PHYSIOLOGICAL, MOLECULAR AND BIOCHEMICAL STUDIES ON EGYPTIAN MICROALGAE ISOLATES FOR POTENTIAL USE IN BIODIESEL PRODUCTION

By

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B.Sc. Agric. Sci. (International Agric.), Fac. Agric., Cairo Univ., 2007
M.Sc. Agric. Sci. (Plant Physiology), Fac. Agric., Cairo Univ., 2015

THESIS
Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences
(Plant Physiology)

Department of Agric. Botany
Plant Physiology Section
Faculty of Agriculture
Cairo University
EGYPT

Format Reviewer **2019** Vice Dean of Postgraduate Studies

APPROVAL SHEET

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SUPERVISION SHEET

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Title of Thesis: Physiological, Molecular and Biochemical Studies on Egyptian Microalgae isolates for Potential Use in Biodiesel Production

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ABSTRACT

Blue-green algae (cyanobacteria) have all the potential to act as a platform for the development of fourth-generation biofuel. Nevertheless, the diversity and eco-physiology of blue-green algae in northern African countries and their biofuel production potential is still understudied. The aim of this thesis was screening eight isolates of blue-green algae belonging to genera of *Anabaena* (*A. circinalis, A. fertilissima, A. sphaerica, A. variabilis, and A. sp.*) and *Nostoc* (*N. commune*, *N. muscorum*, and *N. sp.*) for high lipid productivity, and to select the best isolate to further increase its lipid induction using different treatments. The results revealed that *A. sphaerica* was the superior isolate with good fatty acids profile and with the highest lipids productivity which was attributed to high specific growth rate and high lipid concentration. *A. sphaerica* was then subjected to different treatments including culture operation mode, media duplicity as well as different nutrition strategies in terms of nutrients supplementation or elimination (i.e. two stages mode of nutrition). All studied parameters of *A. sphaerica* were found to be best induced by NO_3 or PO_4 elimination in two stages mode of nutrition, as well as under 60μM $FeCl_3$ supplementation. The environmental adaptability of *A. sphaerica* for lipid production under single or/and combined salt (50, 75, and 100 mM NaCl) and mild heat stress (35 ºC) was evaluated, and it was found that lipids productivity was best induced under all studied NaCl concentrations, while the 35 ºC treatment slightly increased both lipid yield and lipids % of *A. sphaerica*. When NaCl was combined with 35 ºC, all parameters were increased, although to a lower degree than in a single factor. The combined effects of best studied treatments (+50 mM NaCl, -NO_3, -P, +60 μM $FeCl_3$) were evaluated for lipids induction including double, triple as well as quadruple combinations. Generally, triple and quadruple combinations decreased most parameters, whereas –P and +60 μM $FeCl_3$ in a double combination was the only treatment that synergistically induced lipids as compared to single effect of each factor alone. Finally, identification of the *A. sphaerica* strain was based on molecular studies on the 16S rDNA sequence of *A. sphaerica* which was submitted to the NCBI GenBank database (accession No. MK271089) and the strain was named *Anabaena sphaerica* FSR1. Estimated biodiesel properties of *Anabaena sphaerica* FSR1 calculated from 5 previous treatments, i.e. +60 μM $FeCl_3$, –NO_3, +50 mM NaCl, +Fe –P, and +Na+Fe–P were apparently found within the range of the biodiesel standards EN 14213 and EN 14214 (Europe), especially ASTM D6751–08 (United States). We recommend opting for the treatment that induces the highest lipid productivity to be more economically feasible, which was found to be the addition of 50 mM NaCl to the BG-11_0 media. Future studies involving this valuable strain are recommended to screen for useful bio products as well as assessing the success of its open air culturing.

Key words: blue-green algae, *Anabaena sphaerica*, nutrients starvation, stress,16S rRNA, biodiesel.

اسم الطالب: ريهام محمد نجيب أحمد علي البحبوحي **الدرجة:** دكتوراه الفلسفة

عنوان الرسالة: دراسات فسيولوجية جزيئية و حيوية على عزلات الطحالب المصرية لاستخدامها في انتاج الوقود الحيوي.

المشرفون : دكتور: نجيبة كامل الغمراوي
دكتور: محمد رمضان ابو العلا نسيم
دكتور: عصام عبد المعطي درويش

قسم: النبات الزراعي **فرع:** فسيولوجيا النبات **تاريخ منح الدرجة:** / /

المستخلص العربي

تمتلك الطحالب الخضراء المزرقة (cyanobacteria) القدرة على العمل كمنصة لتطوير الجيل الرابع من الوقود الحيوي. ومع ذلك فإن الدراسات التي تهتم بالتنوع والفيزيولوجيا البيئية للطحالب الخضراء المزرقة في بلدان شمال أفريقيا وإمكانية إنتاجها للوقود الحيوي ما زالت قليلة جدا. الهدف من هذه الرسالة هو دراسة ثمانية عزلات من الطحالب الخضراء المزرقة التي تنتمي إلى أجناس أنابينا (*A. circinalis, A. fertilissima, A. sphaerica, A. variabilis,* و *A. sp.*) و نوستوك (*N. commune*, *N. muscorum*, *N. sp.*) من خلال دراسة إنتاجيتها للدهون، واختيار أفضل عزلة منهم ثم زيادة إنتاجية الدهون في هذه العزلة باستخدام بعض المعاملات المختلفة. أوضحت النتائج أن *A. sphaerica* كانت العزلة الأفضل من حيث تفريد الدهون و الأعلى من حيث إنتاجية الدهون والتي كانت تعزى إلى أسرع معدل نمو وأعلى محتوى من الدهون. بعد ذلك تم إخضاع *A. sphaerica* لمعاملات مختلفة بما في ذلك طريقة تنميتها على بيئة مضاعفة في تركيز الأملاح المغذية، بالإضافة إلى استخدام استراتيجيات التغذية المختلفة من حيث تركيز بعض العناصر الغذائية أو إزالتها (التغذية على مرحلتين). وقد أشارت جميع القياسات لل *A. sphaerica* زيادتها باستخدام معاملات إزالة NO_3 أو PO_4 في معاملة التغذية على مرحلتين، وكذلك في معاملة إضافة تركيز ٦٠ ميكرومولر من $FeCl_3$. كذلك تم تقييم القدرة لل *A. sphaerica* على التكيف البيئي وقدرتها على إنتاج الدهون وذلك بمعاملتها بتركيزات مختلفة بملح كلوريد الصوديوم (٥٠ ، ٧٥ ، ١٠٠ مللي مولر) وإجهاد حراري طفيف (٣٥ درجة مئوية) منفردين أو مجتمعين. وقد وجد أن أفضل إنتاجية للدهون تحدث تحت جميع تركيزات كلوريد الصوديوم المدروسة ، في حين أن معاملة ٣٥ درجة مئوية أحدثت زيادة طفيفة في نسبة الدهون ٪ ل *A. sphaerica*. وعندما تم الجمع بين معاملات كلوريد الصوديوم و ٣٥ درجة مئوية، حدثت زيادة في جميع القياسات ولكن بدرجة أقل من تأثير العوامل منفردة. تم أيضا تقييم التأثيرات لأفضل المعاملات (+٥٠ مللي مولر NaCl، $-NO_3$، $-P$، أو ٦٠+ ميكرومولر $FeCl_3$) في زيادة تراكم الدهون، وذلك من خلال المعاملات المزدوجة، الثلاثية أو الرباعية. وبشكل عام قللت المعاملات الثلاثية والرباعية معظم القياسات، في حين أن المعاملة المزدوجة $-P$ + ٦٠ ميكرومولر $FeCl_3$ كانت لها التأثير التضافري على زيادة تراكم الدهون مقارنة بالتأثيرات الفردية لكل منهما. وفي النهاية تم تحديد سلالة *A. sphaerica* استنادًا إلى دراسات جزيئية على تسلسل rDNA لـ 16S من *A. sphaerica* والذي تم تقديمه إلى قاعدة بيانات NCBI GenBank (رقم الانضمام MK271089) وتم تحديد السلالة ب *Anabaena sphaerica* FSR1. أظهرت خواص الوقود الحيوي المقدرة لل *Anabaena sphaerica* FSR1 أنها كانت في نطاق المعايير العالمية للوقود الحيوي EN 14213 و EN 14214 (اوروبا)، و خاصةً ASTM D6751–08 (الولايات المتحدة). ولذلك نوصي باختيار المعاملة التي تحفز أعلى إنتاجية للدهون لتكون أكثر جدوى من الناحية الاقتصادية و هي إضافة ٥٠ مللي مولر كلوريد الصوديوم إلى بيئة $BG\text{-}11_0$. و كذلك توصي النتائج بإجراء دراسات مستقبلية على هذه السلالة القيّمة من أجل فحص المنتجات الحيوية المفيدة بالإضافة إلى تقييم نجاح زراعتها في البيئة الطبيعية.

الكلمات الدالة: ،الطحالب الخضراء المزرقة، *Anabaena sphaerica*، نقص العناصر، اجهاد بيئي، 16SrRNA، الوقود الحيوي.

DEDICATION

Dreams and dedication are a powerful combination. The words were found to express what is inside…

Thanksgiving and appreciation and feelings of love, sincerity and gratitude. I dedicate this thesis and my success to my Sweetheart, to my friend of struggle and success, to my love and life, to Shady.

The love of a family is life's greatest blessing. Family is experience of love and support and patience. With love I dedicate this thesis to Dad, Mom, Ahmed, Waleed, Arwa, Rowan, Hamza, and Cuty Loly.

Nothing in this world can express my love and my immense thanks to all of you.

Together we have it all!!

ACKNOWLEDGEMENT

In the Name of Allah, the Most Beneficent, the Most Merciful. All the praises and thanks be to Allah, all thankful belong to ALLAH who makes everything possible.

My deepest prayers for the late ***Dr. Naguiba El-Ghamrawy****, Late Prof. of Plant Physiology, Plant Physiology Department, Faculty of Agriculture, Cairo University, may Allah rest her soul. I feel immense gratitude for her support during the beginning of this thesis and her friendly advices and encouragement.*

I wish to express my deep appreciation and profound gratitude to ***Prof. Dr. Mohamed Ramadan****, Plant Physiology Department, Faculty of Agriculture, Cairo University, for his great help and dedication in and supervising me, his moral support during this work, close guidance, creative supervision and valuable experience and discussions.*

All respect and thanks for ***Dr. Essam Darwish*** *Associate Prof. of Plant Physiology, Faculty of Agriculture, Cairo University for supervising the work as well as his continual valuable guidance during the work and providing a lot of required facilities to accomplish the analysis and experiments of this thesis.*

Sincere gratitude is also due to ***Prof. Dr. Fekry Mohamed Ghazal****, Prof. of Soil Microbiology, Soil Microbiology Department, Soils, Water and Environment Res. Inst., Agricultural Research Center, for his continual valuable guidance during the work and his constructive criticism during the development of this thesis. I would like to thank him for his patience while training me to think as an algologist, and for encouraging me when I was facing difficulties.*

Special thanks are due to ***Dr. Shady Abdel Mottaleb*** *Lecturer of Plant Physiology, Faculty of Agriculture, Cairo University, for his valuable help and scientific support, along my study, that I can't forget it. Also, all appreciation and respect for his advices, guidance and Spiritual support with each step starting from planning the PhD plan and suggesting the thesis work till finishing this fruitful thesis.*

Deep thanks to ***Dr. Manal Farouk*** *Researcher at Agricultural Genetic Engeneering Research Institute (AGERI), Agricultural Research Center, for her great help and providing facilities to accomplish a required molecular analysis of A. sphaerica isolate.*

Deep thanks to ***Prof. Dr. Essam Agamy*** *Vice Dean of Postgraduate and Research, and* ***Dr. Ayman Yehia Amin,*** *Prof. of Plant Physiology, Faculty of Agriculture, Cairo University for their great help and providing facilities to accomplish required biochemical analysis in my thesis.*

LIST OF ABBREVIATIONS AND INITIALS

Abbreviation	Meaning of abbreviation
ANOVA	Analysis of variance
BG-11	Blue-Green 11 Medium
$BG\text{-}11_0$	Nitrogen-free Blue-Green 11 Medium
BLAST	Basic Local Alignment Search Tool
bp	Base pair
Chl-a	Chlorophyll-a
CTAB	Cetyltrimethylammounium bromide
DHA	Docosahexaenoic acid (C22:6)
EPA	Eicosapentaenoic acid (C20:5)
FAME	Fatty Acid Methyl Ester
K’	Growth rate
MLDP	Major lipid droplet protein
mM	Milli molar
NCBI	National Center for Biotechnology Information
nm	nanometer
No.	Number
O.D	Optical Density
PAR	Photosynthetically active radiation
PCR	Polymerase chain reaction
POS	Primary oleaginous species
PS I	Photosystem I
PS II	Photosystem II
ROS	Reactive oxygen species
SOS	Secondary oleaginous species
TAG	Triacylglyceride
TEM	Transmission electron microscopy
UV	Ultra violet
μ	Specific growth rate
$\mu E\ m^{-2}\ s^{-1}$	Micro Einstein /metre^2/second
μM	Micro molar