

**RELATIONSHIPS AMONGST CHLORELLA ISOLATES
FROM THE TROPICAL, TEMPERATE AND ANTARCTIC
REGIONS BASED ON MORPHOLOGICAL, BIOCHEMICAL
AND MOLECULAR STUDIES**

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**THESIS SUBMITTED IN FULFILLMENT
OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE**

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FACULTY OF SCIENCE
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KUALA LUMPUR**

2011

ABSTRACT

Algae from the genus *Chlorella* are widely distributed on earth, including the Polar Regions. The alga is a model organism for physiology and biochemistry experiments as well as an important organism for biotechnological exploitation. The major objective of this study was to unravel the relationship amongst *Chlorella* isolates from the Antarctic, sub-Antarctic, tropical and temperate regions based on 18S rDNA sequences as well as morphological and biochemical (fatty acid profiles and pigment composition) features. Eleven isolates of *Chlorella*, consisting of two Antarctic isolates, one sub-Antarctic isolate, seven tropical isolates and one temperate isolate from the University of Malaya Algae Culture Collection (UMACC) were included in this study. There were no distinct morphological features that can be used to differentiate the various isolates of *Chlorella* studied. The pigmentation of all the strains was similar, consisting of chlorophyll *a*, chlorophyll *b*, lutein, β -carotene, cis-neoxanthin and violaxanthin. There was no marked difference in terms of their fatty acid profile, which was dominated by 18:3. The morphology, fatty acid profiles and pigmentation of the 11 isolates showed that they belong to the “true” *Chlorella*. This was further supported by molecular analysis based on 18S rDNA, which revealed that they clustered together forming one clade within the Trebouxiophyceae. The phylogenetic analysis revealed that the *Chlorella* isolates from Antarctic were closely related to the tropical and temperate isolates.

ABSTRAK

Mikro alga daripada genus *Chlorella* boleh didapati di pelbagai habitat di seluruh dunia merangkumi kutub utara dan selatan. Ia sangat berguna di dalam bidang fisiologi, biokimia, serta bioteknologi. Objektif kajian ini ialah untuk menguraikan hubungan/kaitan di antara *Chlorella* daripada kawasan Antartika, sub-Antartika, tropika dan temperat (beriklim sederhana) dengan menggunakan 18S rDNA sequence, morfologi dan fisiologi seperti komposisi asid lemak dan pigmentasi. Pertumbuhan, komposisi biokimia, asid lemak dan pigmentasi serta analisis molekul dengan menggunakan gen 18S rDNA telah dikaji. Sebanyak 11 baka *Chlorella* merangkumi 2 baka Antartika, 1 sub-Antartika, 7 tropika dan 1 temperat telah didapati daripada Koleksi Alga Universiti Malaya (UMACC). Dari segi morfologi, tiada sifat istimewa boleh digunakan untuk membezakan *Chlorella* daripada kawasan berlainan. Sebanyak enam komponen pigment telah dikenalpasti seperti klorofil *a*, klorofil *b*, lutein, karotenoid, cis-neoxanthin dan violaxanthin. 11 *Chlorella* tersebut mempunyai komposisi asid lemak yang sama serta komposisi utamanya ialah 18:3. Pigmentasi serta komposisi asid lemak juga tidak menunjukkan sebarang perbezaan di antara *Chlorella* daripada kawasan berlainan malah kedua-dua konsep memberi keputusan/penghasilan yang sama. Pigmentasi, komposisi asid lemak serta genetik analisis menunjukkan bahawa semua *Chlorella* yang dikaji adalah daripada genus *Chlorella* sebenar serta terkumpul di kelas Trebouxiophyceae. Baka *Chlorella* Antartika menunjukkan hubungan yang sangat rapat dengan baka dari tropika dan temperat.

ACKNOWLEDGEMENTS

Here I would like to take this opportunity to express my appreciation to all the people who have helped me to finish this project. First of all, I would like to express my heartiest appreciation to my supervisor Professor. Dr. Phang Siew Moi who has devoted much of her time for invaluable guidance especially on the planning of the study. My grateful thanks extended to my second supervisors Professor. Dr. Chu Wan Loy and Dr. Gan Sook Yee for their guidance, constructive and consistent motivation during the project. I also would like to thank Mr.Raghu, Puan Zubaidah and Puan Vijaya and all the TEM lab assistants who have guided me during the TEM analysis. Special thanks go to Dr. Miyashita who has guided me for pigment analysis.

I would also like to extend my gratitude to the Ministry of Science, Technology and Innovation of Malaysia for their financial support under the Antarctic Biodiversity Grant (8123204). The generous support of the “University Graduate Research Scholarship Universiti Malaya” which enabled me to pursue my study is gratefully acknowledged. Thanks also go to the Institute of Research Management and Consultancy (IPPP), University of Malaya for their Graduate Research Grant. Thanks to the Australian Antarctic Division (AAD) and South African National Antarctic Program (SANAP) for their assistance and logistic support in the collection of algae samples from Antarctica and sub-Antarctica.

I wish to extend my gratitude to my laboratory mates for their cooperation during the laboratory work. I am also grateful to my family members for their encouragement, support and care. Talking about support and motivation, I would like thank my friend Priscilla who cheers me up whenever the results are not promising. All

of your sacrifices and unreserved concern will forever be remembered. Finally, thank
GOD for the success of this project.

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LIST OF ABBREVIATIONS

min	-	minutes
<i>Chl-a</i>	-	Chlorophyll <i>a</i>
<i>Chl-b</i>	-	Chlorophyll <i>b</i>
CO ₂	-	Carbon dioxide
H ₂ O	-	water
O ₂	-	oxygen
Cu	-	Copper
CCAP	-	Culture collection of Algae and protozoa
ND	-	Not detected
PUFA	-	Polyunsaturated fatty acid
MUFA	-	Monounsaturated fatty acid
SFA	-	Saturated fatty acid
DNA	-	Deoxyribonucleic acid
OsO ₄	-	Osmium Tetraoxide
TAE	-	Tris-Acetate-EDTA
MeOH	-	Methanol
CHCl ₃	-	Chloroform
MgCl ₂	-	Magnesium chloride
dNTP	-	Deoxyribonucleotide triphosphate
bp	-	basepairs

LIST OF SYMBOLS AND UNITS

μm	-	micrometer
μ	-	Specific growth rate
OD_{620}	-	Optical density at 620nm
km^2	-	kilometer square
%	-	percentage
cm	-	centimeter
m^{-2}	-	meter square
<	-	less than
$^{\circ}\text{C}$	-	Degree Celsius
mM	-	millimeter
nm	-	nanometer
min	-	minute
rpm	-	revolutions per minute
mL	-	milliliter
s	-	second
h	-	hour
M	-	Molar