



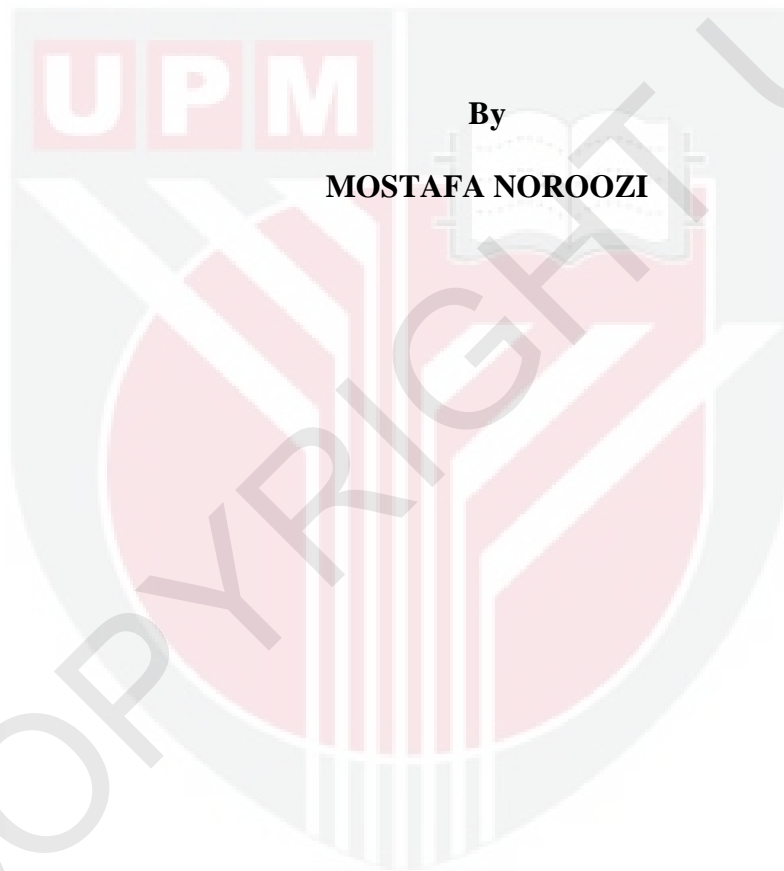
UNIVERSITI PUTRA MALAYSIA

**ISOLATION AND CULTURE OF GREEN ALGAE *HAEMATOCOCCUS*
PLUVIALIS AND ITS MOLECULAR DIVERSITY**

MOSTAFA NOROOZI

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**ISOLATION AND CULTURE OF GREEN ALGAE *HAEMATOCOCCUS
PLUVIALIS* AND ITS MOLECULAR DIVERSITY**



By

MOSTAFA NOROOZI

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

November 2011

DEDICATED TO

To my inspiring mother

To my lovely wife, Saeidah

To my handsome sons, Hossein and Mohammad Hesam

To my supporting siblings



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

ISOLATION AND CULTURE OF GREEN ALGAE *HAEMATOCOCCUS PLUVIALIS* AND ITS MOLECULAR DIVERSITY

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November 2011

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Haematococcus pluvialis (Flotow) is a unicellular green alga from volvocale algae living in temporary shallow freshwater ponds and has many applications for humans, poultry and fishery due to its ability to produce astaxanthin. The main objective of this study was isolation of new strains from the water bodies in Iran and to evaluate the growth of nine strains of *H. pluvialis* obtained from four countries (four strains from Iran, two strains from Finland, one strain from Switzerland and three strains from USA), in distinct growth media and also determine their genetic diversity based on ISSR and RAPD.

The traditional methods and our new found method were used to isolate the four Iranian species. In the new method the algal suspension were exposed to high light intensity to make the cells transform to big, red cyst form which is easy to see and easier to separate from other organisms. The isolated algae were cultured in Bold medium as general algal medium to obtain enough biomass to inoculums to other media. All the cultures were

done under sterile condition using pre-autoclaved chemicals and microbiological filter passed air for aeration. Two methods were used to detect the biomass of cultivated cells including 0.8 micron Milipore filter paper and DMSO method to extract and measure chlorophyll concentration in all media and strains. The astaxanthin concentration was measured by spectrophotometer and HPLC methods which DMSO was used to extract pigment from the cyst of *H. pluvialis*. In this research, the cultured media were centrifuged to precipitate the biomass and DNA was extracted from 10 different strains of *H. pluvialis* using the Dellaporta method with the liquid nitrogen and mortar. Genetic similarity study was carried out using 14 ISSR and 12 RAPD primers.

The biomass obtained from Bold culture were used for the purpose of inoculation to four other media namely: NIES, OHM, Mixotroph and COMBO (the COMBO medium was used for the first time to culture *H. pluvialis*). The results of this study depicted that Mixotroph growth medium gave the best biomass of 0.577 g dry wt/l followed by COMBO and OHM media respectively. Moreover, the strains showed geographical differentiations as a result the Iran4 and USA2 strains produced highest biomass and the Finland2 showed the slowest growth.

Molecular markers are suitable tools for the purpose of finding out genetic variations in organisms; however there is no study conducted on ISSR or RAPD molecular markers regarding this organism. The molecular weights of the bands produced ranged from 3.4 to 0.14 Kb. The PCA and dendrogram clustered the *H. pluvialis* strains into various groups according to their geographical origins. The lowest genetic similarity was between the Iran2 and USA1 strains and the highest genetic similarity was between Finland1 and Finland2. The results showed that ISSR and RAPD markers are useful for the genetic

diversity studies of *Haematococcus* as they showed clustering of strains from similar geographical origins. As a conclusion different strains of *H. pluvialis* shows various abilities in physiological, metabolites and biochemical traits. The various characteristics of strains explain the necessity of survey on new resources to discover more efficient strains.



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

PEMENCILAN, KULTUR ALGA HIJAU ALGA *HAEMATOCOCCUS PLUVIALIS* DAN KEPELBAGAIAN GENETIK MOLEKULARNYA

Oleh

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Haematococcus pluvialis (Flotow) adalah alga hijau alga unisel dari kumpulan volvocale yang biasa ditemui di badan air tawar cetek sementara dan mempunyai banyak kegunaan bagi manusia, ternakan dan perikanan kerana kemampuannya untuk menghasilkan astaxanthin. Tujuan utama dari penelitian ini adalah untuk menilai pertumbuhan sepuluh strain *Haematococcus pluvialis* yang diperolehi daripada empat negara (empat strain dari Iran, dua strain dari Finland, satu strain dari Switzerland dan tiga strain dari Amerika Syarikat) yang dikultur dalam media pertumbuhan yang berbeza dan juga untuk menentukan kepelbagaian genetik berdasarkan kaedah ISSR dan RAPD.

Media Bold digunakan untuk mendapatkan pengeluaran biojisim utama bagi tujuan inokulasi bagi empat media lain iaitu: NIES, OHM, Mixotroph dan COMBO (media COMBO digunakan buat pertama kalinya untuk pengkulturan *H. pluvialis*). Biojisim homogeny yang tumbuh dalam media berbeza ditapis melalui kertas penapis Millipore

0.8µm untuk mendapatkan berat kering. Kepekatan klorofil ditentukan melalui kaedah dimetilsulfoksida. Keputusan kajian ini menunjukkan bahawa media pertumbuhan Miksotrof memberikan biojisim terbaik dengan berat kering 0,577 g / l diikuti oleh medium COMBO dan media OHM. Selain itu, strain menunjukkan perbezaan geografi antra hasil strain Iran4 dan USA2 yang menghasilkan biojisim tertinggi manakala Finland2 menunjukkan pertumbuhan paling lambat.

Penanda molekul adalah alat yang sesuai untuk tujuan mengetahui variasi genetik dalam organisma, namun tiada kajian yang telah dilakukan menggunakan penanda molekul ISSR atau RAPD terhadap organisma ini. Dalam kajian ini, sampel DNA diekstrak dari 10 strain *H. pluvialis* yang berbeza menggunakan kaedah Dellaporta. Kajian persamaan genetik dijalankan dengan menggunakan primer 14 ISSR dan 12 RAPD.

Kesamaan genetik terendah adalah antara strain Iran2 dan USA1 manakala kesamaan genetik tertinggi adalah antara Finland1 dan Finland2. Keputusan kajian menunjukkan bahawa penanda ISSR dan RAPD adalah berguna untuk kajian kepelbagaian genetik *Haematococcus* yang telah menunjukkan hasil kluster strain dari asal geografi yang serupa.

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Finally, this thesis is dedicated to all those who believe in the virtue of learning.

I certify that a Thesis Examination Committee has met 30th November to conduct the final examination of Mostafa Noroozi on his thesis entitled “Isolation and culture of green algae *Haematococcus pluvialis* and its molecular diversity” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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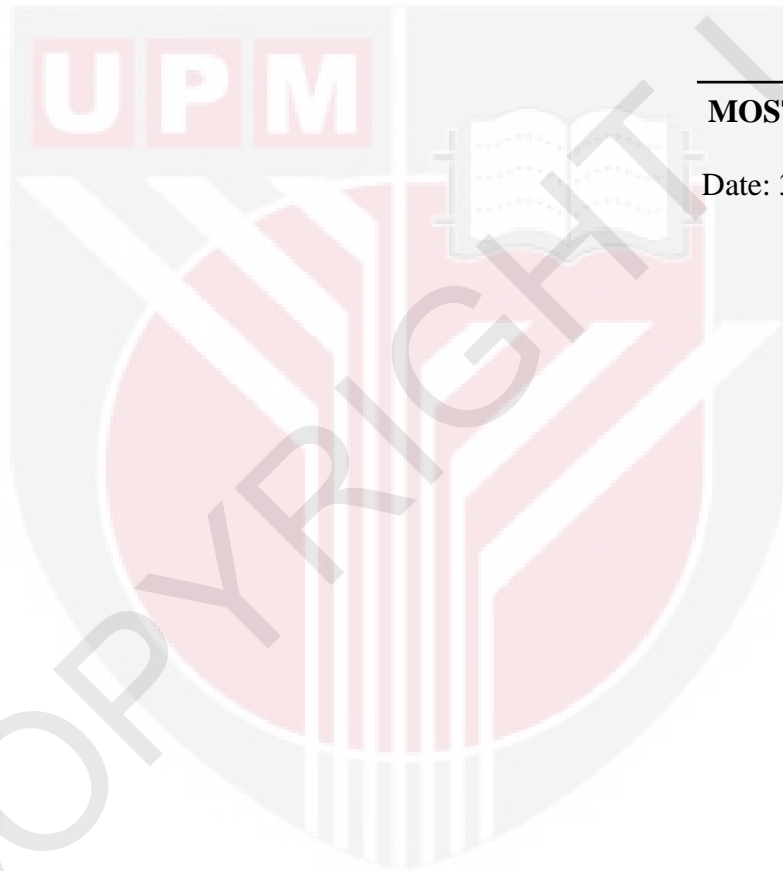
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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.



MOSTAFA NOROOZI

Date: 30 November 2011

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