UNIVERSITI PUTRA MALAYSIA

MORPHOLOGICAL, BIOCHEMICAL AND TRANSCRIPTOMIC CHARACTERISATION OF Chlorella sorokiniana AND Chlorella zofingiensis DURING NORMAL AND STRESS CONDITIONS

SITI NOR ANI BINTI AZAMAN

IB 2018 2

MORPHOLOGICAL, BIOCHEMICAL AND TRANSCRIPTOMIC CHARACTERISATION OF *Chlorella sorokiniana* AND *Chlorella zofingiensis* DURING NORMAL AND STRESS CONDITIONS

By

SITI NOR ANI BINTI AZAMAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

July 2017

This thesis is lovingly dedicated to

My late father Azaman bin Dio
and my mother Aminah binti Jusoh

My beloved husband,
Wan Amirul Faiz bin Wan Muhammad

My dearest kids,
Wan Nurin Auni
and Wan Aisyah Inara

Who leads me with the light of their endless love, support and encourage me throughout my life.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

MORPHOLOGICAL, BIOCHEMICAL AND TRANSCRIPTOMIC CHARACTERISATION OF *Chlorella sorokiniana* AND *Chlorella zofingiensis* DURING NORMAL AND STRESS CONDITIONS

By

SITI NOR ANI BINTI AZAMAN

July 2017

Chairman : Yeap Swee Keong, PhD
Faculty : Institute of Bioscience

Chlorella has been identified as one of the most interesting microalgae species, which has high nutritional values, high growth rate, and is able to produce a wide range of metabolites in response to environmental changes. The objectives of this study are to characterise the morphology and biochemical contents and to identify the genes and miRNAs involved in regulating the production of carotenoids and lipids in *Chlorella sorokiniana* and *Chlorella zofingiensis* when cultured under high light intensity combined with glucose supplementation. In this study, stress was introduced to the *Chlorella* cultures by adding 2% glucose and increasing the light intensity from 10 to 100 µmol photons m^{-1} s^{-1}. Then, the pigments, total phenolic contents, and antioxidant activities of both *Chlorella* species were evaluated. The results showed that both strains grew larger when cultured under stress condition. Although the total carotenoid content was increased under stress condition, reduction of the pigment and total phenolic contents associated with lower antioxidant activity were also recorded. Subsequently, the transcriptome of *C. sorokiniana* was sequenced using Illumina paired-end sequencing, and 198,844,110 raw reads with the length of 100 bp were produced. After pre-processing, ~95% of high quality reads were *de novo* assembled using Trinity software into 18,310 contigs. Analysis of differential gene expression by DESeq2 package showed that a total of 767 genes were upregulated and 948 genes were downregulated in stress conditions. Then, miRNAs that regulate the genes during normal and stress conditions of both *C. sorokiniana* and *C. zofingiensis* were profiled and analysed using CLC Genomic Workbench and OmiRas. From both analysis pipelines, the known and predicted novel miRNAs were identified. Although most of the identified miRNAs were not functionally determined, this study suggests that they were species-specific, which may have roles in regulating genes during stress condition. In conclusion, identifying the genes and the regulation of various metabolite productions under different growth conditions are useful for further strain enhancement of the microalgae.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN MORFOLOGI, BIOKIMIA DAN TRANSKRIPTOMIK BAGI *Chlorella sorokiniana* DAN *Chlorella zofingiensis* SEMASA KEADAAN NORMAL DAN TEKANAN

Oleh

SITI NOR ANI BINTI AZAMAN

Julai 2017

Pengerusi : Yeap Swee Keong, PhD
Fakulti : Institut Biosains

Chlorella telah dikenalpasti sebagai salah satu spesies microalga yang paling menarik dan mempunyai nilai pemakanan dan kadar pertumbuhan yang tinggi, serta dapat menghasilkan pelbagai metabolit sebagai tindak balas kepada perubahan persekitaran. Objektif kajian ini adalah untuk mencirikan kandungan morfologi dan biokimia dan mengenal pasti gen dan miRNA yang terlibat dalam mengawal pengeluaran karotenoid dan lipid dalam *Chlorella sorokiniana* dan *Chlorella zofingiensis* apabila dibiakkan di bawah keamatan cahaya tinggi yang digabungkan dengan penambahan glukosa. Dalam kajian ini, tekanan telah diberikan kepada kultur *Chlorella* dengan menambahkan 2% glukosa dan meningkatkan keamatan cahaya dari 10 ke 100 µmol foton m^{-1} s^{-1}. Kemudian, pigmen, jumlah kandungan fenol, dan aktiviti bahan antioksida bagi kedua-dua spesies *Chlorella* telah dinilai. Keputusan menunjukkan bahawa kedua-dua strain tumbuh lebih besar apabila dikultur dalam keadaan tekanan. Walaupun jumlah kandungan karotenoid meningkat dibawah keadaan tekanan, penurunan pigmen dan jumlah kandungan fenol yang dikaitkan dengan aktiviti bahan antioksidan yang rendah juga direkodkan. Seterusnya, transkriptom *C. sorokiniana* telah dijujukkan menggunakan teknologi penjujukan hujung berpasangan Illumina, dan sebanyak 198,844,110 jujukan nukleotid mentah dengan panjang 100 bp telah dihasilkan. Setelah dipraproses, ~95% bacaan berkualiti tinggi dihimpunkan secara *de novo* menggunakan perisian Trinity menjadi 18,310 kontig. Analisis pembezaan pengekspresan gen oleh pakej DESeq2 menunjukkan sebanyak 767 gen dikawalatur menaik dan 948 gen dikawalatur menurun dalam keadaan tekanan. Kemudian, miRNA yang mengawal gen semasa keadaan normal dan tekanan kedua-dua *C. sorokiniana* dan *C. zofingiensis* diprofilkan dan dianalisis menggunakan CLC Genomic Workbench dan OmiRas. Berdasarkan kedua-dua saluran kaedah analisis ini, miRNA sedia diketahui serta miRNA ramalan novel telah dikenal pasti. Walaupun kebanyakan miRNA yang dikenal pasti ini tidak ditentukan fungsinya, kajian ini mencadangkan bahawa ia adalah khusus kepada spesies tersebut

yang mungkin mempunyai peranan tertentu dalam mengawalatur gen semasa keadaan tekanan. Kesimpulannya, mengenal pasti gen dan pengawalaturan pengeluaran pelbagai metabolit dalam keadaan pertumbuhan yang berbeza adalah sangat berguna bagi peningkatan strain mikroalga ini.

ACKNOWLEDGEMENTS

ALHAMDULILLAH, all praises to Allah for the strengths and His blessing in completing this thesis. First and foremost, I would like to express my gratitude to my supervisor Dr Yeap Swee Keong. Performing the projects described herein and writing this dissertation would not have been possible without his continuing support and guidance. I would like to thank other members of my committee, Prof Dr Fatimah Md Yusoff, Dr Tan Sheau Wei, Dr Norio Nagao, Prof David Hornby and Dr Qaiser I. Sheikh for their guidance and suggestions. Not forget to my collaborator from Columbia Asia University, Dr Darren C. J. Wong. Through meaningful discussions, they helped me to refine and expand upon my projects, resulting in better work. I thank each of them for their patience and their belief in my abilities. Their knowledge in a broad range of disciplines helped me gain a new perspective and direction on this research project. A special deeply thank to my parents and my family members have been a great support to me through their mutual encouragement. My husband has been incredibly patient and supportive throughout my graduate career. These works are dedicated to him because he is both my motivation and my reason to work hard and to try my best in everything I do. I also extend my thanks to my siblings, Siti Nor Ain, Muhammad Nor Adli and Siti Norhani for their support and sacrifice through my entire life. I would like to extend my special thanks to Ministry of Education that supported my work financially. Last but not least, I am also very grateful for the logistical and personnel support from the Institute of Bioscience and the University of Sheffield.

Thank you so much.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Yeap Swee Keong, PhD
Associate Professor
China-ASEAN College of Marine Sciences
Xiamen Universiti Malaysia
(Chairman)

Fatimah Md Yusoff, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Member)

Tan Sheau Wei, PhD
Research Officer
Institute of Bioscience
Universiti Putra Malaysia
(Member)

Norio Nagao, PhD
Research Officer
Institute of Bioscience
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly acknowledgeg;
- ownership of intellectual property from the thesis is as stipulated in the Memorandum of Agreement (MoA), or as according to the Universiti Putra Malaysia (Research) Rules 2012, in the event where the MoA is absent;
- permission from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) are required prior to publishing it (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, Guide to Thesis Preparation 37 Lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012.
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Siti Nor Ani binti Azaman (GS35310JD)

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Yeap Swee Keong

Signature: _____
Name of Member of Supervisory Committee: Fatimah Md Yusoff

Signature: _____
Name of Member of Supervisory Committee: Tan Sheau Wei

Signature: _____
Name of Member of Supervisory Committee: Norio Nagao

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AAE	Ascorbic acid equivalent
acetyl-ACP	Acetyl-acyl carrier protein
ADP	Adenosine diphosphate
AGPAT	1-acylglycerol-3-phosphate O-acyltransferase
ath-miR	*Arabidopsis thaliana*-microRNA
ATP	Adenosine tryphosphate
BBM	Bold basal medium
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BLAST	Basic local alignment search tool
B-tub	Beta-tubulin
BWA	Burrows-wheeler aligner
CAL	Calmodulin
CAM	Crassulacean acid metabolism
CBF/DREBs	C-repeat-binding factor/dehydration-responsive element
CD	Chloroplast division
CDKA	Cyclin dependent kinase A
Cdna	Complementary DNA
CO2	Carbon dioxide
Cq	Quantification cycle
DAG	Diglycerides or diacylglycerol
DEGs	Differentially expressed genes
DGAT1	Diacylglycerol O-acyltransferase
DMAPP	Dimethylallyl pyrophosphate
DNA	Deoxyribonucleic acid
DOXP	Deoxyxylulose-5-phosphate
DPPH	2,2-diphenyl-1-picrylhydrazyl
DREs	Dehydration-responsive element
dsRNA	Double-stranded RNA
EFA	Essential fatty acids
EST	Expressed sequence tag
FADH	Flavin adenine dinucleotide
FDR	False discovery rate
FRAP	Ferric-reducing antioxidant power
GAE	Gallic acid equivalent
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GC content	Guanine-cytosine content
Gdna	Genomic DNA
GO	Gene ontology
GPAT	Glycerol-3-phosphate acyltransferase
GtRNAdb	Genomic tRNA database
HA	Hatching autospore
HCl	Hydrochloric acid
HKG	Housekeeping gene
HSP90	Heat shock proteins 90
IPP	Isopentenyl pyrophosphate
ITS	Internal transcribed spacer

KAAS	KEGG Automatic Annotation Server
KEGG	Kyoto encyclopedia of genes and genomes
Kmer	Short DNA sequence consisting of a fixed number (K) of bases
KO	KEGG orthology
KOALA	KEGG Orthology And Links Annotation
log_2FC	Log 2 fold change
MA	Mature autospore
MAGs	Monoglycerides or monoacylglycerol
Mbp	Mega base pairs
MEP pathway	Non-mevalonate pathway or Methylerythritol 4-phosphate
miRBase	MicroRNA database
miRNA	MicroRNA
mRNA	Mesengger RNA
N content	If a sequencer is unable to make a base call with sufficient confidence then it will normally substitute an N rather than a conventional base call
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
NaOH	Sodium hydroxide
NCBI	National center for biotechnology information
NGS	Next generation sequencing
NTC	Non-template control
OD	Optical density
ORF	Open reading frame
PC	Phosphatidylcholine
PCA	Principal component analysis
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PEP	Phosphoenolpyruvate
PGM	Phosphoglucomutase
piRNA	Piwi-interacting RNA
PPAP2	Phosphatidate phosphatase
PSII	Photosystem II
PUFA	Polyunsaturated fatty acid
Repbase	Repetitive DNA database
rfam	Non-coding RNA (ncRNA) database
RNA	Ribonucleic acid
RNA-SEQ	RNA sequencing
ROS	Reactive oxygen species
RPL19	Ribosomal protein L19
rRNA	Ribosomal RNA
RT-QPCR	Quantitative reverse transcription PCR
SAGE	Serial analysis of gene expression
SEM	Scanning electron microscope
siRNA	Small interfering RNA
snoRNA	Small nucleolar RNAs
snoRNABase	Small nucleolar RNA database
SNP	Single nucleotide polymorphism
snRNA	Small nuclear RNA
spp	Several species
SSU	Small subunit

TAE	Tris base, acetic acid and EDTA
TAG	Triglycerides or triacylglycerol
TCA cycle	Tricarboxylic acid or citric acid cycle
TEM	Transmission electron microscope
TPC	Total phenolic content
tRNA	Transfer RNA
UBQ	Ubiquitin
UCP	Universal chlorophyte primer
UV	Ultraviolet
UV/VIS	Ultraviolet /visible
UV-B	Ultraviolet B
VST	Variance stabilization normalization

CHAPTER 1

GENERAL INTRODUCTION

Microalgae have been identified as good source of lipids for biofuels, protein and health metabolites including polyphenols, vitamins and antioxidants (Mostafa 2012). *Chlorella* sp. has received considerable attention, in view of its relatively high nutritional value, its capacity to modify its metabolites in response to changes in its growth medium, relatively rapid rates of reproduction and the possession of a thick cell wall that protect its nutrients (Iwamoto 2004). The microalgae *Chlorella* can produce an unusually wide range of metabolites during both normal growth and growth under stress (de Morais et al. 2015). It is well known that different microalgae produce different metabolites during stress (Skjanes et al. 2013).

In this study, two species of microalgae *Chlorella sorokiniana* and *Chlorella zofingiensis* were selected and evaluated based on their potential for producing high value metabolites under different culture conditions. The relative concentrations and profiles of various metabolites in microalgae are similar under optimal growth. However, the metabolite profile undergoes considerable changes under sub-optimal growth. Different algae species use different methods for managing these changes in the environment. Depending on their ability to handle various stresses, the microalgae produce different secondary metabolites to increase their chances of survival (Skjanes et al. 2013).

Numerous studies concerning the effects of stress upon the metabolome of microalgae have been documented in the literature (Ip and Chen 2005a; Lemoine and Schoefs 2010). However, our understanding of how microalgae respond to physiological stress at the molecular level is largely confined to model organisms, and the relevant pathways in microalgae have not been fully documented. Furthermore, the size and the appearance of microalgae can change profoundly depending on the environmental conditions and associated levels and types of stress. There is a clear need to improve the set of molecular methods and tools for working with microalgae. Previous studies suggested that, when the microalgae *Chlorella* was grown under different growth condition, the colour of the *Chlorella* changed from green to red or yellow, as a consequences of contributed by the pigment production (Ip et al. 2004; Del Campo et al. 2004; Ip and Chen 2005b; Cordero et al. 2011).

Phenotypic details surrounding the influence of stress on the production of pigments and metabolites that contribute to microalgae survival, can be understood by performing comparative transcriptome profiling (Fu et al. 2014; Sun et al. 2015). Transcriptomic study is an appropriate tool which provides an initial, broad view of the regulation of secondary metabolite biosynthetic pathways during a microalgal stress response. So far, the studies have focused on growth experiments and metabolite content screening, and have yielded

very limited information in respect of gene expression, in microalgae under normal and stress conditions (Doan et al. 2011; Perez-Garcia et al. 2011; Baba and Shiraiwa 2013; Goiris et al. 2015). With the discovery of specific classes of RNA molecules as gene regulatory in addition to the more widely accepted protein based transcriptional regulatory (Bartel 2004), transcriptome sequencing can provide a valuable approach for obtaining microalgae functional genomics information. However, transcriptomes relating to the biosynthetic pathways of lipids, polyphenols and vitamin D of *Chlorella* species remain to be profiled. Therefore, it is important to study the transcriptomic profile as well as the metabolite composition of *Chlorella.* It is important and timely to determine the true potential of these species and to support the potential for genetic engineering of these microalgae as they become an increasing focus for their development as alternative source of biofuel, food and health supplements.

In this study, *C. sorokiniana* and *C. zofingiensis*, two *Chlorella* species that exhibit relatively fast growth rates and have potential to produce high yields of secondary metabolites, including secondary pigments, during mixothropic growth were chosen. By performing systematic studies including both physiological (morphological and biochemical) and molecular response of both *Chlorellas* towards normal and stress conditions, this study would provide necessary information for understanding the molecular basis of some important metabolite biosynthesis pathways such as lipids and carotenoids.

1.1 Statement of the hypotheses

The hypotheses of this study are:

1. High light intensity combined with glucose supplementation that increase pigments and lipids production would alter antioxidant activity in *C. sorokiniana* and *C. zofingiensis*.

2. Transcriptomic analysis would reveal the upregulation of genes involve in carotenoid and lipid biosynthesis pathways of *C. sorokiniana* and *C. zofingiensis* when cultured under high light intensity combined with glucose supplementation.

3. Small RNA transcriptomic analysis would reveal the specific miRNA involve in regulating the production of carotenoid and lipid in *C. sorokiniana and C. zofingiensis* when cultured under high light intensity combined with glucose supplementation.

1.2 Research objectives

The objectives of this study are:

1. To characterise the morphology and biochemical contents (such as pigments, phenolic and antioxidant) of *C. sorokiniana* and *C. zofingiensis* under normal and high light intensity combined with glucose supplementation stress conditions.

2. To identify the genes involved in regulating the production of carotenoids and lipids in *C. sorokiniana and C. zofingiensis* when cultured under high light intensity combined with glucose supplementation through RNA-sequencing technique.

3. To identify the miRNAs involved in regulating the production of carotenoids and lipids in *C. sorokiniana and C. zofingiensis* when cultured under high light intensity combined with glucose supplementation through small-RNA sequencing technique.

REFERENCES

Abdel-Raouf, N., A. Al-Homaidan & I. Ibraheem (2012) Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences,* 19**,** 257-275.

Adams, J. (2008) Transcriptome: connecting the genome to gene function. *Nature Education,* 1**,** 195.

Adelfi, M. G., M. Borra, R. Sanges, M. Montresor, A. Fontana & M. I. Ferrante (2014) Selection and validation of reference genes for qPCR analysis in the pennate diatoms *Pseudo-nitzschia multistriata* and *P. arenysensis*. *Journal of Experimental Marine Biology and Ecology,* 451**,** 74-81.

Ahn, J.-W., K. Hwangbo, S. Y. Lee, H.-G. Choi, Y.-I. Park, J. R. Liu & W.-J. Jeong (2012) A new Arctic *Chlorella* species for biodiesel production. *Bioresource Technology,* 125**,** 340-343.

Alberts, B., A. Johnsen & J. Lewis (2002) *From RNA to Protein*. New York: Garland Science.

Allen, A. E., A. Vardi & C. Bowler (2006) An ecological and evolutionary context for integrated nitrogen metabolism and related signaling pathways in marine diatoms. *Current Opinion in Plant Biology,* 9**,** 264-273.

Armbrust, E. V., J. A. Berges, C. Bowler, B. R. Green, D. Martinez, N. H. Putnam, S. Zhou, A. E. Allen, K. E. Apt, M. Bechner, M. A. Brzezinski, B. K. Chaal, A. Chiovitti, A. K. Davis, M. S. Demarest, J. C. Detter, T. Glavina, D. Goodstein, M. Z. Hadi, U. Hellsten, M. Hildebrand, B. D. Jenkins, J. Jurka, V. V. Kapitonov, N. Kroger, W. W. Lau, T. W. Lane, F. W. Larimer, J. C. Lippmeier, S. Lucas, M. Medina, A. Montsant, M. Obornik, M. S. Parker, B. Palenik, G. J. Pazour, P. M. Richardson, T. A. Rynearson, M. A. Saito, D. C. Schwartz, K. Thamatrakoln, K. Valentin, A. Vardi, F. P. Wilkerson & D. S. Rokhsar (2004) The genome of the diatom Thalassiosira pseudonana: ecology, evolution, and metabolism. *Science,* 306**,** 79-86.

Arnao, M. B. (2000) Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practical case. *Trends in Food Science & Technology,* 11**,** 419-421.

Arya, M., I. S. Shergill, M. Williamson, L. Gommersall, N. Arya & H. R. Patel (2005) Basic principles of real-time quantitative PCR. *Expert Review of Molecular Diagnostics,* 5**,** 209-219.

Avagyan, A (2008) Microalgae production development global prospects and profitable technology wasterwater purification by the use microalgae. In *Water and Wastewater International*.

Baba, M. & Y. Shiraiwa (2013) Biosynthesis of lipids and hydrocarbons in algae. In *Photosynthesis,* ed. Z. Dubinsky, 331-356. Croatia: InTech.

Baker, M. (2010) Next-generation sequencing: adjusting to data overload. *Nature Methods,* 7**,** 495-499.

Ball, S., H.-P. Guan, M. James, A. Myers, P. Keeling, G. Mouille, A. Buléon, P. Colonna & J. Preiss (1996) From glycogen to amylopectin: a model for the biogenesis of the plant starch granule. *Cell,* 86**,** 349-352.

Ballicora, M. A., A. A. Iglesias & J. Preiss (2003) ADP-glucose pyrophosphorylase: a regulatory enzyme for bacterial glycogen synthesis. *Microbiology and Molecular Biology Reviews,* 67**,** 213-225.

Barclay, W., K. Meager & J. Abril (1994) Heterotrophic production of long chain omega-3 fatty acids utilizing algae and algae-like microorganisms. *Journal of Applied Phycology,* 6**,** 123-129.

Bartel, D. P. (2004) MicroRNAs-genomics, biogenesis, mechanism, and function. *Cell,* 116**,** 281-297.

Battchikova, N., E.-M. Aro & P. J. Nixon (2011) Structure and physiological function of NDH-1 complexes in cyanobacteria. In *Bioenergetic Processes of Cyanobacteria,* eds. G. A. Peschek, C. Obinger & G. Renger, 445-467. Springer.

Becker, E. W. (2007) Micro-algae as a source of protein. *Biotechnology Advances,* 25**,** 207-10.

Benemann, J. R. (1992) Microalgae aquaculture feeds. *Journal of Applied Phycology,* 4**,** 233-245.

Berg, J., J. Tymoczko & L. Stryer (2002a) Glycogen breakdown requires the interplay of several enzymes. In *Biochemistry,* ed. W. H. Freeman. New York.

Berg, J., J. Tymoczko & L. Stryer (2002b) The glycolytic pathway is tightly controlled. In *Biochemistry,* ed. W. H. Freeman. New York.

Berthelot, K., Y. Estevez, A. Deffieux & F. Peruch (2012) Isopentenyl diphosphate isomerase: A checkpoint to isoprenoid biosynthesis. *Biochimie,* 94**,** 1621-1634.

Blanc, G., I. Agarkova, J. Grimwood, A. Kuo, A. Brueggeman, D. D. Dunigan, J. Gurnon, I. Ladunga, E. Lindquist, S. Lucas, J. Pangilinan, T. Proschold, A. Salamov, J. Schmutz, D. Weeks, T. Yamada, A. Lomsadze, M. Borodovsky, J. M. Claverie, I. V. Grigoriev & J. L. Van Etten (2012) The genome of the polar eukaryotic microalga *Coccomyxa subellipsoidea* reveals traits of cold adaptation. *Genome Biology,* 13**,** R39.

Blanc, G., G. Duncan, I. Agarkova, M. Borodovsky, J. Gurnon, A. Kuo, E. Lindquist, S. Lucas, J. Pangilinan & J. Polle (2010) The *Chlorella*

variabilis NC64A genome reveals adaptation to photosymbiosis, coevolution with viruses, and cryptic sex. *The Plant Cell Online,* 22**,** 2943-2955.

Bock, C., L. Krienitz & T. Proschold (2011) Taxonomic reassessment of the genus Chlorella (Trebouxiophyceae) using molecular signatures (barcodes), including description of seven new species. *Fottea,* 11**,** 293-312.

Boldt, L., D. Yellowlees & W. Leggat (2008) Measuring *Symbiodinium* sp. gene expression patterns with quantitative real-time PCR. *Proceedings of the 11th ICRS***,** 118-122.

Borowitzka, M. A. (1995) Microalgae as sources of pharmaceuticals and other biologically active compounds. *Journal of Applied Phycology,* 7**,** 3-15.

Borowitzka, M. A. (1999) Commercial production of microalgae: ponds, tanks, tubes and fermenters. *Journal of Biotechnology,* 70**,** 313-321.

Boycott, K. M., M. R. Vanstone, D. E. Bulman & A. E. MacKenzie (2013) Rare-disease genetics in the era of next-generation sequencing: discovery to translation. *Nature Reviews Genetics,* 14**,** 681-691.

Brody, M. & A. E. Vatter (1959) Observations on cellular structures of *Porphyridium cruentum*. *The Journal of Biophysical and Biochemical Cytology,* 5**,** 289-293.

Brown, T. A (2006) Genomes. 3-30. Garland science.

Busi, M. V., J. Barchiesi, M. Martín & D. F. Gomez-Casati (2014) Starch metabolism in green algae. *Starch-Stärke,* 66**,** 28-40.

Bustin, S., H. S. Dhillon, S. Kirvell, C. Greenwood, M. Parker, G. L. Shipley & T. Nolan (2015) Variability of the Reverse Transcription Step: Practical Implications. *Clinical chemistry,* 61**,** 202-212.

Cadoret, J.-P., M. Garnier & B. Saint-Jean (2012) Microalgae, functional genomics and biotechnology. *Advances in Botanical Research,* 64**,** 285-341.

Cagliari, A., R. Margis, F. dos Santos Maraschin, A. C. Turchetto-Zolet, G. Loss & M. Margis-Pinheiro (2011) Biosynthesis of triacylglycerols (TAGs) in plants and algae. *International Journal of Plant Biology,* 2**,** 10.

Cardozo, K. H., T. Guaratini, M. P. Barros, V. R. Falcão, A. P. Tonon, N. P. Lopes, S. Campos, M. A. Torres, A. O. Souza & P. Colepicolo (2007) Metabolites from algae with economical impact. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 146**,** 60-78.

Carrington, J. C. & V. Ambros (2003) Role of microRNAs in plant and animal development. *Science,* 301**,** 336-338.

Carvalho, A. P., S. O. Silva, J. M. Baptista & F. X. Malcata (2011) Light requirements in microalgal photobioreactors: an overview of biophotonic aspects. *Applied Microbiology and Biotechnology,* 89**,** 1275-1288.

Catalanotti, C., W. Yang, M. C. Posewitz & A. R. Grossman (2013) Fermentation metabolism and its evolution in algae. *Frontiers in Plant Science,* 4.

Cerutti, H., X. Ma, J. Msanne & T. Repas (2011) RNA-mediated silencing in algae: biological roles and tools for analysis of gene function. *Eukaryotic cell,* 10**,** 1164-1172.

Chacón-Lee, T. L. & G. E. González-Mariño (2010) Microalgae for "healthy" foods—Possibilities and challenges. *Comprehensive Reviews in Food Science and Food Safety,* 9**,** 655-675.

Chen, C.-Y., K.-L. Yeh, R. Aisyah, D.-J. Lee & J.-S. Chang (2011) Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. *Bioresource Technology,* 102**,** 71-81.

Chen, C.-Y., X.-Q. Zhao, H.-W. Yen, S.-H. Ho, C.-L. Cheng, D.-J. Lee, F.-W. Bai & J.-S. Chang (2013) Microalgae-based carbohydrates for biofuel production. *Biochemical Engineering Journal,* 78**,** 1-10.

Chen, C. Y. & D. G. Celia (1994) Effects of pH on the growth and carbon uptake of marine phytoplankton. *Marine Ecology Progress Series,* 109**,** 83-94.

Cheng, D. & Q. He (2014) Assessment of environmental stresses for enhanced microalgal biofuel production—an overview. *Frontiers in Energy Research,* 2**,** 1-26.

Chia, M. A., A. T. Lombardi & G. Melao Mda (2013) Growth and biochemical composition of *Chlorella vulgaris* in different growth media. *Annals of the Brazilian Academy of Sciences,* 85**,** 1427-1438.

Chiou, T.-J. (2007) The role of microRNAs in sensing nutrient stress. *Plant, Cell & Environment,* 30**,** 323-332.

Chisti, Y. (2007) Biodiesel from microalgae. *Biotechnology advances,* 25**,** 294-306.

Chokshi, K., I. Pancha, K. Trivedi, B. George, R. Maurya, A. Ghosh & S. Mishra (2015) Biofuel potential of the newly isolated microalgae *Acutodesmus dimorphus* under temperature induced oxidative stress conditions. *Bioresource Technology,* 180**,** 162-171.

Chung, C.-C., S.-P. L. Hwang & J. Chang (2008) Nitric oxide as a signaling factor to upregulate the death-specific protein in a marine diatom, *Skeletonema costatum*, during blockage of electron flow in photosynthesis. *Applied and Environmental Microbiology,* 74**,** 6521-6527.

Cifuentes, A. S., M. A. González, I. Inostroza & A. Aguilera (2001) Reappraisal of physiological attributes of nine strains of *Dunaliella* (Chlorophyceae): growth and pigment content across a salinity gradient. *Journal of Phycology,* 37**,** 334-344.

Colleoni, C., D. Dauvillée, G. Mouille, M. Morell, M. Samuel, M.-C. Slomiany, L. Liénard, F. Wattebled, C. d'Hulst & S. Ball (1999) Biochemical characterization of the *Chlamydomonas reinhardtii* α-1, 4 glucanotransferase supports a direct function in amylopectin biosynthesis. *Plant Physiology,* 120**,** 1005-1014.

Conesa, A., P. Madrigal, S. Tarazona, D. Gomez-Cabrero, A. Cervera, A. McPherson, M. W. Szcześniak, D. J. Gaffney, L. L. Elo & X. Zhang (2016) A survey of best practices for RNA-seq data analysis. *Genome Biology,* 17**,** 19.

Cordero, B. F., I. Obraztsova, I. Couso, R. Leon, M. A. Vargas & H. Rodriguez (2011) Enhancement of lutein production in *Chlorella sorokiniana* (Chorophyta) by improvement of culture conditions and random mutagenesis. *Marine Drugs,* 9**,** 1607-1624.

Cox, S., N. Abu-Ghannam & S. Gupta (2010) An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *International Food Research Journal* 17**,** 205-220.

Crane, K. W. & J. P. Grover (2010) Coexistence of mixotrophs, autotrophs, and heterotrophs in planktonic microbial communities. *Journal of Theoretical Biology,* 262**,** 517-527.

D'haene, B. & J. Hellemans (2010) The importance of quality control during qPCR data analysis. *International Drug Discovery***,** 18-24.

da Silva Gorgônio, C. M., D. A. G. Aranda & S. Couri (2013) Morphological and chemical aspects of *Chlorella pyrenoidosa*, *Dunaliella tertiolecta, Isochrysis galbana* and *Tetraselmis gracilis* microalgae. *Natural Science* 5**,** 783-791

Dalrymple, O. K., T. Halfhide, I. Udom, B. Gilles, J. Wolan, Q. Zhang & S. Ergas (2013) Wastewater use in algae production for generation of renewable resources: a review and preliminary results. *Aquatic Biosystems,* 9**,** 2.

de Morais, M. G., B. d. S. Vaz, E. G. de Morais & J. A. V. Costa (2015) Biologically active metabolites synthesized by microalgae. *BioMed Research International*.

Del Campo, J. A., H. Rodriguez, J. Moreno, M. A. Vargas, J. Rivas & M. G. Guerrero (2004) Accumulation of astaxanthin and lutein in *Chlorella zofingiensis* (Chlorophyta). *Applied Microbiology Biotechnology,* 64**,** 848-854.

Derelle, E., C. Ferraz, P. Lagoda, S. Eychenié, R. Cooke, F. Regad, X. Sabau, C. Courties, M. Delseny & J. Demaille (2002) DNA Libraries for Sequencing the Genome of *Ostreococcus tauri* (Chlorophyta, Pasinophyceae): The smallest Free-Living Eukarytoic Cell. *Journal of phycology,* 38**,** 1150-1156.

Dewick, P. M. (2002) The biosynthesis of C 5–C 25 terpenoid compounds. *Natural Product Reports,* 19**,** 181-222.

Dhull, N. P., R. Soni, D. K. Rahi & S. K. Soni (2014) Evaluation of autotrophic and mixotrophic regimen *Chlorella pyrenoidosa* cells in various wastes water for its biochemical composition and biomass production. *Peer Journal,* 2.

Dittami, S. M., I. Riisberg, U. John, R. J. Orr, K. S. Jakobsen & B. Edvardsen (2012) Analysis of expressed sequence tags from the marine microalga *Pseudochattonella farcimen* (Dictyochophyceae). *Protist,* 163**,** 143-161.

Doan, T. T. Y., B. Sivaloganathan & J. P. Obbard (2011) Screening of marine microalgae for biodiesel feedstock. *Biomass and Bioenergy,* 35**,** 2534-2544.

Dong, M., X. Zhang, X. Chi, S. Mou, J. Xu, D. Xu, W. Wang & N. Ye (2012) The validity of a reference gene is highly dependent on the experimental conditions in green alga *Ulva linza*. *Current Genetics,* 58**,** 13-20.

Dongre, S. K., S. Manglawat, P. Singh, M. Yadav & A. Tiwari (2014) Effect of environmental parameters enhancing the microalgal lipid as a sustainable energy source for biodiesel production-A Review. *International Journal of Biological & Pharmaceutical Research,* 5**,** 327-335.

Dubinsky, Z. & N. Stambler (2009) Photoacclimation processes in phytoplankton: mechanisms, consequences, and applications. *Aquatic Microbiol Ecology,* 56**,** 163-176.

Ducat, D. C. & P. A. Silver (2012) Improving carbon fixation pathways. *Current Opinion in Chemical Biology,* 16**,** 337-344.

Eckardt, N. A. (2010) The *Chlorella* genome: big surprises from a small package. *Plant Cell,* 22**,** 2924.

Ekblom, R., & Galindo, J. (2011). Applications of next generation sequencing in molecular ecology of non-model organisms. *Heredity*, 107(1), 1–15. http://doi.org/10.1038/hdy.2010.152

Elsabahy, M., A. Nazarali & M. Foldvari (2011) Non-viral nucleic acid delivery: key challenges and future directions. *Current Drug Delivery,* 8**,** 235-244.

Eussen, S., O. Klungel, J. Garssen, H. Verhagen, H. van Kranen, H. van Loveren & C. Rompelberg (2010) Support of drug therapy using functional foods and dietary supplements: focus on statin therapy. *British Journal of Nutrition,* 103**,** 1260-1277.

Fan, J., Y. Cui, M. Wan, W. Wang & Y. Li (2014) Lipid accumulation and biosynthesis genes response of the oleaginous *Chlorella pyrenoidosa* under three nutrition stressors. *Biotechnology for Biofuels,* 7**,** 1-14.

Fan, J., K. Ning, X. Zeng, Y. Luo, D. Wang, J. Hu, J. Li, H. Xu, J. Huang & M. Wan (2015) Genomic Foundation of Starch-to-Lipid Switch in Oleaginous *Chlorella* spp. *Plant Physiology,* 169**,** 2444-2461.

Feldmesser, E., S. Rosenwasser, A. Vardi & S. Ben-Dor (2014) Improving transcriptome construction in non-model organisms: integrating manual and automated gene definition in *Emiliania huxleyi*. *BMC Genomics,* 15**,** 1-16.

Feng, P., Z. Deng, L. Fan & Z. Hu (2012) Lipid accumulation and growth characteristics of *Chlorella zofingiensis* under different nitrate and phosphate concentrations. *Journal of Bioscience and Bioengineering,* 114**,** 405-410.

Fields, M. W., A. Hise, E. J. Lohman, T. Bell, R. D. Gardner, L. Corredor, K. Moll, B. M. Peyton, G. W. Characklis & R. Gerlach (2014) Sources and resources: importance of nutrients, resource allocation, and ecology in microalgal cultivation for lipid accumulation. *Applied Microbiology and Biotechnology,* 98**,** 4805-4816.

Fillmore, N., O. A. Alrob & G. D. Lopaschuk (2011) Fatty Acid beta-Oxidation.

Fleige, S., & Pfaffl, M. W. (2006). RNA integrity and the effect on the real-time qRT-PCR performance. *Molecular aspects of medicine,* 27(2), 126-139.

Fischer, B. B., A. Krieger-Liszkay, É. Hideg, I. Šnyrychová, M. Wiesendanger & R. I. Eggen (2007) Role of singlet oxygen in chloroplast to nucleus retrograde signaling in *Chlamydomonas reinhardtii*. *FEBS Letters,* 581**,** 5555-5560.

Fryera, R. M., J. Randalla, T. Yoshidaa, L.-L. Hsiao, J. Blumenstock, K. E. Jensen, T. Dimofte, R. V. Jensen & S. R. Gullans (2002) Global Analysis of Gene Expression: Methods, Interpretation, and Pitfalls. *Experimental Nephrology,* 10**,** 64-74.

Fu, X., D. Wang, X. Yin, P. Du & B. Kan (2014) Time course transcriptome changes in *Shewanella algae* in response to salt stress. *PloS one,* 9**,** e96001.

Fučíková, K. & L. A. Lewis (2012) Intersection of *Chlorella, Muriella and Bracteacoccus*: Resurrecting the genus *Chromochloris* KOL et CHODAT (Chlorophyceae, Chlorophyta). *Fottea,* 12**,** 83-93.

Gao, C., Y. Wang, Y. Shen, D. Yan, X. He, J. Dai & Q. Wu (2014) Oil accumulation mechanisms of the oleaginous microalga *Chlorella protothecoides* revealed through its genome, transcriptomes, and proteomes. *BMC Genomics,* 15.

Gallardo-Escárate, C., Valenzuela-Muñoz, V., & Nuñez-Acuña, G. (2014). RNA-Seq analysis using *de novo* transcriptome assembly as a reference for the salmon louse *Caligus rogercresseyi*. *PloS one,* 9(4), e92239.

George, B., I. Pancha, C. Desai, K. Chokshi, C. Paliwal, T. Ghosh & S. Mishra (2014) Effects of different media composition, light intensity and photoperiod on morphology and physiology of freshwater microalgae *Ankistrodesmus falcatus*–A potential strain for biofuel production. *Bioresource Technology,* 171**,** 367-374.

Giorgi, F. M., C. Del Fabbro & F. Licausi (2013) Comparative study of RNA-seq-and Microarray-derived coexpression networks in *Arabidopsis thaliana*. *Bioinformatics,* 29**,** 717-724.

Goiris, K., K. Muylaert, I. Fraeye, I. Foubert, J. De Brabanter & L. De Cooman (2012) Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. *Journal of Applied Phycology,* 24**,** 1477-1486.

Goiris, K., W. Van Colen, I. Wilches, F. León-Tamariz, L. De Cooman & K. Muylaert (2015) Impact of nutrient stress on antioxidant production in three species of microalgae. *Algal Research,* 7**,** 51-57.

Gouveia, L., V. Veloso, A. Reis, H. Fernandes, J. Novais & J. Empis (1996) Evolution of pigment composition in *Chlorella vulgaris*. *Bioresource Technology,* 57**,** 157-163.

Grabherr, M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury & Q. Zeng (2011a) Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology,* 29.

Grabherr, M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury & Q. Zeng (2011b) Trinity-reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nature Biotechnology,* 29**,** 644-652.

Graham, L. E. & L. W. Wilcox (1999) *Algae*. Upper Saddle River, New Jersey: Prentice Hall.

Grünewald, K., J. Hirschberg & C. Hagen (2001) Ketocarotenoid biosynthesis outside of plastids in the unicellular green alga *Haematococcus pluvialis*. *Journal of Biological Chemistry,* 276**,** 6023-6029.

Guarnieri, M. T., A. Nag, S. L. Smolinski, A. Darzins, M. Seibert & P. T. Pienkos (2011) Examination of triacylglycerol biosynthetic pathways via de novo transcriptomic and proteomic analyses in an unsequenced microalga. *PLoS One,* 6**,** e25851.

Guckert, J. B. & K. E. Cooksey (1990) Triglyceride accumulation and fatty acid profile changes in *Chlorella* (Chlorophyta) during high pH-induced cell cycle inhibition. *Journal of Phycology,* 26**,** 72-79.

Guedes, A. C., H. M. Amaro & F. X. Malcata (2011) Microalgae as sources of carotenoids. *Marine Drugs,* 9**,** 625-644.

Guiry, M. D. (2012) How many species of algae are there? *Journal of Phycology,* 48**,** 1057-1063.

Guleria, P., M. Mahajan, J. Bhardwaj & S. K. Yadav (2011) Plant small RNAs: biogenesis, mode of action and their roles in abiotic stresses. *Genomics, Proteomics & Bioinformatics,* 9**,** 183-199.

Guo, R. & J.-S. Ki (2012) Evaluation and validation of internal control genes for studying gene expression in the dinoflagellate *Prorocentrum minimum* using real-time PCR. *European Journal of Protistology,* 48**,** 199-206.

Gupta, S., A. Jha, A. Pal & G. Venkateshwarlu (2007) Use of natural carotenoids for pigmentation in fishes. *Natural Product Radiance,* 6**,** 46-49.

Hall, T. A (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic acids symposium series*, 95-98.

Hammouda, O., A. Gaber & N. Abdelraouf (1995) Microalgae and wastewater treatment. *Ecotoxicology and Environmental safety,* 31**,** 205-210.

He, L. & G. J. Hannon (2004) MicroRNAs: small RNAs with a big role in gene regulation. *Nature Reviews Genetics,* 5**,** 522-531.

Hemaiswarya, S., R. Raja, R. R. Kumar, V. Ganesan & C. Anbazhagan (2011) Microalgae: a sustainable feed source for aquaculture. *World Journal of Microbiology and Biotechnology,* 27**,** 1737-1746.

Hemalatha, A., K. Girija, C. Parthiban, C. Saranya & P. Anantharaman (2013) Antioxidant properties and total phenolic content of a marine diatom, green microalgae. *Advances in Applied Science Research,* 4**,** 115-157.

Hering, D., R. K. Johnson & A. Buffagni (2006) Linking organism groups—major results and conclusions from the STAR project. In *The Ecological Status of European Rivers: Evaluation and Intercalibration of Assessment Methods,* eds. M. T. Furse, D. Hering, K. Brabec, A. Buffagni, L. Sandin & P. Verdonschot, 109-113. Netherlands: Springer.

Herranz, H. & S. M. Cohen (2010) MicroRNAs and gene regulatory networks: managing the impact of noise in biological systems. *Genes & Development,* 24**,** 1339-1344.

Hindák, F. (1982) Taxonomic position of the chlorococcal alga *Chlorella zofingiensis* Dönz 1934 (Chlorophyceae). *Algological Studies,* 40**,** 13-23.

Hirata, S., M. Taya & S. Tone (1996) Characterization of *Chlorella* cell cultures in batch and continuous operations under a photoautotrophic condition. *Journal of Chemical Engineering of Japan,* 29**,** 953-959.

Hong, S.-J. & C.-G. Lee (2015) Microalgal Systems Biology for Biofuel Production. In *Algal Biorefineries*, 3-21. Springer.

Hörtensteiner, S. (1999) Chlorophyll breakdown in higher plants and algae. *Cellular and Molecular Life Sciences,* 56**,** 330-347.

Hoshina, R. & Y. Fujiwara (2013) Molecular characterization of *Chlorella* cultures of the National Institute for Environmental Studies culture collection with description of *Micractinium inermum* sp. nov., *Didymogenes sphaerica* sp. nov., and *Didymogenes soliella* sp. nov.(Chlorellaceae, Trebouxiophyceae). *Phycological Research,* 61**,** 124-132.

Hu, Q (2004) Environmental Effects on Cell Composition. In *Handbook of Microalgal Culture: Biotechnology and Applied Phycology,* ed. A. Richmond, 83-93. Blackwell Publishing Ltd.

Hu, Q., M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert & A. Darzins (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant Journal,* 54**,** 621-639.

Huss, V. A. R. & M. L. Sogin (1990) Phylogenetic position of some *Chlorella* species within Chlorococales based on SSu rRNA. *Journal of Molecular Evolution,* 31**,** 432-442.

Hwang, S., K.-H. Choi, J. Kim & J. Cha (2013) Biochemical characterization of 4-α-glucanotransferase from *Saccharophagus degradans* 2-40 and its potential role in glycogen degradation. *FEMS Microbiology Letters,* 344**,** 145-151.

Ip, P.-F. & F. Chen (2005a) Employment of reactive oxygen species to enhance astaxanthin formation in *Chlorella zofingiensis* in heterotrophic culture. *Process Biochemistry,* 40**,** 3491-3496.

Ip, P.-F. & F. Chen (2005b) Production of astaxanthin by the green microalga *Chlorella zofingiensis* in the dark. *Process Biochemistry,* 40**,** 733-738.

Ip, P.-F., K.-H. Wong & F. Chen (2004) Enhanced production of astaxanthin by the green microalga *Chlorella zofingiensis* in mixotrophic culture. *Process Biochemistry,* 39**,** 1761-1766.

Iwamoto, H (2004) *Chapter 11. Industrial production of microalgal cell-mass and secondary products–major industrial species : Chlorella*. Oxford, UK. : Blackwell Publishing Ltd.

Jia, L., D. Zhang, X. Qi, B. Ma, Z. Xiang & N. He (2014) Identification of the conserved and novel miRNAs in mulberry by high-throughput sequencing. *PloS One,* 9**,** e104409.

Jiang, Y. & F. Chen (2000) Effects of temperature and temperature shift on docosahexaenoic acid production by the marine microalge *Crypthecodinium cohnii*. *Journal of the American Oil Chemists' Society,* 77**,** 613-617.

Jiang, Y. J. & K. R. Feingold (2011) The expression and regulation of enzymes mediating the biosynthesis of triglycerides and phospholipids in keratinocytes/epidermis. *Dermato-endocrinology,* 3**,** 70-76.

Jones-Rhoades, M. W., & Bartel, D. P. (2004). Computational identification of plant microRNAs and their targets, including a stress-induced miRNA. *Molecular cell,* 14(6), 787-799.

Juneja, A., R. M. Ceballos & G. S. Murthy (2013) Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: a review. *Energies,* 6**,** 4607-4638.

Khraiwesh, B., J.-K. Zhu & J. Zhu (2012) Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms,* 1819**,** 137-148.

Knothe, G. (2010) Biodiesel: current trends and properties. *Topics in Catalysis,* 53**,** 714-720.

Koboldt, D. (2014) New challenges of next-gen sequencing. *Sequencing and Genomics,* 2016.

Kollas, A.-K., E. C. Duin, M. Eberl, B. Altincicek, M. Hintz, A. Reichenberg, D. Henschker, A. Henne, I. Steinbrecher & D. N. Ostrovsky (2002) Functional characterization of GcpE, an essential enzyme of the non-mevalonate pathway of isoprenoid biosynthesis. *FEBS Letters,* 532**,** 432-436.

Kramer, D. M. & J. R. Evans (2011) The importance of energy balance in improving photosynthetic productivity. *Plant Physiology,* 155**,** 70-78.

Krienitz, L., E. H. Hegewald, D. Hepperle, V. A. Huss, T. Rohr & M. Wolf (2004) Phylogenetic relationship of *Chlorella* and *Parachlorella* gen. nov.(Chlorophyta, Trebouxiophyceae). *Phycologia,* 43**,** 529-542.

Krienitz, L., V. A. Huss & C. Bock (2015) *Chlorella*: 125 years of the green survivalist. *Trends Plant Sci,* 20**,** 67-9.

Kröger, M. & F. Müller-Langer (2011) Impact of heterotrophic and mixotrophic growth of microalgae on the production of future biofuels. *Biofuels,* 2**,** 145-151.

Kuhn, D. E., Martin, M. M., Feldman, D. S., Terry, A. V., Nuovo, G. J., & Elton, T. S. (2008). Experimental validation of miRNA targets. *Methods,* 44(1), 47-54.

Kumar, S., N. Gupta, S. Kumar, V. Yadav, A. Prakash & H. Gurjar (2014) Metabolites in plants and its classification. *World Journal of Pharmacy and Pharmaceutical Sciences,* 4**,** 287-305.

Kuzuyama, T. (2002) Mevalonate and nonmevalonate pathways for the biosynthesis of isoprene units. *Bioscience, Biotechnology, and Biochemistry,* 66**,** 1619-1627.

Lakeman, M. B., P. von Dassow & R. A. Cattolico (2009) The strain concept in phytoplankton ecology. *Harmful Algae,* 8**,** 746-758.

Lane, A. E. & J. E. Burris (1981) Effects of environmental pH on the internal pH of *Chlorella pyrenoidosa*, *Scenedesmus quadricauda*, and *Euglena mutabilis*. *Plant Physiology,* 68**,** 439-442.

Latasa, M. & E. Berdalet (1994) Effect of nitrogen or phosphorus starvation on pigment composition of cultured *Heterocapsa* sp. *Journal of Plankton Research,* 16**,** 83-94.

Latendresse, M., S. Paley & P. D. Karp (2012) Browsing metabolic and regulatory networks with BioCyc. In *Bacterial Molecular Networks,* eds. J. v. Helden, A. Toussaint & D. Thieffry, 197-216. Springer.

Lemoine, Y. & B. Schoefs (2010) Secondary ketocarotenoid astaxanthin biosynthesis in algae: a multifunctional response to stress. *Photosynthesis research,* 106**,** 155-177.

León, R. & F. Galván (1999) Interaction between saline stress and photoinhibition of photosynthesis in the freshwater green algae *Chlamydomonas reinhardtii*. Implications for glycerol photoproduction. *Plant Physiology and Biochemistry,* 37**,** 623-628.

Leopoldini, M., N. Russo & M. Toscano (2011) The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chemistry,* 125**,** 288-306.

Li, B. & C. N. Dewey (2011) RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics,* 12.

Li, H. & R. Durbin (2010) Fast and accurate long-read alignment with Burrows–Wheeler transform. *Bioinformatics,* 26**,** 589-595.

Li, Y.-R., W.-T. Tsai, Y.-C. Hsu, M.-Z. Xie & J.-J. Chen (2014) Comparison of autotrophic and mixotrophic cultivation of green microalgal for biodiesel production. *Energy Procedia,* 52**,** 371-376.

Li, Y., J. Huang, G. Sandmann & F. Chen (2009) High-Light and Sodium Chloride Stress Differentially Regulate the Biosynthesis of Astaxanthin Inchlorella Zofingiensis(Chlorophyceae). *Journal of Phycology,* 45**,** 635-641.

Li, T., Gargouri, M., Feng, J., Park, J.-J., Gao, D., Miao, C., Gang, D. R, Chen, S. (2015). Regulation of starch and lipid accumulation in a microalga *Chlorella sorokiniana*. Bioresource technology, 180, 250-257.

Li, Q., Liu, J., Zhang, L., & Liu, Q. (2014). De Novo Transcriptome Analysis of an Aerial Microalga *Trentepohlia jolithus*: Pathway Description and Gene Discovery for Carbon Fixation and Carotenoid Biosynthesis. *PloS one,* 9(9), e108488.

Liao, Y., G. K. Smyth & W. Shi (2013) The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. *Nucleic Acids Research,* 41**,** e108-e108.

Liao, Y., G. K. Smyth & W. Shi (2014) FeatureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics,* 30**,** 923-930.

Lichtenthaler, H. K. & C. Buschmann (2001) Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy. *Current Protocols in Food Analytical Chemistry*.

Lin, C.-K. E., Kaptein, J. S., & Sheikh, J. (2017). Differential expression of microRNAs and their possible roles in patients with chronic idiopathic urticaria and active hives. *Allergy & Rhinology,* 8(2), e67.

Liu, B.-H. & Y.-K. Lee (2000) Secondary carotenoids formation by the green alga *Chlorococcum* sp. *Journal of Applied Phycology,* 12**,** 301-307.

Liu, C., G. Wu, X. Huang, S. Liu & B. Cong (2012a) Validation of housekeeping genes for gene expression studies in an ice alga *Chlamydomonas* during freezing acclimation. *Extremophiles,* 16**,** 419-425.

Liu, J., Z. Sun, H. Gerken, Z. Liu, Y. Jiang & F. Chen (2014) *Chlorella zofingiensis* as an alternative microalgal producer of astaxanthin: biology and industrial potential. *Marine Drugs,* 12**,** 3487-515.

Liu, J., Z. Sun, Y. Zhong, J. Huang, Q. Hu & F. Chen (2012b) Stearoyl-acyl carrier protein desaturase gene from the oleaginous microalga *Chlorella zofingiensis*: cloning, characterization and transcriptional analysis. *Planta,* 236**,** 1665-1676.

Liu, J., Mao, X., Zhou, W., & Guarnieri, M. T. (2016). Simultaneous production of triacylglycerol and high-value carotenoids by the astaxanthin-

producing oleaginous green microalga *Chlorella zofingiensis*. *Bioresource technology,* 214, 319-327.

Lodish, H., A. Berk, S. L. Zipursky, P. Matsudaira, D. Baltimore & J. Darnell (2000) Photosynthetic stages and light-absorbing pigments. In *Molecular Cell Biology,* ed. W. H. Freeman. New York: NCBI Bookshelf.

Lohse, M., A. Nagel, T. Herter, P. May, M. Schroda, R. Zrenner, T. Tohge, A. R. Fernie, M. Stitt & B. Usadel (2014) Mercator: a fast and simple web server for genome scale functional annotation of plant sequence data. *Plant, Cell & Environment,* 37**,** 1250-1258.

Lopez, D., D. Casero, S. J. Cokus, S. S. Merchant & M. Pellegrini (2011) Algal Functional Annotation Tool: a web-based analysis suite to functionally interpret large gene lists using integrated annotation and expression data. *BMC Bioinformatics,* 12**,** 282.

Love, M., S. Anders & W. Huber (2014) Differential analysis of count data–the DESeq2 package. *Genome Biology,* 15**,** 1-54.

Love, M. I., S. Anders, V. Kim & W. Huber (2015) RNA-Seq workflow: gene-level exploratory analysis and differential expression. *F1000Research,* 4**,** 1070. http://www-huber.embl.de/ (last accessed.

Lu, C., S. S. Tej, S. Luo, C. D. Haudenschild, B. C. Meyers & P. J. Green (2005) Elucidation of the small RNA component of the transcriptome. *Science,* 309**,** 1567-1569.

Lu, S., J. Wang, Q. Ma, J. Yang, X. Li & Y.-J. Yuan (2013) Phospholipid metabolism in an industry microalga *Chlorella sorokiniana*: the impact of inoculum sizes. *PloS one,* 8**,** e70827.

Lynch, D. V. & G. A. Thompson (1982) Low temperature-induced alterations in the chloroplast and microsomal membranes of *Dunaliella salina*. *Plant physiology,* 69**,** 1369-1375.

Machu, L., L. Misurcova, J. Vavra Ambrozova, J. Orsavova, J. Mlcek, J. Sochor & T. Jurikova (2015) Phenolic content and antioxidant capacity in algal food products. *Molecules,* 20**,** 1118-1133.

Magi, A., M. Benelli, A. Gozzini, F. Girolami, F. Torricelli & M. L. Brandi (2010) Bioinformatics for next generation sequencing data. *Genes,* 1**,** 294-307.

Martinez, N. J., M. C. Ow, M. I. Barrasa, M. Hammell, R. Sequerra, L. Doucette-Stamm, F. P. Roth, V. R. Ambros & A. J. Walhout (2008) A *C. elegans* genome-scale microRNA network contains composite feedback motifs with high flux capacity. *Genes & Development,* 22**,** 2535-2549.

Masojıdek, J., M. Koblızek & G. Torzillo (2004) Photosynthesis in microalgae. In *Handbook of Microalgal Culture: Biotechnology and Applied Phycology,* ed. A. Richmond, 20. Blackwell Publishing.

Matsukawa, R., M. Hotta, Y. Masuda, M. Chihara & I. Karube (2000) Antioxidants from carbon dioxide fixing *Chlorella sorokiniana*. *Journal of Applied Phycology* 12**,** 263–267.

Mayer, A. M. & M. T. Hamann (2005) Marine pharmacology in 2001–2002: marine compounds with anthelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems and other miscellaneous mechanisms of action. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 140**,** 265-286.

McCarthy, D. J., Y. Chen & G. K. Smyth (2012) Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Research***,** 1-10.

Merchant, S. S., S. E. Prochnik, O. Vallon, E. H. Harris, S. J. Karpowicz, G. B. Witman, A. Terry, A. Salamov, L. K. Fritz-Laylin & L. Maréchal-Drouard (2007) The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. *Science,* 318**,** 245-250.

Molnar, A., A. Bassett, E. Thuenemann, F. Schwach, S. Karkare, S. Ossowski, D. Weigel & D. Baulcombe (2009) Highly specific gene silencing by artificial microRNAs in the unicellular alga *Chlamydomonas reinhardtii*. *The Plant Journal,* 58**,** 165-174.

Molnár, A., F. Schwach, D. J. Studholme, E. C. Thuenemann & D. C. Baulcombe (2007) miRNAs control gene expression in the single-cell alga *Chlamydomonas reinhardtii*. *Nature,* 447**,** 1126-1129.

Morell, M. K., Z. Li, A. Regina, S. Rahman, C. d'Hulst & S. G. Ball (2008) Control of starch biosynthesis in vascular plants and algae. In *Control of Primary Metabolism in Plants,* eds. W. C. Plaxton & M. T. McManus, 258. Blackwell Publishing.

Moroney, J. V. & A. Somanchi (1999) How do algae concentrate CO_2 to increase the efficiency of photosynthetic carbon fixation? *Plant Physiology,* 119**,** 9-16.

Mostafa, S. S (2012) *Microalgal biotechnology: prospects and applications*. INTECH Open Access Publisher.

Mozaffarian, D., A. Ascherio, F. B. Hu, M. J. Stampfer, W. C. Willett, D. S. Siscovick & E. B. Rimm (2005) Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. *Circulation,* 111**,** 157-164.

Mulders, K. J., Janssen, J. H., Martens, D. E., Wijffels, R. H., & Lamers, P. P. (2014). Effect of biomass concentration on secondary carotenoids and triacylglycerol (TAG) accumulation in nitrogen-depleted *Chlorella zofingiensis*. *Algal Research*, 6, 8-16.

Müller, S., L. Rycak, P. Winter, G. Kahl, I. Koch & B. Rotter (2013) omiRas: a Web server for differential expression analysis of miRNAs derived from small RNA-Seq data. *Bioinformatics,* 29**,** 2651-2652.

Murata, N., S. Takahashi, Y. Nishiyama & S. I. Allakhverdiev (2007) Photoinhibition of photosystem II under environmental stress. *Biochimica et Biophysica Acta (BBA)-Bioenergetics,* 1767**,** 414-421.

Naik, S. N., V. V. Goud, P. K. Rout & A. K. Dalai (2010) Production of first and second generation biofuels: a comprehensive review. *Renewable and Sustainable Energy Reviews,* 14**,** 578-597.

Nakamura, Y. (1983) Change in molecular weight distribution in starch when degraded at different temperatures in *Chlorella vulgaris*. *Plant Science Letters,* 30**,** 259-265.

Nakamura, Y. & S. Miyachi (1982) Effect of temperature on starch degradation in *Chlorella vulgaris* 11h cells. *Plant and Cell Physiology,* 23**,** 333-341.

Nakano, S., H. Takekoshi & M. Nakano (2010) *Chlorella pyrenoidosa* supplementation reduces the risk of anemia, proteinuria and edema in pregnant women. *Plant foods for human nutrition,* 65**,** 25-30.

Ndhlala, A. R., M. Moyo & J. Van Staden (2010) Natural antioxidants: fascinating or mythical biomolecules? *Molecules,* 15**,** 6905-6930.

Negi, S., A. N. Barry, N. Friedland, N. Sudasinghe, S. Subramanian, S. Pieris, F. O. Holguin, B. Dungan, T. Schaub & R. Sayre (2016) Impact of nitrogen limitation on biomass, photosynthesis, and lipid accumulation in *Chlorella sorokiniana*. *Journal of Applied Phycology,* 28**,** 803-812.

Nemcova, Y. & T. Kalina (2000) Cell wall development, microfibril and pyrenoid structure in type strains of *Chlorella vulgaris, C. kessleri, C. sorokiniana* compared with *C. luteoviridis* (Trebouxiophyceae, Chlorophyta). *Archiv für Hydrobiologie , Supplement,* 136**,** 95-106.

Nishida, I. & N. Murata (1996) Chilling sensitivity in plants and cyanobacteria: the crucial contribution of membrane lipids. *Annual Review of Plant Biology,* 47**,** 541-568.

Ntambi, J. M. & M. Miyazaki (2004) Regulation of stearoyl-CoA desaturases and role in metabolism. *Progress in Lipid Research,* 43**,** 91-104.

Nunes-Nesi, A., W. L. Araújo, T. Obata & A. R. Fernie (2013) Regulation of the mitochondrial tricarboxylic acid cycle. *Current opinion in plant biology,* 16**,** 335-343.

Obulesu, M., M. R. Dowlathabad & P. Bramhachari (2011) Carotenoids and Alzheimer's disease: an insight into therapeutic role of retinoids in animal models. *Neurochemistry International,* 59**,** 535-541.

Okada, K. (2009) PetH is rate-controlling in the interaction between PetH, a component of the supramolecular complex with photosystem II, and PetF, a light-dependent electron transfer protein. *Biochemical and biophysical research communications,* 389**,** 394-398.

Palenik, B., J. Grimwood, A. Aerts, P. Rouzé, A. Salamov, N. Putnam, C. Dupont, R. Jorgensen, E. Derelle & S. Rombauts (2007) The tiny eukaryote *Ostreococcus* provides genomic insights into the paradox of plankton speciation. *Proceedings of the National Academy of Sciences,* 104**,** 7705-7710.

Paniagua-Michel, J., Olmos-Soto, J., & Ruiz, M. A. (2012). Pathways of carotenoid biosynthesis in bacteria and microalgae. *Microbial Carotenoids from Bacteria and Microalgae: Methods and Protocols,* 1-12.

Perez-Garcia, O. & Y. Bashan (2015) Microalgal heterotrophic and mixotrophic culturing for bio-refining: From metabolic routes to techno-economics. In *Algal biorefineries*, 61-131. Springer.

Perez-Garcia, O., F. M. Escalante, L. E. de-Bashan & Y. Bashan (2011) Heterotrophic cultures of microalgae: metabolism and potential products. *Water Research,* 45**,** 11-36.

Pérez-Jiménez, J., S. Arranz, M. Tabernero, M. E. Díaz-Rubio, J. Serrano, I. Goñi & F. Saura-Calixto (2008) Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Research International,* 41**,** 274-285.

Petkov, G. & G. Garcia (2007) Which are fatty acids of the green alga *Chlorella*? *Biochemical Systematics and Ecology,* 35**,** 281-285.

Pignolet, O., S. Jubeau, C. Vaca-Garcia & P. Michaud (2013) Highly valuable microalgae: biochemical and topological aspects. *Journal of Industrial Microbiol & Biotechnology,* 40**,** 781-96.

Plaza, M., M. Herrero, A. Cifuentes & E. Ibáñez (2009) Innovative natural functional ingredients from microalgae. *Journal of Agricultural and Food Chemistry,* 57**,** 7159-7170.

Prior, R. L., X. Wu & K. Schaich (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry,* 53**,** 4290-4302.

Priyadarshani, I. & B. Rath (2012) Commercial and industrial applications of micro algae–A review. *Journal of Algal Biomass Utilization,* 3**,** 89-100.

Prochnik, S. E., J. Umen, A. M. Nedelcu, A. Hallmann, S. M. Miller, I. Nishii, P. Ferris, A. Kuo, T. Mitros & L. K. Fritz-Laylin (2010) Genomic analysis of organismal complexity in the multicellular green alga *Volvox carteri*. *Science,* 329**,** 223-226.

Proffitt, A (2013) Next generation sequencing survey. BIO-IT World.

Provan, J., S. Murphy & C. A. Maggs (2004) Universal plastid primers for Chlorophyta and Rhodophyta. *European Journal of Phycology,* 39**,** 43-50.

Qin, Z., C. Li, L. Mao & L. Wu (2014) Novel insights from non-conserved microRNAs in plants. *Frontiers in Plant Science,* 5**,** 586.

Raven, J. A. & R. J. Geider (1988) Temperature and algal growth. *New phytologist,* 110**,** 441-461.

Remias, D., U. Karsten, C. Lütz & T. Leya (2010) Physiological and morphological processes in the Alpine snow alga *Chloromonas nivalis* (Chlorophyceae) during cyst formation. *Protoplasma,* 243**,** 73-86.

Rismani-Yazdi, H., B. Z. Haznedaroglu, K. Bibby & J. Peccia (2011) Transcriptome sequencing and annotation of the microalgae *Dunaliella tertiolecta*: pathway description and gene discovery for production of next-generation biofuels. *BMC Genomics,* 12**,** 148.

Rodriguez-Garcia, I. & J. L. Guil-Guerrero (2008) Evaluation of the antioxidant activity of three microalgal species for use as dietary supplements and in the preservation of foods. *Food Chemistry,* 108**,** 1023-1026.

Rosenberg, J. N., G. A. Oyler, L. Wilkinson & M. J. Betenbaugh (2008) A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. *Current Opinion in Biotechnology,* 19**,** 430-436.

Rosic, N. N., M. Pernice, M. Rodriguez-Lanetty & O. Hoegh-Guldberg (2011) Validation of housekeeping genes for gene expression studies in *Symbiodinium* exposed to thermal and light stress. *Marine Biotechnology,* 13**,** 355-365.

Safafar, H., J. van Wagenen, P. Møller & C. Jacobsen (2015) Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. *Marine Drugs,* 13**,** 7339-7356.

Safi, C., B. Zebib, O. Merah, P.-Y. Pontalier & C. Vaca-Garcia (2014) Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review. *Renewable and Sustainable Energy Reviews,* 35**,** 265-278.

Sakai, N., Y. Sakamoto, N. Kishimoto, M. Chihara & I. Karube (1995) *Chlorella* strains from hot springs tolerant to high temperature and high CO 2. *Energy Conversion and Management,* 36**,** 693-696.

Saranya, C., A. Hemalatha, C. Parthiban & P. Anantharaman (2014) Evaluation of antioxidant properties, total phenolic and carotenoid content of *Chaetoceros calcitrans*, *Chlorella salina* and *Isochrysis galbana International Journal of Current Microbiology and Applied Science,* 3**,** 365-377.

Sasaki, Y. & Y. Nagano (2004) Plant acetyl-CoA carboxylase: structure, biosynthesis, regulation, and gene manipulation for plant breeding. *Bioscience, biotechnology, and biochemistry,* 68**,** 1175-1184.

Sharma, K. K., H. Schuhmann & P. M. Schenk (2012) High Lipid Induction in Microalgae for Biodiesel Production. *Energies,* 5**,** 1532-1553.

Shi, X., H. Zhang & S. Lin (2013) Tandem repeats, high copy number and remarkable diel expression rhythm of form II RuBisCO in P*rorocentrum donghaiense* (dinophyceae). *PloS One,* 8**,** e71232.

Shukla, S. P., J. Kviderova, J. Tříska & J. Elster (2013) *Chlorella mirabilis* as a potential species for biomass production in low-temperature environment. *Frontiers in Microbiology,* 4.

Siaut, M., S. Cuiné, C. Cagnon, B. Fessler, M. Nguyen, P. Carrier, A. Beyly, F. Beisson, C. Triantaphylidès & Y. Li-Beisson (2011) Oil accumulation in the model green alga *Chlamydomonas reinhardtii*: characterization, variability between common laboratory strains and relationship with starch reserves. *BMC Biotechnology,* 11**,** 7.

Skjanes, K., C. Rebours & P. Lindblad (2013) Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. *Critical Reviews in Biotechnology,* 33**,** 172-215.

Smith-Unna, R., C. Boursnell, R. Patro, J. Hibberd & S. Kelly (2016) TransRate: reference free quality assessment of de novo transcriptome assemblies. *Genome Research***,** gr. 196469.115.

Spolaore, P., C. Joannis-Cassan, E. Duran & A. Isambert (2006) Commercial applications of microalgae. *Journal of Bioscience and Bioengineering,* 101**,** 87-96.

Stanke, M., O. Keller, I. Gunduz, A. Hayes, S. Waack & B. Morgenstern (2006) AUGUSTUS: ab initio prediction of alternative transcripts. *Nucleic Acids Research,* 34**,** W435-W439.

Steinbrenner, J. & H. Linden (2001) Regulation of two carotenoid biosynthesis genes coding for phytoene synthase and carotenoid hydroxylase during stress-induced astaxanthin formation in the green alga *Haematococcus pluvialis*. *Plant Physiology,* 125**,** 810-817.

Stolz, P. & B. Obermayer (2005) Manufacturing microalgae for skin care. *Cosmetics and Toiletries,* 120**,** 99-106.

Sun, N., Y. Wang, Y.-T. Li, J.-C. Huang & F. Chen (2008) Sugar-based growth, astaxanthin accumulation and carotenogenic transcription of heterotrophic *Chlorella zofingiensis* (Chlorophyta). *Process Biochemistry,* 43**,** 1288-1292.

Sun, P., Y. Mao, G. Li, M. Cao, F. Kong, L. Wang & G. Bi (2015) Comparative transcriptome profiling of *Pyropia yezoensis* (Ueda) M.S. Hwang & H.G. Choi in response to temperature stresses. *BMC Genomics,* 16**,** 1-16.

Sun, X., W. Wang, J. Shen & N. Xu (2014) Transcriptome sequencing of the marine microalga, *Chlorella pyrenoidosa* (Chlorophyta), and analysis of carbonic anhydrase expression under salt stress. *Botanica Marina,* 57**,** 403-412.

Sun, Z., Y. F. Chen & J. Du (2016) Elevated CO2 improves lipid accumulation by increasing carbon metabolism in *Chlorella sorokiniana*. *Plant Biotechnology Journal,* 14**,** 557-566.

Sweetlove, L. J., & Fernie, A. R. (2005). Regulation of metabolic networks: understanding metabolic complexity in the systems biology era. *New phytologist, 168*(1), 9-24.

Taga, M. S., E. E. Miller & D. E. Pratt (1984) Chia seeds as a source of natural lipid antioxidants *Journal of the American Oil Chemists' Society,* 61.

Takaichi, S. (2011). Carotenoids in algae: distributions, biosyntheses and functions. *Marine Drugs,* 9(6), 1101-1118. doi: 10.3390/md9061101.

Takeda, H. (1988) Classification of *chlorella* strain by means of sugar components in cell wall. *Biochemical Systematics and Ecology,* 16**,** 367-371.

Takeshita, T., S. Ota, T. Yamazaki, A. Hirata, V. Zachleder & S. Kawano (2014) Starch and lipid accumulation in eight strains of six *Chlorella* species under comparatively high light intensity and aeration culture conditions. *Bioresource Technology,* 158**,** 127-34.

Takeuchi, K. & K. Reue (2009) Biochemistry, physiology, and genetics of GPAT, AGPAT, and lipin enzymes in triglyceride synthesis. *American Journal of Physiology-Endocrinology And Metabolism,* 296**,** E1195-E1209.

Tamura, K., G. Stecher, D. Peterson, A. Filipski & S. Kumar (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution,* 30**,** 2725-2729.

Thimm, O., O. Bläsing, Y. Gibon, A. Nagel, S. Meyer, P. Krüger, J. Selbig, L. A. Müller, S. Y. Rhee & M. Stitt (2004) Mapman: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *The Plant Journal,* 37**,** 914-939.

Thompson, G. A. (1996) Lipids and membrane function in green algae. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism,* 1302**,** 17-45.

Thompson, J. D., T. Gibson & D. G. Higgins (2002) Multiple sequence alignment using ClustalW and ClustalX. *Current Protocols in Bioinformatics***,** 2.3. 1-2.3. 22.

Tjahjono, A. E., Y. Hayama, T. Kakizono, Y. Terada, N. Nishio & S. Nagai (1994) Hyper-accumulation of astaxanthin in a green alga *Haematococcus pluvialis* at elevated temperatures. *Biotechnology Letters,* 16**,** 133-138.

Tomaselli, L (2004) The Microalgal Cell. In *Handbook of microalgal culture: Biotechnology and applied phycology,* ed. A. Richmond, 3-19. Blackwell Publishing.

Tomaz, T., M. Bagard, I. Pracharoenwattana, P. Lindén, C. P. Lee, A. J. Carroll, E. Ströher, S. M. Smith, P. Gardeström & A. H. Millar (2010) Mitochondrial malate dehydrogenase lowers leaf respiration and alters photorespiration and plant growth in Arabidopsis. *Plant physiology,* 154**,** 1143-1157.

Tripathi, U., R. Sarada & G. Ravishankar (2002) Effect of culture conditions on growth of green alga—*Haematococcus pluvialis* and astaxanthin production. *Acta Physiologiae Plantarum,* 24**,** 323-329.

Tsang, J., J. Zhu & A. van Oudenaarden (2007) MicroRNA-mediated feedback and feedforward loops are recurrent network motifs in mammals. *Molecular Cell,* 26**,** 753-767.

Uma, R., V. Sivasubramanian & S. N. Devaraj (2011) Evaluation of in vitro antioxidant activities and antiproliferative activity of green microalgae, *Desmococcus olivaceous* and *Chlorococcum humicola*. *Journal of Algal Biomass Utilization,* 2**,** 82-93.

Vachali, P., P. Bhosale & P. S. Bernstein (2012) Microbial carotenoids. In *Microbial Carotenoids from Fungi,* ed. J.-L. Barredo, 41-59. Humana Press.

Vandesompele, J., K. De Preter, F. Pattyn, B. Poppe, N. Van Roy, A. De Paepe & F. Speleman (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology,* 3**,** 1-12.

Varfolomeev, S. & L. Wasserman (2011) Microalgae as source of biofuel, food, fodder, and medicines. *Applied Biochemistry and Microbiology,* 47**,** 789-807.

Vílchez, C., E. Forján, M. Cuaresma, F. Bédmar, I. Garbayo & J. M. Vega (2011) Marine carotenoids: biological functions and commercial applications. *Marine Drugs,* 9**,** 319-333.

Vonshak, A. & G. Torzillo (2004) Environmental stress physiology. In *Handbook of Microalgal Culture: Biotechnology and Applied Phycology,* ed. A. Richmond, 57-82. Blackwell Publishing.

Wang, L., Z. Feng, X. Wang, X. Wang & X. Zhang (2010a) DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics,* 26**,** 136-138.

Wang, L., M. Min, Y. Li, P. Chen, Y. Chen, Y. Liu, Y. Wang & R. Ruan (2010b) Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. *Applied Biochemistry and Biotechnology,* 162**,** 1174-1186.

Wang, Y. & T. Chen (2008) The biosynthetic pathway of carotenoids in the astaxanthin-producing green alga *Chlorella zofingiensis*. *World Journal of Microbiology and Biotechnology,* 24**,** 2927-2932.

Wang, Z., M. Gerstein & M. Snyder (2009) RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews Genetics,* 10**,** 57-63.

Wanke, M., K. Skorupinska-Tudek & E. Swiezewska (2001) Isoprenoid biosynthesis via 1-deoxy-D-xylulose 5-phosphate/2-C-methyl-D-erythritol 4-phosphate (DOXP/MEP) pathway. *Acta Biochimica Polonica,* 48**,** 663-672.

Wei, S., M. Y. Gruber, B. Yu, M.-J. Gao, G. G. Khachatourians, D. D. Hegedus, I. A. Parkin & A. Hannoufa (2012) *Arabidopsis* mutant sk156 reveals complex regulation of SPL15 in a *miR156*-controlled gene network. *BMC Plant Biology,* 12**,** 1-17.

Westerhoff, P., Q. Hu, M. Esparza-Soto & W. Vermaas (2010) Growth parameters of microalgae tolerant to high levels of carbon dioxide in batch and continuous-flow photobioreactors. *Environmental technology,* 31**,** 523-532.

White, T. J., T. Bruns, S. Lee & J. Taylor (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications,* 18**,** 315-322.

Wolf, J. B. (2013) Principles of transcriptome analysis and gene expression quantification: an RNA-seq tutorial. *Molecular Ecology Resources,* 13**,** 559-72.

Worden, A. Z., J.-H. Lee, T. Mock, P. Rouzé, M. P. Simmons, A. L. Aerts, A. E. Allen, M. L. Cuvelier, E. Derelle & M. V. Everett (2009) Green evolution and dynamic adaptations revealed by genomes of the marine picoeukaryotes *Micromonas*. *Science,* 324**,** 268-272.

Wu, L., J. Fan & J. G. Belasco (2006) MicroRNAs direct rapid deadenylation of mRNA. *Proceedings of the National Academy of Sciences of the United States of America,* 103**,** 4034-4039.

Wu, Q., T. Liu, H. Liu & G. Zheng (2009) Unsaturated fatty acid: metabolism, synthesis and gene regulation. *African Journal of Biotechnology,* 8.

Xu, H., X. Miao & Q. Wu (2006) High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. *Journal of Biotechnology,* 126**,** 499-507.

Xu, X.-Q. & J. Beardall (1997) Effect of salinity on fatty acid composition of a green microalga from an antarctic hypersaline lake. *Phytochemistry,* 45**,** 655-658.

Xu, Z., D. Zou & K. Gao (2010) Effects of elevated CO2 and phosphorus supply on growth, photosynthesis and nutrient uptake in the marine macroalga *Gracilaria lemaneiformis* (Rhodophyta). *Botanica Marina,* 53**,** 123-129.

Yamamoto, M., M. Fujishita, A. Hirata & S. Kawano (2004) Regeneration and maturation of daughter cell walls in the autospore-forming green alga *Chlorella vulgaris* (Chlorophyta, Trebouxiophyceae). *Journal of Plant Research,* 117**,** 257-64.

Yamamoto, M., I. Kurihara & S. Kawano (2005) Late type of daughter cell wall synthesis in one of the Chlorellaceae, *Parachlorella kessleri* (Chlorophyta, Trebouxiophyceae). *Planta,* 221**,** 766-75.

Yang, S., M. T. Guarnieri, S. Smolinski, M. Ghirardi & P. T. Pienkos (2013) De novo transcriptomic analysis of hydrogen production in the green alga *Chlamydomonas moewusii* through RNA-Seq. *Biotechnology for Biofuels,* 6**,** 17.

Yang, T., Xu, R., Chen, J., & Liu, A. (2016). β-Ketoacyl-acyl Carrier Protein Synthase I (KASI) Plays Crucial Roles in the Plant Growth and Fatty Acids Synthesis in Tobacco. *International Journal of Molecular Sciences*, 17(8), 1287. http://doi.org/10.3390/ijms17081287

Yao, C., J. Ai, X. Cao, S. Xue & W. Zhang (2012) Enhancing starch production of a marine green microalga *Tetraselmis subcordiformis* through nutrient limitation. *Bioresource Technology,* 118**,** 438-44.

Yu, J. & P. Takahashi (2007) Biophotolysis-based hydrogen production by cyanobacteria and green microalgae. *Communicating current research and educational topics and trends in applied microbiology,* 1**,** 79-89.

Zakar, T., Laczko-Dobos, H., Toth, T. N., & Gombos, Z. (2016). Carotenoids assist in cyanobacterial Photosystem II assembly and function. *Frontiers Plant Science,* 7.

Zhao, B., L. Ge, R. Liang, W. Li, K. Ruan, H. Lin & Y. Jin (2009) Members of miR-169 family are induced by high salinity and transiently inhibit the NF-YA transcription factor. *BMC Molecular Biology,* 10**,** 1-10.

Zhao, B., R. Liang, L. Ge, W. Li, H. Xiao, H. Lin, K. Ruan & Y. Jin (2007a) Identification of drought-induced microRNAs in rice. *Biochemical and Biophysical Research Communications,* 354**,** 585-590.

Zhao, L., W.-c. Chang, Y. Xiao, H.-w. Liu & P. Liu (2013) Methylerythritol phosphate pathway of isoprenoid biosynthesis. *Annual Review of Biochemistry,* 82**,** 497-530.

Zhao, R., Y. Cao, H. Xu, L. Lv, D. Qiao & Y. Cao (2011) Analysis of expressed sequence tags from the green alga *Dunaliella Salina* (Chlorophyta). *Journal of Phycology,* 47**,** 1454-1460.

Zhao, T., G. Li, S. Mi, S. Li, G. J. Hannon, X. J. Wang & Y. Qi (2007b) A complex system of small RNAs in the unicellular green alga *Chlamydomonas reinhardtii*. *Genes & Development,* 21**,** 1190-1203.

Zheng, H.-Q., Y.-F. Chiang-Hsieh, C.-H. Chien, B.-K. J. Hsu, T.-L. Liu, C.-N. N. Chen & W.-C. Chang (2014) AlgaePath: comprehensive analysis of metabolic pathways using transcript abundance data from next-generation sequencing in green algae. *BMC genomics,* 15**,** 196.

Zheng, Y., Z. Chi, B. Lucker & S. Chen (2012) Two-stage heterotrophic and phototrophic culture strategy for algal biomass and lipid production. *Bioresource Technology,* 103**,** 484-488.

Zhou, X., G. Wang & W. Zhang (2007) UV-B responsive microRNA genes in *Arabidopsis thaliana*. *Molecular Systems Biology,* 3**,** 103.

Zhu, S., W. Huang, J. Xu, Z. Wang, J. Xu & Z. Yuan (2014) Metabolic changes of starch and lipid triggered by nitrogen starvation in the microalga *Chlorella zofingiensis*. *Bioresource Technology,* 152**,** 292-8.