under different light spectra (BL and RL) and there was no significant difference in gene expression upon CO_2 enrichment. Fucoxanthin chlorophyll *a/c*-binding and early light-induced proteins

were up-regulated under GL. Meanwhile, similar responses of *K. alvarezii* were found under BL and RL that both fucoxanthin chlorophyll *a/c*-binding and high light-induced proteins were down-regulated. Different light spectra appeared to induce the same effect on phycobilisome and photosystem proteins in *K. alvarezii*. Meanwhile, five DEGs encoding photosystem proteins were influenced by the ambient CO_2 concentration. Transcripts encoding phytochrome-like protein were observed expressed only under WL. On the other hand, 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. Red light (8.1±1.4% day^{-1}) had the most significant impact on the growth rate of *K. alvarezii* as compared to those treated with blue light (3.5±1.2% day^{-1}). The FTIR fingerprint of the ground seaweed was found to be identical to that of commercial *Kappa*-carrageenan (Sigma). All the samples produced similar FTIR spectral profiles, demonstrated that different wavelengths of light and supplementation with CO_2 have no influence to the chemical structure of *Kappa*-carrageenan in *K. alvarezii*. The vast majority of transcripts had similar mRNA levels. GL had more pronounced effect on transcriptome as compared to BL and RL did. GL was found more efficient in triggering the biosynthesis of light-harvesting complex as the up-regulation of fucoxanthin and early light-induced proteins. Regulation of gene expression by CO_2 enrichment appears to be more extensive than expected. All the genes necessary to encode the enzymes involved in photosynthetic inorganic carbon fixation were identified in *K. alvarezii*, especially C_4-CCM pathway. Interestingly, most of the key enzymes of C_3 were highly expressed under CO_2 enrichment cultures. These results suggested CO_2 enhancement may alter carbon metabolism and lead to C_3-type carbon metabolism in *K. alvarezii*. The better growth rate in RL as compared to BL is probably due to higher photosynthetic efficiency and quantum yield associated with RL as RL favours the excitation of photosystem II rather than photosystem I. Carrageenan obtained from *in vitro K. alvarezii* belonged to *Kappa*-carrageenan. 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. Blue, green and red light all have demonstrated roles in modulating light responses, such as changes in gene expression and increase in the growth rate of thalli. A small proportion genes were significantly differentially expressed, suggested that light-regulated gene expression in *K. alvarezii* is not a unique/single light response. The findings of this project will contribute to our understanding of molecular mechanisms underlying light-induced responses in lower plants as well as facilitate our understanding in inorganic carbon fixation in red algae. The transcriptome of *K. alvarezii* has large number of hypothetical proteins. The transcriptome studies of the seaweeds are still scarce caused insufficient sequence data available for genome annotation. It is suggested that the proteogenomics could be utilized to improve genome annotations. This study also recommends that seaweed cultivators could utilize red light to enhance the growth rate of *K. alvarezii* during seedling productions.

ABSTRAK

ANALISIS TRANSKRIPTOM DAN KARAGEENAN KE ATAS Kappaphycus alvarezii DI BAWAH GELOMBANG CAHAYA YANG BERBEZA DENGAN KARBON DIOKSIDA YANG DIPERKAYA

Kappaphycus alvarezii merupakan rumpai laut merah diklasifikasikan dalam kumpulan Gigartinales yang mempunyai nilai kormesial yang tinggi dan ditanam dengan Kappaphycus striatus dan Eucheuma denticulatum. K. alvarezii dipilih untuk kajian ini kerana rumpai laut ini mempunyai strategi adaptasi yang boleh menyesuaikan diri pada ekologi yang disulami dengan warna cahaya yang berbeza. Proses asimilasi karbon dalam alga ditadbir oleh pelbagai faktor, tetapi kebanyakan maklumat adalah dari alga hijau unisel. Transkriptom berkaitan dengan proses fotosintesis dan penetapan karbon telah menawarkan mekanisme peraturan gen dalam tumbuhan daratan; namun maklumat tentang makroalga/alga merah adalah terhad. Kajian ini bertujuan untuk mencirikan transkriptom K. alvarezii di bawah kualiti cahaya tertentu dan pengayaan gas CO_2. Hipotesis adalah bahawa K. alvarezii akan bertindak balas terhadap panjang gelombang cahaya yang berbeza pada peringkat molekul dan terdapat perubahan molekul dalam rumpai laut yang dirawat serta mendapatkan pemahaman penetapan karbon dalam alga merah. Transkriptom pendedahan K. alvarezii kepada cahaya biru (BL,492-455 nm), hijau (GL, 577-492 nm), merah (RL, 780-622 nm) dan putih diprofilkan dengan menggunakan RNA-penjujukan pasangan hujung ke hujung jujukan. Di samping itu, transkriptom dari pengayaan CO_2 juga dianalisis untuk mengenalpasti gen pengekspresan berlainan. Penjujukan tertakluk kepada kualiti penapisan dan pengelompokan dengan penggunaan Trinity. Pertumbuhan K. alvarezii dari setiap eksperimen ditentukan dan analisis sehala varians digunakan untuk menilai perbezaan kadar pertumbuhan dan perbezaan adalah bererti jika nilai $p < 0.05$. Selain itu, struktur kimia Kappa-karageenan terdapat dari sampel K. alvarezii yang tertakluk kepada spektrum fotosintesis yang berbeza dianalisis dengan menggunakan Fourier Transform Infrared (FTIR) untuk menentukan kesan cahaya dan pengayaan CO_2 kepada composisi Kappa-karageenan. De novo pengelompokan transkriptom K. alvarezii menghasilkan 76,871 transkrip dengan purata panjang 979bp dan panjang N50 1,707bp serta 55.83% transkrip telah dianotasi. Sebanyak 28,079 transkrip telah dikelompokan kepada 49 kumpulan berfungsi dan 3,264 GO istilah. Analisis KEGG mendapati 10,460 transkrip dipetakan kepada 273 laluan. Penetapan CO_2 telah dikaji dan gen utama pengekodan enzim yang terlibat dalam penetapan karbon di dalam K. alvarezii seperti PPC, PEPC, PRK, PGK, PPDK, membuktikan keaktifan gen laluan C_3 dan C_4 di dalam K. alvarezii. Sejumlah 315 gen ekspresi berlainan terdapat pada penetapan FDR kurang daripada 1e-10 dan log2 gandaan perubahan pada 2 sebagai penggalan. Gen ekspresi berlainan dikesan di bawah pengaruh kualiti cahaya yang berbeza (biru, hijau, merah dan putih) dan CO_2 menyumbang 3.46% daripada gen ekspresi dikenalpasti. Berdasarkan corak gen pengekspresan antara empat cahaya, gen ekspresi berlainan dibahagikan kepada empat kategori: biru-selia, hijau-selia, merah-selia atau biru, hijau, merah-selia. Transkrip pengekspresan light-harvesting protein menurun di bawah cahaya biru

dan hijau manakala tiada perbezaan di bawah pengayaan CO_2. Pengekspresan fucoxanthin klorofil a/c-pengikat and early light-induced protein meningkat di bawah cahaya hijau. Sementara itu, tindakbalas K. alvarezii yang serupa ditemui di bawah cahaya biru dan merah bahawa pengekspresan fucoxanthin klorofil a/c-pegikat dan high light-induced protein menurun. Spektrum cahaya yang berlainan terpapar merangsangkan kesan yang sama pada phycobilisome dan fotosistem protein di dalam K. alvarezii. Lima gen pengekodkan fotosistem protein mempunyai pengekspresan yang ketara disebabkan kepekatan CO_2. Pengekspresan transkrip pengekodkan phytochrome protein hanya diperhatikan di bawah cahaya putih. Selain itu, pertumbuhan K. alvarezii bergantung kepada kepanjangan gelombang cahaya, semakin panjang gelombang cahaya semakin tinggi kadar pertumbuhan. Cahaya merah (8.1±1.4% day^{-1}) mempunyai kesan paling ketara ke atas kadar pertumbuhan K. alvarezii berbanding dengan cahaya biru (3.5±1.2% day^{-1}). FTIR profil dari semua sampel adalah serupa, panjang gelombang cahaya yang berbeza dan pembekalan CO_2 tidak mempengaruhi struktur kimia Kappa-karageenan dari K. alvarezii. Sebahagian besar transckrip mempunyai tahap pengekspresan mRNA yang sama. Cahaya hijau mempunyai kesan yang lebih ketara pada transkriptom berbanding cahaya biru dan merah. Cahaya hijau mendapati lebih berkesan dalam pencetusan light-harvesting kompleks kerana pengekspresan fucoxanthin dan early light-induced protein yang tinggi. Peraturan gen di bawah pengayaan CO_2 lebih ekstensif daripada yang dijangkakan. Gen yang terlibat dalam penetapan CO_2 telah dikenal pasti, terutama C_4-CCM. Kebanyakan C_3 enzim mempunyai pengekspresan yang tinggi di bawah pengayaan CO_2. Keputusan ini mencadangkan peningkatan CO_2 boleh mengubah metabolisme karbon kepada jenis C_3. Kadar pertumbuhan di bawah cahaya merah lebih tinggi daripada di bawah cahaya biru mungkin disebabkan kecekapan fotosintesis dan hasil kuantum kerana cahaya merah mempunyai eksitasi atas fotosistem II berbanding fotosistem I. Karageenan yang diperolehi dari in vitro K. alvarezii adalah Kappa-karageenan. Semua sampel menghasilkan FTIR profil specktrum yang sama, ia dicadangkan gen yang berkaitan dengan biosintesis karageenan tidak terjejas oleh panjang gelombang cahaya yang berlainan ataupun pengayaan CO_2. Hasil kajian ini menyokong rumpai laut diperolehi dari kaedah in vitro serupa dengan jenis liar yang di laut. Tindakbalas rumpai laut terhadap cahaya biru, hijau dan merah seperti perubahan gen pengekspresan dan kadar pertumbuhan thalli. Terdapat sebahagian kecil gen yang berekspres ketara menyarankan bahawa gen pengekspresan tersebut bukan gen yang unik kepada perubahan cahaya terhadap K. alvarezii.Hasil kajian ini akan menyumbang kepada pemahaman kita tentang tindakbalas rumpai laut terhadap cahaya serta penetapan karbon di dalam alga merah. Sebahagain besar transkriptom K. alvarezii adalah protein ramalan. Kajian transkriptom rumpai laut jarang didapati malah menyebabkan kekurangan data sedia ada untuk penanotasi genom bagi rumpai laut. Ia dicadangkan bahawa proteogenom boleh digunakan untuk meningkatkan penjelasan genom dan kemungkinan penemuan gen baru. Kajian ini juga mencadangkan penanam rumpai laut mengguna cahaya merah untuk meningkatkan kadar pertumbuhan K. alvarezii semasa penanaman benih.

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LIST OF ABBREVIATIONS

BL	Blue light
CA	Carbonic anhydrase
CAM	*Crassulacean acid metabolism*
CCM	CO_2 concentrating mechanism
CO_2	Carbon dioxide
Cq	Quantification cycle
DEG	Differentially expressed gene
DGR	Daily growth rate
FAO	Food and Agriculture Organization
FDR	False discovery rate
FPKM	Fragments per kilobase per million mapped reads
FTIR	Fourier Transform Infrared
GL	Green light
GO	Gene Ontology
GOI	Gene of interested
FCPs	Fucoxanthin chlorophyll *a/c*-binding proteins
K-carrageenan	*Kappa*-carrageenan
KEGG	Kyoto Encyclopedia of Genes and Groups
KO	KEGG orthology
LHC	Light harvesting complexes
Nr	Non-redundant
PBS	Phycobilisomes
PCR	Polymerase chain reaction
PES	Provasoli's enriched seawater

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PK	Protein kinase
PS I	Photosystem I
PS II	Photosystem II
rbcL	Ribulose bisphosphate carboxylase large chain
RIN	RNA integrity number
RL	Red light
RNA-Seq	RNA sequencing
ROS	Reactive oxygen scavenging
RPKM	Reads per kilobase per million mapped read
RUBSICO	Ribulose bisphophate carboxylase
WL	White light

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LIST OF SYMBOLS

cm	Centimeter
h	Hour
m	Meter
min	Minute
mL	Milliliter
ng	Nanogram
nm	Nanometer
R^2	Correlation coefficients
µg	Microgram
µL	Microliter
µM	Micromolar
µmol	Micromole
s	Second
%	Percent
$	Dollar
Λ	Lambda
°C	Degree Celcius
Ø	Diameter
≥	Greater than or equal to
≤	Less than or equal to
>	Greater than
<	Less than

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Kappaphycus alvarezii (Doty) Doty ex P.C.Silva (formerly *Eucheuma cottonii*), a marine red alga belonging to the order Gigartinales (Rhodophyta), is one of the most valuable and cultivated *Kappaphycus* species along with *Kappaphycus striatum* and *Eucheuma denticulatum* (Yong *et al.*, 2014: 26). This industry has gained significance as an economic driver due to the important roles of carrageenan (Hayashi *et al.*, 2010: 15). *K. alvarezii* is the single most important source of *kappa*-carragenan (*k*-carrageenan). *K*-carrageenan gels are hard, delicate and synergetic (exude water) and extensively used in foods, beauty products and pharmaceutical industries (McHugh, 2003).

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K. alvarezii was selected for this study owing to the fact that it has adapted to survive in an ecological niche which is characterised by varying light wavelengths. Light has significant effect on the growth and development of marine algae including *K. alvarezii*. In the sea, light is attenuated due to absorption of light and scattering. Light that penetrates the ocean is altered in both wavelength and quantity as the light spectral distribution changes at different depths in seawater (Figure 1.1). Commercial cultivation of *K. alvarezii* (approximately 0.5 m water depth) at open sea is subjected to low (1 m) and high tides (5 m) depend on their environments. The seawater will filter off some of the wavelengths of light and reduce the intensity. Some algae will carry out photosynthesis with very little light. Basic knowledge about the primary metabolic processes in macroalgae is essential for the understanding of the impact of light spectra on growth performance and physiology of these organisms.

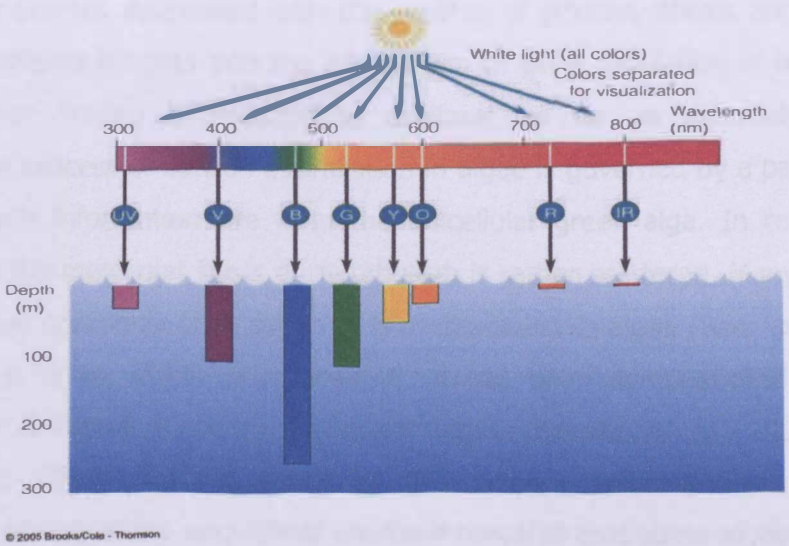

Figure 1.1 : The wavelength of light changes at different levels of the sea because the differential absorption of light by seawater. The figure demonstrates the maximum depth (represented by color-coded lures) at which different colors are no longer visible due to the absorption and scattering of different wavelengths of light.

Source : https://sites.google.com/site/davidbirdprovidedencehigh/Home/course/marine-biology/day-12

Date :19 September 2017

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Recently, insights in the light-mediated physiological responses and molecular mechanisms have been published in brown alga, *Saccharina japonica* (Deng *et al.*, 2012: 7; Wang *et al.*, 2013: 237) and suggested that different light qualities may play significant roles in the physiology and biology of brown algae. On the other hand, Konotchick *et al.* (2013: 198) reported that different abiotic factors (light, nutrients and temperature) caused the shift in transcriptome in *Macrocystis pyrifera* and revealed novel gene families. Furthermore, high genetic similarity was found between *M. pyrifera* and other brown seaweeds, *Ectocarpus* and *Laminaria*. The important discovery in brown algae may give insight in ecology, evolution, physiology, reproduction and so on which are crucial in seaweed genetic engineering.