

BIOSYNTHESIS OF OMEGA-3 FATTY ACIDS IN *Acutodesmus obliquus*

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BIOSYNTHESIS OF OMEGA-3 FATTY ACIDS IN *Acutodesmus obliquus*

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DEDICATION

Alhamdulillah for every blessing Allah has given me; hidden and apparent.
No matter what hardships we are facing, never lose faith in Allah.

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ABSTRACT

Omega-3 fatty acids (Omega-3 FAs) are known for their tremendous advantages to the health of body and brain. Omega-3 FAs have been extensively utilized in nutraceutical and aquaculture sectors. Microalgae has been recognized as a promising alternative vegetarian source to fish; the use of microalgae can reduce the risk of exposure to mercury contamination in fish. This study aims to identify new potential microalga strain to produce omega-3 FAs by optimisation of the culture condition, random mutation, and transcriptomic study. In this study, three microalgae namely, *Acutodesmus obliquus*, *Chlorella* sp., and *Chlorella vulgaris* were tested. The *Acutodesmus obliquus* was identified as a fast-growing oleaginous microalga with a growth rate of 0.09 day⁻¹. It was also found that *Acutodesmus obliquus* had a high ability of producing omega-3 FAs as it contained 47% lipid and 38% ALA. To enhance omega-3 FAs production in *Acutodesmus obliquus*, nine sets of optimisation experiments involving four factors (sodium acetate, sodium nitrate, sodium chloride, and dipotassium hydrogen phosphate) with three levels of concentrations each, were conducted and analysed using the Taguchi method. The optimum culture condition was determined at 0.5 g/L of sodium acetate, 0.015 g/L of sodium nitrate, 0.58 g/L of sodium chloride and 0.0002 g/L of dipotassium hydrogen phosphate. Sodium acetate was identified as the primary limiting factor to produce omega-3 FAs. It accumulates a very long chain of omega-3 FAs which is eicosatetraenoic acid (ETA; C20:4) (7.3%) and rarely found in *Acutodesmus obliquus*. To genetically enhance omega-3 FAs (especially ETA), an ethyl methane sulfonate (EMS) based chemical mutagenesis approach followed by selective screening using quizalofop were conducted which led to the discovery of a novel mutant strain designated as Q2-12E. Not only this mutant showed a significantly improved growth than the wild type (original strain), but it also demonstrated a two-fold higher ETA production (17%) than the wild type (8%). Since the pathways and molecular mechanisms that triggered ETA accumulation in this species are still unknown, a transcriptome analysis has been performed. Based on the results, it is primarily discovered that there is the up-regulation of elongase expression in omega-3 FAs biosynthesis pathway in the mutants of *Acutodesmus* species. This explains the reason for the overaccumulation of ETA in mutant over the wild type in *Acutodesmus* species. The pioneer transcriptome analysis conducted leads to a better understanding on ETA metabolism in this EMS-quizalofop mutant strain, and subsequently, provides a new perspective of the genetic manipulation of nutritional supplement-producing microalgae. Overall, this study has identified that *Acutodesmus obliquus* has rapid growth, and high lipid content under optimised nutrient concentration results in enhanced ALA and ETA production. Moreover, random mutation leads to further enhancement of ETA content due to the upregulated expression of elongase.

ABSTRAK

Asid lemak Omega-3 (Omega-3 FA) terkenal dengan kelebihan yang luar biasa kepada kesihatan badan dan otak. Omega-3 FA telah digunakan secara meluas dalam sektor nutraseutikal dan akuakultur. Mikroalga telah diiktiraf sebagai sumber alternatif omega-3 FA kepada vegetarian; penggunaan mikroalga dapat mengurangkan risiko pendedahan manusia kepada pencemaran merkuri yang terdapat dalam sumber ikan. Kajian ini bertujuan untuk mengenal pasti mikroalga baru yang berpotensi untuk menghasilkan omega-3 FA dengan mengoptimalkan keadaan kultur, diikuti dengan mutasi rawak, dan kajian transkriptomi. Dalam kajian ini, tiga mikroalga yang telah diuji adalah *Acutodesmus obliquus*, *Chlorella* sp., dan *Chlorella vulgaris*. *Acutodesmus obliquus* dikenal pasti sebagai mikroalga yang dapat menghasilkan minyak dalam kepekatan yang tinggi dengan kadar pertumbuhan yang pantas iaitu 0.09 per hari. Selain itu, *Acutodesmus obliquus* juga telah dikenal pasti mempunyai keupayaan yang tinggi dalam menghasilkan omega-3 FA dengan mempunyai 47% kandungan minyak dan 38% kandungan ALA. Untuk meningkatkan pengeluaran omega-3 FA dalam *Acutodesmus obliquus*, sembilan set eksperimen pengoptimuman yang melibatkan empat faktor (natrium asetat, natrium nitrat, natrium klorida, dan dipotassium hidrogen fosfat) dengan tiga tahap kepekatan yang berbeza-beza, telah dijalankan dan dianalisis menggunakan kaedah Taguchi. Keadaan kultur yang optimum telah dikenal pasti pada 0.5 g/L natrium asetat, 0.015 g/L natrium nitrat, 0.58 g/L natrium klorida dan 0.0002 g/L dipotassium hidrogen fosfat. Natrium asetat telah dikenal pasti sebagai faktor terpenting dalam penghasilan asid lemak omega-3 FA. Ia menghasilkan rangkaian asid lemak omega-3 FA yang sangat panjang dan jarang dihasilkan oleh spesies ini iaitu ETA (C20:4) (7.3%). Untuk peningkatan kandungan asid lemak omega-3 FA (terutamanya ETA) dari sudut genetik, kaedah mutasi menggunakan bahan kimia, etil metanesulfonat (EMS) diikuti dengan pemilihan selektif menggunakan quizalofop telah membawa kepada penemuan mutan baru yang dinamakan sebagai Q2-12E. Selain daripada pertumbuhan mutan yang lebih pantas daripada jenis liar, ia juga menunjukkan penghasilan ETA sebanyak dua kali ganda (17%) berbanding dengan jenis *Acutodesmus obliquus* yang tidak mengalami mutasi (8%). Oleh kerana laluan dan mekanisme molekul yang bertanggungjawab untuk penghasilan ETA dalam spesies ini masih belum diketahui, analisis transkriptome telah dilakukan. Berdasarkan keputusan yang telah diterima, ini adalah kajian pertama yang menemukan peningkatan ekspresi elongase dalam laluan biosintesis asid lemak omega-3 FA dalam mutan spesies *Acutodesmus*. Kajian ini menunjukkan punca yang telah menyebabkan peningkatan pengumpulan ETA dalam mutan berbanding *Acutodesmus obliquus* jenis liar. Analisis transkriptome dalam kajian ini adalah kajian pertama yang membawa kepada pemahaman yang lebih baik mengenai metabolisme ETA dalam mutan EMS-quizalofop ini, dan seterusnya, memberikan perspektif yang baru tentang manipulasi genetik mikroalga ini sebagai penghasil makanan tambahan. Secara keseluruhan, kajian ini telah mengenal pasti bahawa *Acutodesmus obliquus* mempunyai pertumbuhan yang pesat, dan kandungan lipid yang tinggi dengan kepekatan nutrien yang dioptimumkan, yang telah berjaya meningkatkan penghasilan ALA dan ETA. Selain itu, mutasi secara rawak telah membawa kepada peningkatan kandungan ETA kerana peningkatan ekspresi elongase.

TABLE OF CONTENTS

	TITLE	PAGE
	DECLARATION	iii
	DEDICATION	iv
	ACKNOWLEDGEMENT	v
	ABSTRACT	vi
	ABSTRAK	vii
	TABLE OF CONTENTS	viii
	LIST OF TABLES	xiii
	LIST OF FIGURES	xv
	LIST OF ABBREVIATIONS	xviii
	LIST OF SYMBOLS	xxi
	LIST OF APPENDICES	xxii
CHAPTER 1	INTRODUCTION	1
	1.1 Background of study	1
	1.2 Problem Statement	3
	1.3 Objectives of study	6
	1.4 Scope of study	6
	1.5 Significance of study	7
CHAPTER 2	LITERATURE REVIEW	9
	2.1 Microalgae	9
	2.2 Fatty acids (FAs)	11
	2.2.1 Polyunsaturated fatty acid (PUFA)	11
	2.2.1.1 Omega-3 FAs	12
	2.3 Microalgae producing omega-3 FAs	16
	2.3.1 <i>Acutodesmus obliquus</i>	19
	2.4 Optimisation of microalgae culture condition for enhanced omega-3 FAs production	20

2.4.1	Culture condition for omega-3 FAs production in microalgae	20
2.4.1.1	Carbon source	21
2.4.1.2	Nitrogen source	22
2.4.1.3	Temperature	22
2.4.1.4	Salinity stress	23
2.4.1.5	Phosphorus source	24
2.4.1.6	Light intensity	24
2.4.2	Combination of carbon, nitrogen, salinity and phosphorus sources for enhanced omega-3 FAs production	25
2.4.3	Optimisation of omega-3 FAs using Taguchi method	26
2.5	Chemical mutation and a selection strategy using herbicide	31
2.5.1	Mutagen	31
2.5.1.1	Ethyl methanesulfonate (EMS)	31
2.5.2	Quizalofop	32
2.5.3	Random mutagenesis and selection method	33
2.6	RNA sequencing (RNA-Seq)	34
CHAPTER 3	RESEARCH METHODOLOGY	39
3.1	Microalgae strains	39
3.1.1	Culture condition of microalgae	39
3.2	Experimental Design	41
3.2.1	Morphology observation	43
3.2.2	Growth of microalgae	43
3.2.3	Determination of biomass	44
3.2.4	Lipid staining	44
3.2.5	Lipid Extraction	45
3.2.6	Fatty acid methyl esters (FAMES) extraction	45
3.2.7	Gas Chromatography Analysis	46
3.3	Optimisation of omega-3 FAs production in <i>A. obliquus</i> using Taguchi method	47

3.3.1	Taguchi method (Orthogonal array design and analytic strategy)	47
3.3.2	Culture condition of <i>A. obliquus</i> in optimised medium	48
3.3.3	Fatty acids analysis for <i>A. obliquus</i> cultured using optimised media	49
3.3.4	Statistical analysis	49
3.4	Improved omega-3 FAs production by random chemical mutagenesis and selective screening	50
3.4.1	Random chemical mutagenesis using EMS	50
3.4.2	<i>A. obliquus</i> sensitivity to the quizalofop	51
3.4.3	EMS mutagenesis with quizalofop as selective pressure	51
3.4.4	Screening of <i>A. obliquus</i> mutants	52
3.4.4.1	Screening mutant with high growth	52
3.4.4.2	Screening ETA producing mutant	52
3.5	RNA-sequencing analysis involved in omega-3 FAs biosynthesis from a mutant of <i>A. obliquus</i>	53
3.5.1	Growth condition of wild type and mutant cultures as preparation for RNA-sequencing analysis	54
3.5.2	Sample collection and preparation for transcriptome analysis	54
3.5.2.1	Harvesting of wild type and mutant cultures	54
3.5.2.2	RNA extraction and sequencing	54
3.5.3	Data analysis of transcriptome	55
3.5.3.1	Quality control	55
3.5.3.2	<i>De novo</i> transcriptome assembly	56
3.5.3.3	Gene functional annotation	56
3.5.3.4	Quantification of gene expression levels	56
3.5.3.5	Differential expression analysis	57
3.5.3.6	GO enrichment analysis	57
3.5.3.7	KEGG pathway enrichment analysis	58

3.5.3.8	Transcriptome mining of genes involved in omega-3 FAs biosynthesis	58
CHAPTER 4	RESULTS AND DISCUSSIONS	59
4.1	Morphology observation	59
4.2	Growth of microalgae	60
4.3	Nile red staining for identification of lipid content	63
4.4	Lipid extraction	65
4.5	Fatty acid methyl esters (FAMES) profiles	67
4.6	Optimisation of <i>A. obliquus</i> culture condition for the enhancement of the omega-3 FAs production using Taguchi Method	71
4.6.1	Taguchi design analysis for the optimal omega-3 FAs production	72
4.6.2	Analysis of variance (ANOVA)	77
4.6.3	Interactions within the process variables	78
4.6.4	Confirmation of the experiment	79
4.7	Improvement and screening of omega-3 FAs producing mutants by using chemical mutagenesis and herbicide	81
4.7.1	Determination of optimum EMS concentration for mutagenesis study	81
4.7.2	Determination of quizalofop concentration for screening EMS mutant	83
4.7.3	Isolation of omega-3 FAs producing EMS mutants followed by selective screening using quizalofop	86
4.7.3.1	Isolation of enhanced growth EMS-quizalofop mutants	86
4.7.3.2	Screening of potential mutant with enhanced omega-3 FAs production	88
4.8	<i>De novo</i> transcriptome analysis of omega-3 FAs hyper-producing <i>A. obliquus</i> mutant obtained by EMS mutation and quizalofop screening	93
4.8.1	RNA quality and quantity	94
4.8.2	Illumina sequencing and <i>de novo</i> transcriptome assembly analysis of <i>A. obliquus</i>	95
4.8.3	Functional annotation of genes	96

4.8.3.1	Gene functional annotation	96
4.8.3.2	Gene annotation results through Nr database	97
4.8.4	Differentially expressed genes (DEGs) analysis	98
4.8.5	Enrichment analysis of annotated unigenes and pathway analysis	100
4.8.5.1	Gene ontology (GO) enrichment analysis of annotated unigene	101
4.8.5.2	KEGG enrichment analysis	103
4.8.6	Transcriptome and pathway analysis involved in ETA biosynthesis	106
4.8.6.1	Comparison of previous and current studies	112
CHAPTER 5	CONCLUSION AND RECOMMENDATIONS	114
5.1	Conclusion	114
5.2	Recommendations	116
REFERENCES		117
LIST OF PUBLICATIONS		163

LIST OF TABLES

TABLE NO.	TITLE	PAGE
Table 2.1	Beneficial properties of algae	10
Table 2.2	Important physiological functions of omega-3 FAs	16
Table 2.3	Commercially used microalgae for omega-3 FAs production	18
Table 2.4	The ALA content detected in the genus of <i>Acutodesmus</i> sp.	20
Table 2.5	The suggested optimum culture condition to enhance omega-3 FAs production	25
Table 2.6	The advantages of Taguchi method	28
Table 2.7	The optimisation method that has been used in the genus of <i>Acutodesmus</i>	30
Table 2.8	Comparison method for producing mutants of microalgal strain with chemical mutagen followed by screening method (selection of mutants).	34
Table 2.9	Transcriptome studies that have been conducted for fatty acid biosynthesis	37
Table 3.1	Composition of AF6 medium (Kawachi et al., 2013)	40
Table 3.2	Composition of BG-11 medium (Kawachi et al., 2013)	41
Table 3.3	Factors and levels for optimisation of omega-3 fatty acids production using Taguchi method	47
Table 3.4	L ₉ orthogonal array of Taguchi method	48
Table 4.1	Specific growth rate and doubling time of the microalgae in AF6 medium	62
Table 4.2	Biomass production by <i>A. obliquus</i> and comparison with the previously reported work	63
Table 4.3	The lipid content by <i>A. obliquus</i> in this study and the comparison with other studies.	67
Table 4.4	The major fatty acid compositions (%) of <i>A. obliquus</i> , <i>C. vulgaris</i> , and <i>Chlorella</i> sp.	68
Table 4.5	The fatty acid profile (FA) (% to total fatty acids) of freshwater microalgae	69

Table 4.6	The ALA content in <i>A. obliquus</i> compared to other <i>Acutodesmus</i> species in previously reported work	71
Table 4.7	Nine experimental runs using L ₉ (3 ⁴) orthogonal array for the percentage of omega-3 FAs molecules and S/N ratio using Taguchi method.	73
Table 4.8	Response Table for S/N Ratios (Larger is better) obtained through the Taguchi analysis	76
Table 4.9	ANOVA results for the parameters involved in omega-3 FAs production	77
Table 4.10	Confirmation of experiment for ETA content by using the Taguchi optimisation method. <i>A. obliquus</i> was cultured under normal AF6 medium (control) and optimised AF6 medium. ETA content was then obtained by extracting FAMES from the microalgae biomass and was detected using GC/MS analysis	79
Table 4.11	The comparison in the production of ETA in the <i>Acutodesmus</i> genus between this study and other studies	80
Table 4.12	Comparison for ETA content in mutants for the family <i>Scenedesmaceae</i> in this and previous studies	90
Table 4.13	Comparison for ETA produced in mutagenic strains of microalgae in this study and previous studies	91
Table 4.14	Comparison of ETA content in mutants in this study and previous studies other than microalgae	92
Table 4.15	RNA quality and quantity of <i>A. obliquus</i> for RNA sequencing	94
Table 4.16	Summary of RNA sequencing and <i>de novo</i> transcriptome assembly	96
Table 4.17	Transcription changes for annotation of significantly different unigenes associated with the ETA biosynthesis pathway of mutant Q2-12E compared to wild type of <i>A. obliquus</i>	107

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
Figure 2.1	Biosynthesis pathway of omega-3 FAs. ALA: α -linolenic acid, SDA: stearidonic acid, ETA: eicosatetraenoic acid, EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid. (Adopted from Ryckebosch et al., 2012)	13
Figure 2.2	The chemical structure of ETA (Adopted from Okuda et al., 2015)	14
Figure 2.3	The chemical structure of EPA (Adopted from Asmarai and EL Kadmiri, 2020)	15
Figure 2.4	The chemical structure of DHA (Adopted from Asmarai and EL Kadmiri, 2020)	15
Figure 2.5	Diagrammatic representation showing microalgae being the primary source of omega-3 FAs and the link in the human food chain	17
Figure 2.6	The chemical structure of EMS (Modified from Joya-Dávila and Gutiérrez-Miceli, 2020)	31
Figure 2.7	The molecular structure of quizalofop (Modified from Xiao-Hong et al., 2007)	32
Figure 2.8	Central Dogma of Molecular Biology	36
Figure 3.1	An overview of the study	42
Figure 4.1	Morphological observation of microalgae with 400X magnification (a: <i>A. obliquus</i> , b: <i>Chlorella</i> sp., c: <i>C. vulgaris</i>)	59
Figure 4.2	Comparison of microalgae growth and the biomass yield by <i>A. obliquus</i> , <i>Chlorella</i> sp. and <i>C. vulgaris</i> . The growth of the microalgae was monitored every alternate day	61
Figure 4.3	Nile red staining of the microalgae. Three microalgae cells were harvested every 10 days and stained with Nile red. Fluorescence signals of the stained cells were then detected using fluorescence spectrometer (LS55, Perkin Elmer, USA)	64
Figure 4.4	The lipid content of microalgae after growing until the stationary phase. The biomass of the microalgae was used to extract the lipid content by Modified Folch's method	66

Figure 4.5	The control factor effects on omega-3 FAs production identified from the Taguchi method. The level for each factor with the highest S/N ratios was considered as the optimum condition.	75
Figure 4.6	The interaction effects plot for S/N ratios. This figure was obtained through the Taguchi analysis	78
Figure 4.7	The survival rate of <i>A. obliquus</i> cells (%) vs EMS concentration (M). <i>A. obliquus</i> culture that was treated with EMS were spread on AF6 agar plate. After one month, the colonies that were formed on the AF6 agar plate were counted. The survival rate of <i>A. obliquus</i> cells was calculated by comparing the number of mutant colonies that formed on the AF6 agar plate with the control (wild type) colonies	82
Figure 4.8	The effect of quizalofop treatment on the colony formation of <i>A. obliquus</i> . <i>A. obliquus</i> culture cells were spread on two types of AF6 agar plates; containing different quizalofop concentration and without quizalofop (control); (a) 0 μ M (Control); (b) 200 μ M; (c) 250 μ M; (d) 300 μ M; (e) 350 μ M; (f) 400 μ M; (g) 450 μ M; (h) 500 μ M. Colonies of <i>A. obliquus</i> were formed after 2 weeks of treatment with quizalofop. The control sample indicates the colony formed without being treated with quizalofop	85
Figure 4.9	Comparison of growth between wild type and EMS mutant screened using quizalofop at stationary phase of growth. The growth profile of 23 mutants can be found in Appendix N.	87
Figure 4.10	Comparison of ETA content in the wild type and mutant of <i>A. obliquus</i> . The wild type and mutants were cultured using optimised AF6 media	89
Figure 4.11	Functional annotation of unigenes in databases	97
Figure 4.12	Species classification	98
Figure 4.13	The distribution of genes expressed in the wild type and mutant of <i>A. obliquus</i>	99
Figure 4.14	The number of up-regulated and down-regulated DEGs of <i>A. obliquus</i> between wild type and mutant Q2-12E strains	100
Figure 4.15	GO enrichment analysis of DEGs. The X-axis shows the GO term in the sub-level of the GO three main domains. The Y-axis shows the number of the DEGs annotated in the GO category. From left to right is the three main GO domains: biological process (BP), cellular component (CC) and molecular function (MF) that are distinguished by different colour code.	102

Figure 4.16 The KEGG enrichment scatter plot of DEGs between mutant Q2-12E and wildtype strains. X-axis: Rich factor. Y-axis: Enriched KEGG pathways. A rich factor refers to the ratio of the DEGs enriched number in the pathway to the number of all unigenes annotated in the pathway. The different colours of the points represent a different q-value ranges. The size of the dots is positively correlated to the number of DEGs in the pathway

104

Figure 4.17 Regulation of ETA biosynthesis pathway from transcriptome data. Red labelled enzymes signify the up-regulated enzyme. Blue labelled enzymes represent the normally expressed enzyme. The upwards solid arrows indicate the upregulation of respective enzymes. Enzyme abbreviations: ACC (Acetyl-CoA carboxylase); MCAT (Malonyl-CoA-acyl carrier protein transacylase); FAS (Fatty acid synthase); FAT (Fatty acyl-ACP thioesterase B).

108

LIST OF ABBREVIATIONS

ALA	- α -linolenic acid
<i>A. obliquus</i>	- <i>Acutodesmus obliquus</i> CN01
ACCase	- Acetyl-CoA carboxylase
ANOVA	- Analysis of variance
ATP	- Adenosine triphosphate
BLAST	- Basic Local Alignment Search Tools
bp	- Base pairs
BP	- Biological Process
<i>C. vulgaris</i>	- <i>Chlorella vulgaris</i> NIES-1269
CC	- Cellular Component
<i>Chlorella</i> sp.	- <i>Chlorella</i> sp. Carolina-15-2069
CO ₂	- Carbon dioxide
DDBJ	- DNA Data Bank of Japan
DEGs	- Differentially expressed genes
DGE	- Digital Gene Expression libraries
DHA	- Docosahexaenoic acid
DNA	- Deoxyribonucleic acid
dNTPs	- Deoxynucleotide triphosphate
DPA	- Docosapentaenoic acid
EAR	- Enoyl-ACP reductase
EMBL	- European Molecular Biology Laboratory
EMS	- Ethyl methanesulfonate
ENU	- N-ethyl-N-nitrosourea
EPA	- Eicosapentaenoic acid
ETA	- Genetic Algorithm
ETA	- Eicosatetraenoic acid
FACS	- Fluorescence activated cell sorting
FAMES	- Fatty acid methyl ester
FAs	- Fatty acids
FAS	- Fatty acid synthase
FAT	- Fatty acyl-ACP thioesterase B
FPKM	- Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced
GC/MS	- Gas chromatograph with mass spectrometer detector
GO	- Gene Ontology
HAD	- 3-hydroxyacyl-ACP dehydratase
HMM	- Hidden Markov Models
KAR	- β -ketoacyl-ACP reductase
KAR	- 3-oxoacyl-ACP reductase,
KAS III	- β -ketoacyl-acyl-carrier-protein synthase III
KCS	- 3-ketoacyl-CoA synthase
KEGG	- Kyoto Encyclopedia of Genes and Genome
KO	- Kyoto Encyclopedia of Genes and Genomes Ortholog Database
KOG	- Eukaryotic Orthologous Groups

LB	- Larger-the-better
LC-PUFA	- Long chain PUFA
MCAT	- Malonyl-CoA-acyl carrier protein transacylase
MF	- Molecular Function
MMS	- Methyl methanesulfonate
MNNG	- N-methyl-N'-nitro-N-nitrosoguanidine
MNU	- N-methyl-N-nitrosourea
mRNA	- Messenger RNAs
MUFA	- Monounsaturated fatty acids
NB	- Nominal-the-better
NCBI	- National Center for Biotechnology Information
NGS	- Next-generation sequencing
NGS	- Next-generation sequencing
Nr	- NCBI non-redundant protein sequences
Nt	- NCBI non-redundant nucleotide sequences
NTG	- Nitrosomethyl guanidine
OA	- Orthogonal array
OAs	- Orthogonal arrays
OD	- Optical density
Omega-3 FAs	- Omega-3 fatty acids
PCBs	- Polychlorinated biphenyls
PCR	- Polymerase chain reaction
PCR	- Polymerase chain reaction
PDB	- Protein Data Bank
Pfam	- protein family
PHS1	- very-long-chain (3 R)-3-hydroxyacyl-CoA dehydratase
PIR	- Protein Information Resource
PRF	- Protein Research Foundation
PUFA	- Polyunsaturated fatty acids
PUFA	- Polyunsaturated fatty acids
Q-PCR	- Quantitative PCR
RIN	- RNA integrity number
RNA	- Ribonucleic acid
RNA-seq	- RNA-sequencing
ROS	- Reactive oxygen species
rpm	- Revolutions per minute
RSEM	- RNA-Seq by Expectation-Maximization
RSM	- Response surface methodology
RT-PCR	- Reverse transcription-PCR
S/N ratio	- Signal-to-noise ratio
SB	- Smaller-the-better
SDA	- Stearidonic acid
SDS	- Sodium Dodecyl Sulfate
SFA	- Saturated fatty acids
T-DNA	- Transfer deoxyribonucleic acid
TAG	- Triacylglycerol
UTM	- Universiti Teknologi Malaysia
UV	- Ultraviolet
UV-VIS	- Ultraviolet-visible
VLCPUFA	- Very long-chain PUFA

WHO - World Health Organization

LIST OF SYMBOLS

ω	-	Methyl
%	-	Percentage
α	-	Alpha
G	-	Guanine
C	-	Cytosine
A	-	Adenine
T	-	Thymine
μ	-	Specific growth rate
nm	-	Wavelength of light in nanometre
°C	-	Degree celcius
a.u.	-	Fluorescence intensity
g	-	Relative centrifuge force
μmol	-	Micromol
ln	-	Natural logarithm
t_d	-	Doubling time
v/v	-	Volume per volume
C17:0	-	heptadecanoic acid
L	-	Latin square
p	-	P-value
M	-	Molarity
w/v	-	Weight per volume
Q	-	Phred score
day^{-1}	-	Specific growth rate
EC	-	Enzyme commission
logFC	-	Fold change

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
Appendix A	GC-MS chromatograms of FAME derived from freeze-dried biomass of freshwater green algae <i>A. obliquus</i>	141
Appendix B	GC-MS chromatograms of FAME derived from freeze-dried biomass of freshwater green algae <i>Chlorella</i> sp.	142
Appendix C	GC-MS chromatograms of FAME derived from freeze-dried biomass of freshwater green algae <i>C. vulgaris</i>	143
Appendix D	GC-MS chromatograms of ETA (C20:4) and ALA (C18:3) derived from freeze-dried biomass of freshwater green algae <i>A. obliquus</i> from first set of experiment of Taguchi method	144
Appendix E	GC-MS chromatograms of ETA (C20:4) and ALA (C18:3) derived from freeze-dried biomass of freshwater green algae <i>A. obliquus</i> from second set of experiment of Taguchi method	145
Appendix F	GC-MS chromatograms of ETA (C20:4) and ALA (C18:3) derived from freeze-dried biomass of freshwater green algae <i>A. obliquus</i> from third set of experiment of Taguchi method	146
Appendix G	GC-MS chromatograms of ETA (C20:4) and ALA (C18:3) derived from freeze-dried biomass of freshwater green algae <i>A. obliquus</i> from fourth set of experiment of Taguchi method	147
Appendix H	GC-MS chromatograms of ETA (C20:4) and ALA (C18:3) derived from freeze-dried biomass of freshwater green algae <i>A. obliquus</i> from fifth set of experiment of Taguchi method	148
Appendix I	GC-MS chromatograms of ETA (C20:4) and ALA (C18:3) derived from freeze-dried biomass of freshwater green algae <i>A. obliquus</i> from sixth set of experiment of Taguchi method	149
Appendix J	GC-MS chromatograms of ETA (C20:4) and ALA (C18:3) derived from freeze-dried biomass of freshwater green algae <i>A. obliquus</i> from seventh set of experiment of Taguchi method	150
Appendix K	GC-MS chromatograms of ETA (C20:4) and ALA (C18:3) derived from freeze-dried biomass of freshwater green	

	algae <i>A. obliquus</i> from eighth set of experiment of Taguchi method	151
Appendix L	GC-MS chromatograms of ETA (C20:4) and ALA (C18:3) derived from freeze-dried biomass of freshwater green algae <i>A. obliquus</i> from ninth set of experiment of Taguchi method	152
Appendix M	Screening of EMS mutant with 400 μ M quizalofop	153
Appendix N	Growth of EMS mutant screened using quizalofop. <i>A. obliquus</i> culture treated with EMS was spread on the AF6 agar plate containing quizalofop. 23 of EMS mutants that showed distinguish morphology on the AF6 agar plate containing quizalofop were isolated. The growth profile of 23 mutants were determined by monitoring the growth every alternate days.	154
Appendix O	GC/MS chromatogram of ETA (C20:4) detected in wild type sample	155
Appendix P	GC/MS chromatogram of ETA (C20:4) detected in mutant Q2-6C	156
Appendix Q	GC/MS chromatogram of ETA (C20:4) detected in mutant Q2-12B sample	157
Appendix R	GC/MS chromatogram of ETA (C20:4) detected in mutant Q2-12D sample	158
Appendix S	GC/MS chromatogram of ETA (C20:4) detected in mutant Q2-12E sample	159
Appendix T	FPKM Density Distribution	160
Appendix U	EC number for enzyme involved in omega-3 FAs biosynthesis pathway	161
Appendix V	t table	162

CHAPTER 1

INTRODUCTION

1.1 Background of study

Recent advances in food supplements due to the virus pandemic have emphasised the beneficial impact of omega-3 fatty acids (omega-3 FAs) on human health and longevity (Yu et al., 2021). Polyunsaturated fatty acids (PUFA) are essential nutrients that cannot be synthesised by humans and need to be consumed in the diet. Prominent derivative products of PUFA are omega-3 FAs and omega-6 fatty acids (Abyor et al., 2011). However, this study does not associate with omega-6 fatty acids as the focus will be solely on omega-3 FAs.

Omega-3 FAs are known as PUFA that have the first double bond located between the third and fourth carbon atom counting from the methyl end of the fatty acid (Ryckebosch et al., 2012). The shortest chain of omega-3 FAs is referred to as α -linolenic acid (ALA, C18:3) (Sayeda et al. 2015). On the other hand, the main long chain of polyunsaturated omega-3 FAs are eicosapentaenoic acid (EPA; 20:5), docosapentaenoic acid (DPA; 22:5) and docosahexaenoic acid (DHA; 22:6) (Liu et al., 2016).

Omega-3 FAs have played a pivotal role in brain development, cardiovascular function and immune system regulation (Liu et al., 2016). Apart from that, there is evidence that shows omega-3 FAs also play a crucial role in treating diseases such as arteriosclerosis, cancer, and rheumatoid arthritis (Fan et al., 2001).

In this new global economic era, the increase in the human population has become a central issue for the need for new sources of omega-3 FAs (Lin et al., 2019). At present, the main commercial source of omega-3 FAs is marine fish (Fan et al., 2001; Kosasih et al., 2021). However, evidence suggests that due to the increase of

human population, marine fish is deemed as not sufficient for the world's requirement of omega-3 FAs. Hence, a much-debated question about whether marine fish will be used as a source of food for humans or as a source for production of omega-3 FAs supplement for humans' health care has increased dramatically. Therefore, efforts have been made to seek for eukaryotic microorganisms such as microalgae, which is an alternative sources for omega-3 FAs production (Fan et al., 2001).

Microalgae is known as an essential food for zooplankton, larvae and juvenile stages of molluscs, crustaceans, and certain herbivorous fish (Dörner et al., 2014a). Recently, there has been renewed interest in microalgal biomass dubbed as a novel "green technology" (Xin et al., 2010; Li et al., 2020). Previous studies have reported that microalgae pose advantages of biomass with its rich source of nutrients such as omega-3 FAs, omega-6 fatty acids, proteins, minerals, and other essential nutrients (Tokuşoglu and Ünal, 2003). In addition, there is a growing body of literature that recognises microalgae as a suitable alternative source of omega-3 FAs with high economic potential (Xin et al., 2010) due to the continuity of their raw materials, easily developed in the tropics, simple cultivation conditions (Li et al., 2020), prevalence to adverse environmental factors (Borowitzka, John, Raven, 2016; Jo et al., 2020) and not susceptible to chemical contamination (Abyor et al., 2011). Existing research also recognizes the crucial role played by microalgae, which include anti-inflammatory, antidiabetic, antioxidant, anticoagulant, immunomodulatory, antimicrobial effects, ultraviolet (UV) protective and tyrosinase inhibitor (Sirikhachornkit et al., 2018).

Acutodesmus obliquus CN01 that was selected as the main microalgae species in this study is widely known as fresh-water (Jo et al., 2020) oleaginous (Li et al., 2020; León-Saiki et al., 2020) unicellular green microalgae that can produce α -linolenic acid (ALA: omega-3 FAs) (Minhas et al., 2020). There is a big possibility in this algal strain but there is not enough data to utilize for omega-3 FAs production. *Acutodesmus obliquus* is a mesophilic microalga that can grow at temperature in the range between 15 and 40 °C (Xin et al., 2010) and has a very short (less than 24 hours) cell cycle (Kim et al., 2014). Existing research recognizes *Acutodesmus* sp. as one of the ideal oleaginous freshwater microalgal species for research due to luxurious growth (Makareviciene et al., 2011) with easy cultivation (Liu et al., 2012; Mandotra

et al., 2014; Martinez, Jimenez, El Yousfi, 1999; Mujtaba et al., 2012; Salama et al., 2013; Wu et al., 2013; Xin, Hong-ying, Jia, 2010; Xin et al., 2010; Xin et al., 2010; Xin, Hong-Ying, Yu-Ping, 2011; Han et al., 2018; Jo et al., 2020; Minhas et al., 2020), highly immune to bacterial infection (Liu et al., 2012), dominate other algal species and can flexibly adapt to wide range of environmental condition (Mandotra et al., 2014).

This present study, therefore, first sets out to assess biochemical composition of three different freshwater microalgae, which are *Acutodesmus obliquus* CN01, *Chlorella* sp. Carolina-15-2069 and *Chlorella vulgaris* NIES-1269. Additionally, this study seeks to shed some light on the omega-3 FAs produced by the newly isolated freshwater green alga *Acutodesmus obliquus* CN01 originated from Malaysia (Yahya et al., 2018) which could give nutritional added value to microalgae oil. Then, the yield of omega-3 FAs produced by the selected microalgae will be enhanced by optimising various culture conditions using statistical Taguchi method with simpler method due to its minimum number of experiments, less time consuming and cost-saving. Optimisation of microalgae cultivation has emerged as powerful platforms to produce biocomponents with high quality that can enhance the production efficiency of microalgae and reduce its cost (Dao et al., 2018). This study also prompted to conduct random mutation induced using ethyl methanesulfonate (EMS) followed by quizalofop selective screening that will be used to improve the algal strain with enhanced omega-3 FAs content and to manipulate the fatty acid metabolism in the selected microalgae. Lastly, *de novo* transcriptome profiling will be conducted to characterize the molecular responses to EMS-induced random mutation followed by quizalofop selective screening and to understand the role of important genes that are involved in fatty acid synthesis in the selected microalgae.

1.2 Problem Statement

Marine fish is currently the commercial source to produce omega-3 FAs. However, fish may not be the best source of omega-3 FAs due to its scarcity, odour, geographical and seasonal variations in quality (Liu and Lin, 2001). Apart from that,

there is an increasing interest in recent years among vegetarians for omega-3 FAs from non-animal sources (Tsai et al., 2016). Growing demand for new sources of omega-3 FAs is also a continuing safety concern within the accumulation of heavy metals and lipo-soluble compounds, such as organic mercury compounds and polychlorinated biphenyls (PCBs) from the contaminants in the marine fish oil (Tsai et al., 2016).

Microalgae is a suitable alternative source for the production of omega-3 FAs due to its ability to grow well in the tropics, and produce in a carefully controlled environment and is suitable for vegetarian dieters (Winwood, 2013; Jo et al., 2020; Li et al., 2020; Minhas et al., 2020). Therefore, microalgae can become an alternative and suitable source for omega-3 FAs production (Jo et al., 2020). *Acutodesmus obliquus* are oxygenic photosynthetic organisms that is of interest (Li et al., 2020) due to its fast growth and easy to cultivate (Sirikhachornkit et al., 2018; Jo et al., 2020; Minhas et al., 2020). Recently, *Acutodesmus obliquus* has been used as a model organism to produce omega-3 FAs that has the potential to meet nutraceutical demand with additional environmental benefits (Jo et al., 2020).

Moreover, existing research has recognized the need to enhance the omega-3 FAs content in the microalgae for the commercial exploitation as a direct supplement to the human diet (Chaturvedi et al., 2004). Herein, it is necessary and imperative to develop practical technology for the cultivation of microalgae (Zhang et al., 2018). Therefore, it has been reported that optimisation of variables for cultivation conditions is effective in altering the biochemical composition of microalgae, by increasing the fatty acid content (Moha-León et al., 2019; Sijil et al., 2019; Kumar et al., 2020; Jo et al., 2020; Minhas et al., 2020; Loh et al., 2021; Teh et al., 2021; San Cha et al., 2021). As a result, optimising the culture conditions can boost the omega-3 FAs content in *Acutodesmus obliquus*.

Recent evidence has demonstrated the need to implement genetic manipulation into microalgae to meet the growing market demand for microalgae-based natural products (Moha-León et al., 2019). Genetic manipulation via random mutation is a feasible and sustainable alternative to produce compounds with commercial interest (Moha-León et al., 2019) such as omega-3 FAs. Random mutation by chemical

mutagenesis has been used in various microalgae as an effective strategy for strain development in microalgae to significantly increased PUFA production especially omega-3 FAs (Robertson et al., 2013; Kumar et al., 2020). In addition, previous literature highlighted the significance of random mutation followed by subsequent selection using herbicides to identify genetic variants in mutants with desirable changes in accumulation of biomolecule (Noochanong, Jirakranwong, and Chanprame, 2018), such as enhanced fatty acid synthesis (Moha-León et al., 2019). EMS is a random, easy and powerful technique in generating several mutants by modifying A-T to G-C in the microalgal genome (Al-Saedi et al., 2019). Therefore, EMS-based mutation followed by quizalofop as selective screening was considered as the promising strategy to enhance omega-3 FAs content in *Acutodesmus obliquus*.

Interestingly, it is found that there is a notable paucity of studies investigating the complete identification of molecular mechanisms controlling omega-3 FAs biosynthesis in oleaginous species such as *Acutodesmus obliquus*. Hence, the effect of random mutation using EMS followed by quizalofop selection on the molecular mechanism for omega-3 FAs production enhancement in *Acutodesmus obliquus* is still being understood poorly. It is noteworthy to understand and identify the genes involved in omega-3 FAs biosynthesis through transcriptomic study for *Acutodesmus obliquus*.

1.3 Objectives of study

The main objectives of the research are as follows:

- 1) To compare the production of omega-3 FAs in freshwater green alga *Acutodesmus obliquus*, *Chlorella* sp., and *Chlorella vulgaris*.
- 2) To determine the optimised culture conditions for omega-3 FAs enhancement in *Acutodesmus obliquus* by using the Taguchi method.
- 3) To isolate mutants of *Acutodesmus obliquus* with enhanced growth and omega-3 FAs content using EMS and quizalofop.
- 4) To characterize the molecular response to EMS mutation screened with quizalofop which induced omega-3 FAs accumulation in *Acutodesmus obliquus* by *de novo* transcriptome of RNA-sequencing (RNA-seq) data.

1.4 Scope of study

Three species of freshwater microalgae (*Acutodesmus obliquus*, *Chlorella* sp. and *Chlorella vulgaris*) were first cultured under standardised conditions in the specific media and the biochemical composition of each microalgae was identified. Growth of the microalgae, dry cell weight, lipid content and fatty acid methyl ester (FAMES) profile were assessed to determine the biochemical composition of the three microalgae species. Next, microalgae with the highest omega-3 FAs content was selected for further study. Then, the culture conditions of the selected microalgae were optimised based on four factors: carbon, nitrogen, salinity and phosphorus. The optimal culture condition for omega-3 FAs production was further assessed using Taguchi's experimental design method. EMS with different dose and treatment time was used as the chemical mutagen to enhance omega-3 FAs production and improve the tolerance of the selected microalgae. In the present study, the mutant strains with high tolerance to EMS are screened out using quizalafop as the selective technique. The growth as well as the omega-3 FAs content of mutants were investigated. Mutant strains with higher growth than the wild type have been isolated and further studied

for omega-3 FAs composition. Mutant with higher growth and omega-3 FAs content than the wild type was selected for further study in this work. To get detailed explanation in terms of the gene involved, *de novo* transcriptome analysis was applied to the RNA-sequencing data to provide basic understanding of the molecular responses to mutation induced by EMS followed by quizalofop screening in the selected microalgae. This study also seeks to provide a better understanding on the role of important genes involved in fatty acid biosynthesis that were triggered by random mutagenesis and selective screening.

1.5 Significance of study

The prevalence of dietary complications has been increasing at an alarming rate in all age groups. As a result, there is a pressing need in Malaysian diet intakes to reduce dietary complications by increasing the intake of omega-3 FAs and to prevent obesity and noncommunicable diseases. Recently, there has been a surge of interest in omega-3 FAs derived from microalgae, which are thought to be convenient and cost-effective. Omega-3 FAs that have a wide range of plausible applications in biotechnology can be derived from *Acutodesmus obliquus*. Up to now, very little is known about omega-3 FAs produced from microalgae in Malaysia. Therefore, this study attempts to shed new light on the suitable microalgae species as promising omega-3 FAs producer that has better application in nutraceutical field and further commercial exploitation. Major obstacles of freshwater microalgae such as *Acutodesmus obliquus* as omega-3 FAs producer is the lower content of omega-3 FAs. To enhance the omega-3 FAs production, the best nutrient concentration in the culture medium has been identified by the application of Taguchi's method that can solve major obstacles to obtain a pure source of omega-3 FAs. To the best of our knowledge, this is the first study to demonstrate the application of Taguchi's method to enhance omega-3 FAs production in *Acutodesmus obliquus*. Previous studies have established that the biosynthesis of fatty acids in microalgae were enhanced by mutation-selection method. Surprisingly, there have been limited studies conducted in relation to mutation-selection method for enhancement of omega-3 FAs production in *Acutodesmus obliquus*. Therefore, this study also provides a major significant to

obtain new strains of *Acutodesmus obliquus* with genetic improvement with higher contents of omega-3 FAs using mutation-selection method. There has been a limited source that provides a better understanding for omega-3 FAs regulation in *Acutodesmus obliquus* especially following random mutagenesis and selective selection method. Hence, this is the first study that seeks to fill the literature gap in this field. Thus, this research sheds new light on understanding mechanism that random mutation-selective selection method triggered and provides a new insight in the gene expression of omega-3 FAs pathway in *Acutodesmus obliquus*.

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LIST OF PUBLICATIONS

Journal with Impact Factor

1. **Othman, F. S.**, Jamaluddin, H., Ibrahim, Z., Hara, H., Yahya, N. A., & Mohamad, K. I. S. E. (2019). Production of α -linolenic Acid by an Oleaginous Green Algae *Acutodesmus obliquus* Isolated from Malaysia. *J Pure Appl Microbiol*, 13(3), 1297-1306. <https://doi.org/10.22207/JPAM.13.3.01> (**Q4, IF: 0.073**)
2. **Othman, F. S.**, Hashim, M., Omar, A. H., Reba, M. N. M., Muhamad Said, M. F., Soeed, K., Shahir, S., Abdul-Wahab, M.F., Iwamoto, K., Mohamad, S.E. & Ibrahim, Z. (2020). Isolation and Identification of Psychrotrophic Photosynthetic Bacterium from Antarctic Seawater with Antibacterial Activity. *Journal of Pharmacy and Pharmacology*, 8, 200-206. <https://doi:10.17265/2328-2150/2020.07.002> (**Q2, IF: 3.765**)

Indexed Journal

1. **Othman, F. S.**, Ibrahim, Z., Dollah, R., Yoshizaki, S., Homma, K., Hara, H., Suzuki, I., Iwamoto, K., & Mohamad S. E. (2021). *Acutodesmus obliquus* CN01 as new potential source of eicosatetraenoic acid: Enhancing omega-3 fatty acids production by *Acutodesmus obliquus* CN01 by medium optimization using Taguchi method. *European Journal of Molecular & Clinical Medicine*, 8(3), 4464-4473. (**Indexed by SCOPUS**)
2. Kuwabara, T., Iwamoto, K., Hara, H., Yamaguchi, T., Mohamad, S. E., Abdullah, N., Khudzari, J.M. & **Othman, F. S.** (2021, February). Prevention of Soil Erosion Using Microalgae in Malaysia. In *IOP Conference Series: Materials Science and Engineering*, 1051(1), 12047. IOP Publishing. (**Indexed by Scopus**)

Non-Indexed Conference Proceedings

1. **Othman, F. S.**, Salleh, A. A., Ibrahim, N. N., Tharek, A., Ibrahim Z., & Mohamad, S. E. (2017). Production of omega-3 from freshwater and marine microalgae. In *8th Asian Pacific Phycological Forum (APPF 2017)* (pp. 143).
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3. **Othman, F. S.**, Abdullah, N., Ibrahim Z., Jamaluddin, H., Hara, H., Iwamoto, K., Mohamad, S. E. (2018). Omega-3 Fatty Acids Production from. Microalgae. In *SCEJ 83rd Annual Meeting (Osaka, 2018)*(C303).
 4. **Othman, F. S.**, Ibrahim Z., Dolah, R., Yoshizaki, S., Homma, K., Hara, H., Suzuki, I., Iwamoto, K., & Mohamad, S. E. (2020). *Acutodesmus obliquus* CN01 as new potential source of eicosatetraenoic acid: Enhancing omega-3 fatty acids production by *Acutodesmus obliquus* CN01 by medium optimization using Taguchi method. In *International Conference on Innovative Research in Science and Technology (ICIRST-2020)*(pp. 11).