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

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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, ß-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 menunjukkkan hubungan yang sangat rapat dengan baka dari tropika dan temperat.

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LIST OF ABBREVIATIONS

min	-	minutes
Chl-a	-	Chlorophyll *a*
Chl-b	-	Chlorophyll *b*
CO_2	-	Carbon dioxide
H_2O	-	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
CHCl3	-	Chloroform
MgCl2	-	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
^oC	-	Degree Celsius
mM	-	millimeter
nm	-	nanometer
min	-	minute
rpm	-	revolutions per minute
mL	-	milliliter
s	-	second
h	-	hour
M	-	Molar