THE UNIVERSITY of ADELAIDE

ELUCIDATION AND ISOLATION OF SPECIFIC BIOACTIVE COMPOUND IN CYANOBACTERIA ISOLATES

by

Chee Kuan Kwei

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Learn from yesterday, live for today, hope for tomorrow. The important thing is not to stop questioning.

Albert Einstein

ABSTRACT

Sulfoquinovosyldiacylglyceride (SQDG), one of the bioactive compounds isolated from *Spirulina*, has been proven to act as an inhibitor of reverse-transcriptase, and has potential for the use in the Combination Anti-Retroviral Therapy (cART). A number of researchers report that the many potential uses of *Spirulina*, with the most widely used strains being *Spirulina platensis* and *S. maxima*, require further investigation to determine their actual usefulness in medical applications.

In this work, four commercial isolates marketed as *Spirulina* and an Australian isolate, *Spirulina* sp. CS-785/01 (CSIRO), were selected to determine their bio-composition and applicability as a source of bioactive compounds. During the course of the investigation, it was determined that the four commercial isolates were in fact *Arthrospira* and the Australian isolate is *Halospirulina*. However, this insight occurred towards the end of the study and the '*Spirulina*' has been adopted as common name when referring to the isolates during the experimental programme that has been reported in the thesis. The commercial isolates used in this research were *Spirulina* (J), *Spirulina* (M), *Spirulina* (P) and *Spirulina* (S), while the Australian isolate *Spirulina* sp. was also used throughout the investigation.

The identification of these isolates was examined based on their molecular and the chemotaxonomic classification. Furthermore, the genes responsible for the production of SQDG were isolated to assess the potential therapeutic value of these isolates in treating disease, such as HIV (Human immunodeficiency virus). Since SQDG is a compound of current interest, a suitable extraction technique was developed to optimise its production. Different extraction techniques, such as microwave-assisted sonication and homogenisation, coupled with various forms of organic solvents, were examined in this study. Preliminary phytochemical analysis was also undertaken to reveal the potential use of the investigated isolates for the further development of pharmaceuticals due to the presence of specific phyto-constituents. Finally, sequential extracts and the isolated compound of SQDG were used to determine their bioactivity against a range of microbes and HIV.

The results revealed that the Australian isolate was not highly productive in respect to growth. This is due to its limited adaptability to changes in temperature and culture media type. The Australian isolate is limited to a specific temperature range and culture media type. Additionally, it has substantial biochemical variation compared to the commercial isolates. The diversity of these isolates could be explained by their molecular and chemotaxonomic classification, which revealed that the Australian isolate belongs to the genus of *Halospirulina,* while the commercial isolates belong to the genus *Arthrospira*. The carbohydrate, protein, lipid and fatty acid content of the commercial isolates also indicated that they have higher nutritional value when compared to the *Spirulina* sp. This strongly suggested that the commercial isolates belonged to a different genus to the *Spirulina* sp.

In spite of the diversity of the classification, all investigated isolates showed the presence of SQDG. Overall, the results showed that *Spirulina* (S) has a high potential for synthesis of SQDG, due to its high content of C16:0 and C18:2, while the SQDG content of Australian isolate was lowest among the investigated isolates. By applying this data, the Australia isolate is unlikely to be suitable for large-scale production. It also showed lower appreciable amounts of SQDG and gamma linolenic acid (GLA). The highest yield of SQDG from *Spirulina* (M) was from using a chloroform:methanol (2:1, v/v) extraction solvent system. However, a methanol extraction solvent system is suggested, due to its high recovery of SQDG and low toxicity. Because SQDG is a potent inhibitor of HIV-reverse transcriptase, it can be concluded that commercial isolates are good sources for drug production because of their high content of SQDG and rapid biomass production.

STATEMENT OF ORIGINALITY

This work contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution from Chee Kuan Kwei and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Chee Kuan Kwei

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ABBREVIATION

^{o}C	Degree Celsius
α	Alpha
AIDS	Acquired Immunodeficiency Syndrome
AFDW	Ash free dry weight
ATCC	American Type Culture Collection
BLAST	Basic Local Alignment Search Tool
bp	Base pairs
BuOH	Butanol
C	Cytosine
$CaCl_2$	Calcium chloride
cART	Combination Anti-retroviral Therapy
$CHCl_3$	Chloroform
chl	Chlorophyll
CSIRO	Commonwealth Scientific and Industrial Research Organization
DGDG	Digalactosyldiacylglycerol
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide trisphosphate
EDTA	Ethylenediaminetetraacetic acid
ELSD	Evaporative light scattering detector
EPA	Eicosapentaenoic acid
EtOH	Ethanol
FAME	Fatty acid methyl esters
g	Gram
G	Guanine
GC	Gas chromatography
GLA	Gamma linolenic acid
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HIV	Human immunodeficiency virus

HPLC	High pressure liquid chromatography
HSV	Herpes simplex virus
IMVS	Institute of Medical and Veterinary Science
IUPAC	International Union of Pure and Applied Chemistry
kb	Kilobases
L	Litre
m	Milli
MeOH	Methanol
mg	Milligrams
$MgCl_2$	Magnesium chloride
MGDG	Monogalactosyldiacylglycerol
min	Minute
MS	Mass spectrometry
NaAc	Sodium acetate
NaCl	Sodium chloride
NCBI	National Centre for Biotechnology Information
NMR	Nuclear magnetic resonance
NNRTIs	Non-nucleoside reverse-transcriptase inhibitors
NRPS	Non-ribosomal peptide synthase
NRTIs	Nucleoside reverse-transcriptase inhibitors
PCC	Pasteur culture collection
PCR	Polymerase chain reaction
PKS	Polyketide synthase
PUFA	Poly unsaturated fatty acids
rRNA	Ribosomal RNA
RNA	Ribonucleic acid
rpm	Revolution per minute
s	Second
SARDI	South Australian Research and Development Institute
SDS	Sodium dodecyl sulphate
SQDG	Sulfoquinovosyldiacylglycerol
TAE	Tris-acetate-EDTA
TE	Tris-EDTA

Tris	Tris hydroxymethyl aminomethane
TLC	Thin layer chromatography
UDP	Uridine diphosphate
UNSW	University of New South Wales
UV	Ultraviolet
V	Volt
v/v	Volume per volume
WH	Woods Hole
w/v	Weight per volume
XS	Xanthogenate-SDS