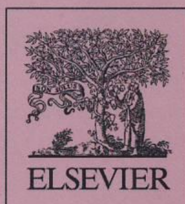
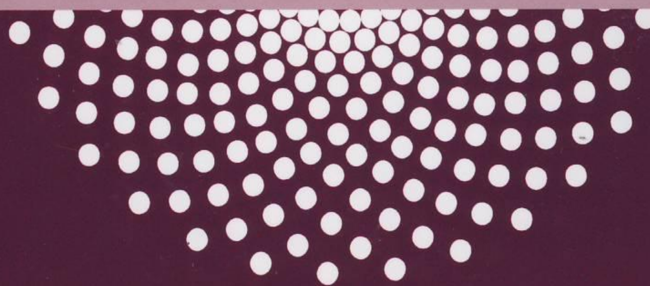
Volume 14 • 2015 ISSN 1876-6196



Procedia Chemistry



2nd Humboldt Kolleg in Conjunction with International Conference on Natural Sciences 2014, HK-ICONS 2014

Editors:

Roy Hendroko Setyobudi, Hugo Scheer, Leenawaty Limantara, Yuzo Shioi, Leszek Fiedor, Tatas H.P. Brotosudarmo and Monika N.U. Prihastyanti

Available online at www.sciencedirect.com

ScienceDirect

Available online at www.sciencedirect.com
ScienceDirect
Procedia Chemistry
Procedia Chemistry 14 (2015) i

2nd Humboldt Kolleg in Conjunction with International Conference on Natural Sciences 2014, HK-ICONS 2014

Editors:
Roy Hendroko Setyobudi, Hugo Scheer,
Leenawaty Limantara, Yuzo Shioi,
Leszek Fiedor, Tatas H.P. Brotosudarmo
and Monika N.U. Prihastyanti

ELSEVIER

Amsterdam - Boston - London - New York - Oxford - Paris - Philadelphia - San Diego - St Louis

© 2015 Elsevier Ltd.

This journal and the individual contributions contained in it are protected under copyright by Elsevier Ltd. and the following terms and conditions apply to their use:

Photocopying
Single photocopies of single articles may be made for personal use as allowed by national copyright laws. Permission of the Publisher and payment of a fee is required for all other photocopying, including multiple or systematic copying, copying for advertising or promotional purposes, resale, and all forms of document delivery. Special rates are available for educational institutions that wish to make photocopies for non-profit educational classroom use.

For information on how to seek permission visit www.elsevier.com/permissions or call: (+44) 1865 843830 (UK) / (+1) 215 239 3804 (USA).

Derivative Works
Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution. Permission of the Publisher is required for all other derivative works, including compilations and translations (please consult www.elsevier.com/permissions).

Electronic Storage or Usage
Permission of the Publisher is required to store or use electronically any material contained in this journal, including any article or part of an article (please consult www.elsevier.com/permissions).

Except as outlined above, no part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without prior written permission of the Publisher.

Notice
No responsibility is assumed by the Publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein. Because of rapid advances in the medical sciences, in particular, independent verification of diagnoses and drug dosages should be made.

Although all advertising material is expected to conform to ethical (medical) standards, inclusion in this publication does not constitute a guarantee or endorsement of the quality or value of such product or of the claims made of it by its manufacturer.

ELSEVIER

Preface
L. Limantara. . . . .
The Condition of *Uncaria* C
A. Rauf, Rahmawaty, A.
Hepatoprotective Effects of
A. Dwijayanti, A. Frethe
Coral Diseases in Panjang I
A. Sabdono, P.H. Sawon
The Routes of Administrati
A.D. Wijayanti, G.D. Sa
Apoptosis in Mice Liver C
A.A. Maramis, M. Ami
An α-Glucosidase Inhibito
A.P.G. Macabeo, P.Y.M
Isolation and Characteriza
A.P. Subroto, C. Utomo
Microwave Assisted Extra
A.C. Kumoro, I. Hartat
Hydrothermal Carbonatio
A.T. Mursito, A. Yuliya
Biosolid Management Op
A. Ghimire, R. Sen, A.
Identifying Sumatran Pea
A. Wibowo, H. Sloterd
Pathotyping of Local Isol
A. Haryanto, M. Purw
Process Improvement of
Avicenna, M. Mel, S.I
Preliminary Evaluation o
C.J. Hui, M.N.U. Prih
Inhibition of the Non-Me
C. Lienau, S. Konzuc
Design of Expression Ca
C. Utomo, A.P. Subro
Micropropagation of Em
C.L.M. Marbun, N. T
Numerical Simulation a
Wave Energy Conversio
D.T. Ba, N.D. Anh, P
Thermal Stability of α-P
D. Markovic, S. Böh
Expression Analysis of
D.A. Listiawan, R. T
The Level of Glutamic
Rr. E. Susetyarini . .
Genotypic Characteriza
E.A. Corcolon, A.C.
Oxygen Ion-Conductiv
Biodiesel-Fuel Cells
F. Rahmawati, W. Fa

t by Elsevier Ltd. and the

copyright laws. Permission
iple or systematic copying,
. Special rates are available
oom use.

call: (+44) 1865 843830

cts for internal circulation
on outside the institution.
ns and translations (please

d in this journal, including

eval system or transmitted
vise, without prior written

or property as a matter of
, products, instructions or
, in particular, independent

nclusion in this publication
of the claims made of it by

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Procedia Chemistry 14 (2015) iii–v

Procedia Chemistry

Contents

Preface
L. Limantara 1

The Condition of *Uncaria Gambir* Roxb. as One of Important Medicinal Plants in North Sumatra Indonesia
A. Rauf, Rahmawaty, A.Z. Siregar 3

Hepatoprotective Effects of *Acalypha Indica* and *Centella Asiatica* in Rat's Liver Against Hypoxia
A. Dwijayanti, A. Frethernety, N.S. Hardiany, E.H. Purwaningsih 11

Coral Diseases in Panjang Island, Java Sea: Diversity of Anti-Pathogenic Bacterial Coral Symbionts
A. Sabdono, P.H. Sawonua, A.G.D. Kartika, J.M. Amelia, O.K. Radjasa 15

The Routes of Administration of Amikacin as Consideration in Reptile Therapy
A.D. Wijayanti, G.D. Satria, A.E.T.H. Wahyuni 22

Apoptosis in Mice Liver Cells Caused by Formalin-containing Food: Normalization of HSP70 Overexpression by Chlorophyllin
A.A. Maramis, M. Amin, Sumarno, A.D. Corebima 27

An α-Glucosidase Inhibitor from *Drepananthus Philippinensis*
A.P.G. Macabeo, P.Y.M. Rubio, G.J.D. Alejandro, M. Knorn 36

Isolation and Characterization of Oil Palm Wrinkled 1 (WRI1) Gene
A.P. Subroto, C. Utomo, C. Darmawan, Z.A. Tanjung, T. Liwang 40

Microwave Assisted Extraction of Dioscorin from Gadung (*Dioscorea Hispida* Dennst) Tuber Flour
A.C. Kumoro, I. Hartati 47

Hydrothermal Carbonation of K-Rich Ash, Value Added Energy Engineering and CO_2 Mineral Sequestration
A.T. Mursito, A. Yuliyanti, Jakah 56

Biosolid Management Options in Cassava Starch Industries of Thailand: Present Practice and Future Possibilities
A. Ghimire, R. Sen, A.P. Annachhatre 66

Identifying Sumatran Peat Swamp Fish Larvae through DNA Barcoding, Evidence of Complete Life History Pattern
A. Wibowo, H. Sloterdijk, S.P. Ulrich 76

Pathotyping of Local Isolates Newcastle Disease Virus from Field Specimens by RT-PCR and Restriction Endonuclease Analysis
A. Haryanto, M. Purwaningrum, S. Verawati, S.H. Irianingsih, N. Wijayanti 85

Process Improvement of Biogas Production from Anaerobic Co-digestion of Cow Dung and Corn Husk
Avicenna, M. Mel, S.I. Ihsan, R.H. Setyobudi 91

Preliminary Evaluation of the Pigments Content from *Rhodobacter Sphaeroides* at Stages during Photosynthetic Growth
C.J. Hui, M.N.U. Prihastyanti, T.H.P. Brotosudarmo 101

Inhibition of the Non-Mevalonate Isoprenoid Pathway by Reverse Hydroxamate Analogues of Fosmidomycin
C. Lienau, S. Konzuch, T. Gräwert, B. Illarionov, A. Bacher, M. Fischer, N. Tanaka, T. Kurz 108

Design of Expression Cassettes for Inverted Repeat and Intron-Spliced Inverted Repeat of PATE Gene Silencing
C. Utomo, A.P. Subroto, C. Darwaman, T. Liwang 117

Micropropagation of Embryogenic Callus of Oil Palm (*Elaeis Guineensis* Jacq.) Using Temporary Immersion System
C.L.M. Marbun, N. Toruan-Mathius, Reflini, C. Utomo, T. Liwang 122

Numerical Simulation and Experimental Analysis for a Linear Trigonal Double-Face Permanent Magnet Generator Used in Direct Driven Wave Energy Conversion
D.T. Ba, N.D. Anh, P.V. Ngoc 130

Thermal Stability of α-Phycoerythrocyanin
D. Markovic, S. Böhm, K.-H. Zhao, H. Scheer 138

Expression Analysis of Transcription Factors Involved in Chloroplast Differentiation
D.A. Listiawan, R. Tanoue, K. Kobayashi, T. Masuda 146

The Level of Glutamic Acid in the Semen of Male White Rat (*Ratus Norwegicus*) after Being Treated with Tannin of *Pluchea Indica*
Rr. E. Susetyarini 152

Genotypic Characterization of Turmeric (*Curcuma Longa* L.) Accessions from Mindanao, Philippines Using RAPD Markers
E.A. Corcolon, A.C. Laurena, M.L. Dionisio-Sese 157

Oxygen Ion-Conductivity and Chemical Stability of Ceria Based-Electrolyte in Composite with Sodium Carbonate as Electrolyte for Direct Biodiesel-Fuel Cells
F. Rahmawati, W. Fajriati, E. Heraldy, D.G. Syarif 164

Function of Tetrapyrroles, Regulation of Tetrapyrrole Metabolism and Methods for Analyses of Tetrapyrroles
H. Schlicke, A. Richter, M. Rothbart, P. Brzezowski, B. Hedtke, B. Grimm 17
Biliproteins and their Applications in Bioimaging
H. Scheer, X. Yang, K.-H. Zhao 17
Mathematical Model of the Hydrotropic Microwave Assisted Extraction of Anti Malarial Agent from *Andrographis Paniculata*
I. Hartati, L. Kurniasari, Y. Anas 18
Composition of Photosynthetic Pigments in a Red Alga *Kappaphycus Alvarezi* Cultivated in Different Depths
Indriatmoko, Heriyanto, L. Limantara, T.H.P. Brotosudarmo 19
Separation of Photosynthetic Pigments by High-performance Liquid Chromatography: Comparison of Column Performance, Mobile Phase, and Temperature
Indriatmoko, Y. Shioi, T.H.P. Brotosudarmo, L. Limantara 20
Potentially Antihyperglycemic from Biomass and Phycocyanin of *Spirulina Fusiformis* Voronikhin by in Vivo Test
I. Setyaningsih, M. Bintang, N. Madina 21
Flower Bracts of Temulawak (*Curcuma Xanthorrhiza*) for Skin Care: Anti–Acne and Whitening Agents
I. Batubara, I. Julita, L.K. Darusman, A.M. Muddathir, T. Mitsunaga 21
Analysis on the Chlorophyll Content of Commercial Green Leafy Vegetables
L. Limantara, M. Dettling, R. Indrawati, Indriatmoko, T.H.P. Brotosudarmo........ 22
Identification, Isolation and Antioxidant Activity of Pheophytin from Green Tea (*Camellia Sinensis* (L.) Kuntze)
L. Kusmita, I. Puspitaningrum, L. Limantara 23
In Vitro Selection of *Jatropha Curcas* Linn. Hybrids Using Polyethylene Glycol to Obtain Drought Tolerance Character
Maftuchah, A. Zainudin........ 23
Application of Simple Multispectral Image Sensor and Artificial Intelligence for Predicting of Drought Tolerant Variety of Soybean
M.A.S. Adhiwibawa, Y.E. Setiawan, Y. Setiawan, K.R. Prilianti, T.H.P. Brotosudarmo 24
Whole Cell Hydrolysis of Sardine (*Sardinella Lemuru*) Oil Waste Using *Mucor Circinelloides* NRRL 1405 Immobilized in Poly-Urethane Foam
M.G.M. Purwanto, M.V. Maretha, M. Wahyudi, M.T. Goeltom 25
Sensory Characteristics of Seasoning Powders from Overripe Tempeh, a Solid State Fermented Soybean
M.D.P.T. Gunawan-Puteri, T.R. Hassanein, E.K. Prabawati, C.H. Wijaya, A.N. Mutukumira........ 26
Indigenous Herbal Recipes for Treatment of Liver Cirrhosis
M.P.B. Wijayagunawardane, C.U.B. Wijerathne, C.B. Herath 27
Financial Prospects in the Application of Decanter System for the Treatment of Sludge Wastewater in Water Treatment Plant System
Mohajit........ 27
Detection of Gamma-Irradiated Mutant of Rodent Tuber (*Typhonium Flagelliforme* Lodd.) in Vitro Culture by RAPD Molecular Marker
N.F. Sianipar, Ariandana, W. Maarisit........ 28
Performance of Tetraphenylsulfonato Disilane in Catalytic Transesterification of Crude Palm Oil and Esterification of Fatty Acids with Secondary Alcohols
N. Bangun, W. Manullang, L. Panggabean, S.B. Sembiring, R. Simangunsong, P. Bali, F.R. Panjaitan 29
The Potency of Guava *Psidium Guajava* (L.) Leaves as a Functional Immunostimulatory Ingredient
N. Laily, R.W. Kusumaningtyas, I. Sukarti, M.R.D.K. Rini 30
Formulating Two Steps Loan Scheme to Support Emission Reduction Projects in Indonesia
N. Syaifudin, H. Amir, S. Wianwiwat 30
The Performance of *Jatropha Curcas* Linn. Capsule Husk as Feedstocks Biogas in One Phase Anaerobic Digestion
P.G. Adinurani, S.R. Hendroko, S.K. Wahono, A. Nindita, M. Mairziwan, A. Sasmito, Y.A. Nugroho, T. Liwang 31
Bioremediation of Aged Petroleum Oil Contaminated Soil: From Laboratory Scale to Full Scale Application
Q. Helmy, R. Laksmono, E. Kardena 32
Integrated Geographic Information System and Global Positioning System for Mapping of Forest Plants in Supporting Natural Resources Protection
Rahmawaty, E.K. Sari, A. Syofyan, A. Rauf........ 33
Peptide-derived from Seahorse Exerts a Protective Effect Against Cholinergic Neuronal Death in *in vitro* Model of Alzheimer's Disease
R. Pangestuti, S.-K. Kim 34
Encapsulation of Brown Seaweed Pigment by Freeze Drying: Characterization and its Stability during Storage
R. Indrawati, H. Sukowijoyo, Indriatmoko, R.D.E. Wijayanti, L. Limantara........ 35
Genetic Diversity of *Celosia* Variants in East Java Based on Polyphenol Oxidase-PPO Genes
R. Mastuti, E.L. Arumingtyas, A.A. Fatinah........ 36
Potential of Ciplukan (*Physalis Angulata* L.) as Source of Functional Ingredient
R.W. Kusumaningtyas, N. Laily, P. Limandha........ 36
Preliminary Study the Potency of Macroalgae in Yogyakarta: Extraction and Analysis of Algal Pigments from Common Gunungkidul Seaweeds
R. Haryatfrehni, S.C. Dewi, A. Meilianda, S. Rahmawati, I.Z.R. Sari 37
Removal of Lead and Copper from Contaminated Water Using EAPR System and Uptake by Water Lettuce (*Pistia Stratiotes* L.)
R.S. Putra, F. Cahyana, D. Novarita........ 38
Biological Purification System: Integrated Biogas from Small Anaerobic Digestion and Natural Microalgae
Salafudin, R.H. Setyobudi, S.K. Wahono, A. Nindita, P.G. Adinurani, Y.A. Nugroho, A. Sasmito, T. Liwang........ 38
Serial Endothelial Cell Count of Donor Corneal Buttons in Optisol-GS
S.A.B. Sibayan, Ma. C.P. Garcia-Arenal, K.D. Corpus, J.R.G. Manlongat, M.B.B. Ibañez IV, Ma. D.B. Padilla, J.U. Dy Liacco........ 39

Vitamin A Fortification Efforts Require Accurate Monitoring of Population Vitamin A Status to Prevent Excessive Intakes
S.A. Tanumihardjo 398

Second Generation Bioethanol from Arabica Coffee Waste Processing at Smallholder Plantation in Ijen Plateau Region of East Java
S.S. Harsono, Salahuddin, M. Fauzi, G.S. Purwono, D. Soemarno, Kissinger 408

Adaptation of the Photosynthetic Unit of Purple Bacteria to Changes of Light Illumination Intensities
T.H.P. Brotosudarmo, L. Limantara, Heriyanto, M.N.U. Prihastyanti 414

The Chlorophyll *b* Reductase NOL Participates in Regulating the Antenna Size of Photosystem II in *Arabidopsis Thaliana*
T. Jia, H. Ito, A. Tanaka 422

Heavy Metals in Water of Stream Near an Amalgamation Tailing Ponds in Talawaan – Tatelu Gold Mining, North Sulawesi, Indonesia
T.M. Palapa, A.A. Maramis 428

Effects of Pigment–Protein Fraction from *Nannocloropsis Oculata* on TNFα and IL-6 which Act as an Anti-inflammatory Against Viral Nervous Necrosis (VNN) Infection
U. Yanuhar 437

A Survey of Photosynthetic Pigments from Surface to Oligotrophic Deep Seawater in Suruga Bay, Japan
W. Miyata, T. Suzuki, B.E. Casareto, Y. Suzuki, Y. Shioi 444

Effects of Cyclohexane/Acetone Ratio on Bixin Extraction Yield by Accelerated Solvent Extraction Method
W. Rahmalia, J.-F. Fabre, Z. Mouloungui 455

Anti-Cancer Effect of Kaffir Lime (*Citrus Hystrix* DC) Leaf Extract in Cervical Cancer and Neuroblastoma Cell Lines
W.A. Sri Tunjung, J. Cinatl Jr., M. Michaelis, C.M. Smales 465

Agrobacterium Tumefaciens Mediated Transformation of *rolC::Hd3a-GFP* in Black Rice (*Oryza Sativa* L. cv. Cempo Ireng) to Promote Early Flowering
Y.A. Purwestri, R.D.K. Sari, L.N. Anggraeni, A.B. Sasongko 469

The Photosensitizer Stabilities of Tookad® on Aggregation, Acidification, and Day-light Irradiation
Y.A. Handoko, F.S. Rondonuwu, L. Limantara 474

Status of Drug Discovery Research Based on Marine Organisms from Eastern Indonesia
Y.H. Tapilatu 484

Sweet Potato Production for Bio-Ethanol and Food Related Industry in Indonesia: Challenges for Sustainability
Y. Widodo, S. Wahyuningsih, A. Ueda 493

Peroxidase Enzyme Activity of Rhizobacteria-introduced Shallots Bulbs to Induce Resistance of Shallot towards Bacterial Leaf Blight (*Xanthomonas Axonopodis* pv *Allii*)
Y. Yanti 501

Partial Oxidation of Methane to Methanol over Heterogeneous Catalyst Co/ZSM-5
Y.K. Krisnandi, B.A.P. Putra, M. Bahtiar, Zahara, I. Abdullah, R.F. Howe 508

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Procedia Chemistry

Procedia Chemistry 14 (2015) 101 – 107

2nd Humboldt Kolleg in conjunction with International Conference on Natural Sciences, HK-ICONS 2014

Preliminary Evaluation of the Pigments Content from *Rhodobacter sphaeroides* at Stages during Photosynthetic Growth

Chan Jia Hui[a], Monika Nur Utami Prihastyanti[b], Tatas Hardo Panintingjati Brotosudarmo[b]*

[a]*Department of Cell and Molecular Biology, Faculty of Biotechnology and Science Biomolecule, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

[b]*Ma Chung Research Center for Photosynthetic Pigments, Villa Puncak Tidar N-01, Malang 65151, Indonesia*

Abstract

Under photosynthetic growth, purple non–sulfur photosynthetic bacterium *Rhodobacter sphaeroides* can use a diverse array of substrates for the source of carbon donor. Substrates such as acetate and succinate are most commonly used to study energetic and metabolic networks, especially in the production and consummation of NADPH during the citric acid cycle (TCA cycle) and ethylmalonyl–CoA pathway, respectively. Although the utilization of both substrate, the bacterium will grow at different growth rate and this also influence the biosynthesis of photosynthetic pigments as important components for overall photosynthesis. For this study, *Rhodobacter sphaeroides* strain 2.4.1, GA and G1C have been grown in acetate and succinate. Here, preliminary results on the evaluation the pigment ratio at different stages of the growth is reported, especially on the growth in succinate substrate.

© 2015 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Peer-review under responsibility of the Scientific Committee of HK-ICONS 2014

Keywords: Bacteriochlorophylls; carotenoids; pigment ratio; *Rhodobacter sphaeroides*.

Nomenclature

LH	light–harvesting complexes	EM–CoA	ethylmalonyl–Coenzyme A
RC–LH	core complexes	Cars	carotenoids
Bchl	bacteriochlorophylls	NADPH	nicotinamide adenine dinucleotide phosphate

* Corresponding author. Tel.: +62 821 4149 0052; fax: +62 341 550 175.
E-mail address: tatas.brotosudarmo@machung.ac.id

1876-6196 © 2015 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Peer-review under responsibility of the Scientific Committee of HK-ICONS 2014
doi:10.1016/j.proche.2015.03.015

1. Introduction

R. sphaeroides is a purple photosynthetic bacterium, which can grow under several condition, such as aerobic and anaerobic respiration, fermentation, and also anoxygenic photosynthesis[1]. Several group of carbon sources have been determined to be the most utilized carbon in the growth of *Rhodobacter sphaeroides*. D–glucose is one of the carbon sources used by *R. sphaeroides* to support the growth due to the ability to produce NADPH through assistance from glucose–6–phosphate dehydrogenase enzyme based on glycolysis pathway. Other example of carbon source is acetate which requires anabolic process to convert acetate into ethylmalonyl–CoA in Em–CoA pathway. This process also involves the consumption of NADPH . Thus, it takes time to increase optical density of the cells. The third group of carbon source is succinate which is able to enter TCA cycle and make the cells have a rapid growth compared to cells grown in acetate[1]. On the other hand, *R. sphaeroides* is able to produce high amount of fatty acid, photopigments and other components of the photosynthetic apparatus[2].

Under anaerobic condition, cytoplasmic membrane of *R. sphaeroides* undergoes changes where invagination process occurred and developing intracytoplasmic membrane system, which is also known as chromatophore. The intracytoplasmic membrane is physically continuous with cytoplasmic membrane but both of them performing the different functions and structures. In these resulting specialized domains, containing the pigments and proteins that are essential for light reaction of photosynthesis. Basically, chromatophore is light–reflecting organelles that can be found in the membrane of photosynthetic microorganisms, which can absorb light energy by using light–absorbing antenna complexes. For some organisms, there are two types of antenna complexes in the chromatophore, which are known as light harvesting complex 1 (LH1) and 2 (LH2)[3]. In the last 25 years, the antenna system from this group of bacteria has been studied extensively and perhaps the best-analyzed antenna complex is LH2.

R. sphaeroides contains two antenna complexes which absorb at different wavelengths. The LH1 complex is core antenna complex, which exists as an integral membrane pigment-protein complex. They form a ring that physically surrounding the reaction center, presenting RC–LH1 complex. From the intact bacterial cell of *R. sphaeroides*, it exhibits an intense band at ~875 nm. A single subunit of LH1 is consisting of a pair of α and β protein subunits. It also contains two bacteriochlorophyll (bchl) molecules which have an absorbance value at around 820 nm. However, there is a shifting of absorbance band to ~875 nm which representing the aggregation of subunits forming the intact LH1, these are known as B875 pigments

LH2 integral membrane antenna complex in purple photosynthetic bacteria is similar but clearly distinct to LH1. Bacteriochlorophyll pigments in LH2 exhibit spectrally different compared to those from LH1. There are two well-separated absorption bands which centered at around 800 nm and 850 nm, referring to B800 and B850 pigments. For B800 pigments, the bacteriochlorophyll exist in monomeric form and forming a ring that parallel to the plane of the membrane that the complex embedded in, while for the B850 pigments, each subunit consists of two bacteriochlorophylls, in the form of arrangement of coupled dimer.

2. Materials and methods

2.1. Isolation of bacterial membrane

Three different strains of *Rhodobacter sphaeroides*, namely *R. sphaeroides* 2.4.1, Ga, and G1C were incubated at room temperature, 2 500 lux (1 lux = 1 lm · m^{-2}) under anaerobic condition in four rectangular bottles respectively which containing succinate medium[4] with sodium succinate ($C_4H_4Na_2O_4$) or sodium acetate (CH_3COONa) as carbon source. The bacterial cultures which have achieved initial log phase and late log phase were harvested respectively by centrifugation at 6 000 rpm (60 rpm = 1 hertz) for 10 min at 4 °C after 3 d to 4 d and 5 d to 7 d after inoculation. The harvested bacterial cells were resuspended well with 20 mM of Tris HCl (pH 8.0) buffer, and disrupted by using a sonicator for 10 min. Then, the bacterial membrane which is in the pellet form of each strain of *R. sphaeroides* was collected by carrying out centrifugation at 11 500 rpm for 15 min at 4 °C.

2.2. Pigment extr

For pigment
experiment. Solv
Three mL of solv
process was carri

2.3. Determinatio

The electronic
Spectrophotomet
diluted with 4.5 n
were subjected to
Pigment ratio
carried out by usi
the stationary pha
solution (0.25 M
60 : 20 v / v / v
pyridine solution
milli–Q (Millipor
until the pH was
of 0.25 M. All p
methanol and a
equipment dwell

3. Results and

3. 1. Growth ana

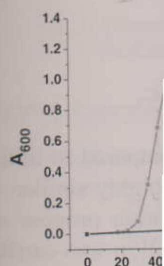


Fig.1.

In order to e
comparable gro
supporting phot
all three strains
compared to th
strain before lo

2.2. Pigment extraction

For pigment extraction, 0.1 g of bacterial membranes was taken. Rapid extraction was carried out in this experiment. Solvent used to extract pigment is acetone-methanol which was prepared in 7 : 3 (v / v) proportions. Three mL of solvent was added to bacterial membrane sample and vortex for 2 min in a conical tube. Extraction process was carried out at room temperature under green light condition.

2.3. Determination of pigment ratio

The electronic absorbance spectra of the pigment component were recorded, at room temperature, by using a Spectrophotometer UV-Vis-NIR (UV1700–Shimadzu). Sample was prepared in 1 : 10 ratio (0.5 ml of sample diluted with 4.5 ml of solvent). The crude pigments extracted from both initial and late log phases of *R. sphaeroides* were subjected to HPLC analysis.

Pigment ratio was determined using HPLC equipped with photodiode array detector. Analytical separations were carried out by using a Waters Symmetry C_8 COLUMN (150 mm × 4.6 mm, 3.5 µm particle size, 100 Å pore size) as the stationary phase. While for the mobile phases, eluent A was a mixture of methanol:acetonitrile: queous pyridine solution (0.25 M pyridine) (50 : 25 : 25 v / v / v) while eluent B was mixture of methanol:acetonitrile:acetone (20 : 60 : 20 v / v / v). Organic solvent that was employed to prepare mobile phases were HPLC–grade. The aqueous pyridine solution (0.25 M) was prepared by 10 mL of acetic acid and 20 mL of pyridine were added to 900 mL of milli–Q (Millipore) water in a 1 L flask and mixed by using a magnetic stirrer. Acetic acid was then added dropwise until the pH was 5.0. The mixture was diluted to 100 mL with distilled water to achieve final pyridine concentration of 0.25 M. All procedures were carried out in a fume hood. The pyridine solution was filtered after mixing with methanol and acetonitrile (eluent A). Different gradient profiles were adjusted for minimizing differences of equipment dwell volume. The flow rate was fixed at 1 mL · min^{-1} [5].

3. Results and discussion

3.1. Growth analysis

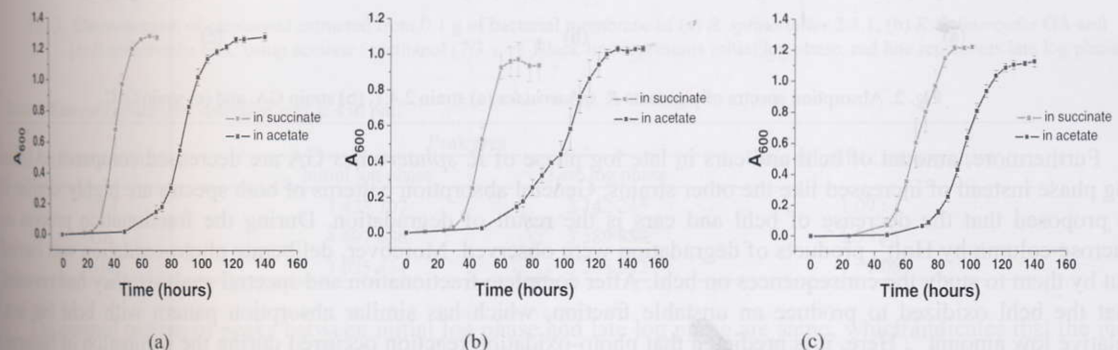


Fig.1. Growth curves of *R. sphaeroides* strain (a) 2.4.1., (b) GA, (c) G1C with succinate and acetate as carbon sources

In order to extract pigment from bacteria of different growth phases, growth analysis was carried out to generate comparable growth curves for both carbon sources. Acetate and succinate are carbon sources that capable of supporting photosynthetic growth of *R. sphaeroides* along with some differences on metabolic activity. Growth of all three strains of *R. sphaeroides* when using acetate as carbon source, were significantly impaired (Figure 1) compared to those which were cultured with succinate as carbon source. A long lag phase was exhibited by every strain before log phase started. It is because growth of *R. sphaeroides* on acetate occurred via the ethylmalonyl-CoA

(EM-CoA) pathway. In this metabolic pathway, longer time is needed for acetate to be converted into succinyl-Co and condensed with glyoxylate, which is the EM-CoA pathway product[6,7], resulting in formation of malate befo entering tricarboxylic cycle (TCA cycle). It should be noted, however, there is shorter lag phase before grow commenced. Succinate is able to directly enter the TCA cycle without passing through EM-CoA cycle.

3.2. Absorption spectra

By referring to growth curves from Figure 1 as comparable data, crude pigment was extracted from bacteri culture with succinate as carbon source at both initial log and late log phases by using solvent acetone:methan (7 : 3 v / v). Figure 2 shows the electronic absorption spectra of extracted crude pigments from three strains o *R. sphaeroides* respectively. Their Q_y absorptions appear at same wavelength 770 nm, which represents the bc while their carotenoids (cars) absorb at different wavelength ranging from 400 nm to 500 nm.

Amount of bchl and cars are found to be higher in late log phase. Figure 2 shows an evident that the quantity o bchl and cars produced increases as cell density is increased from initial log phase to late log phase[8]. However, should be noted that the increasing of bchl is not significant because of shading effect occurred during the bacteri growth. The shading effect is the result due to the increasing cell densities, thus reduce the effectiveness of the lig intensity. It is predicted that the ability of bacteria to develop chromatophore is reduced when the effectiveness o light intensity is also reduced and repressed both of the amount of intracytoplasmic membrane synthesized an pigment produced[1].

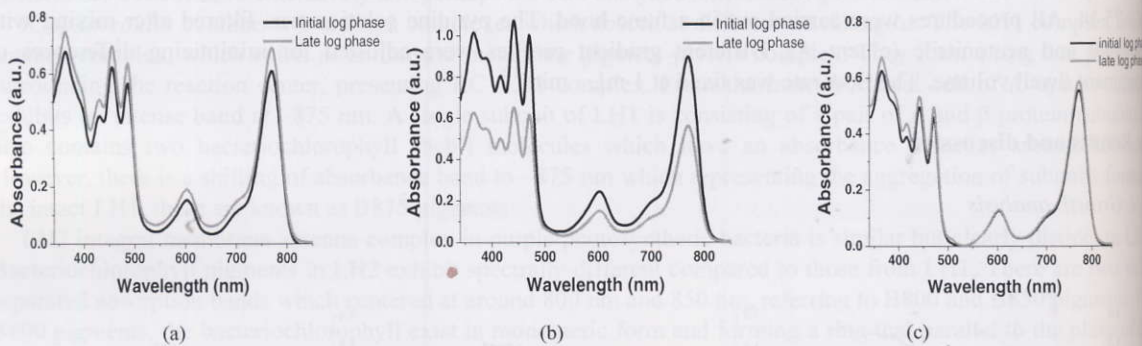


Fig. 2. Absorption spectra of pigments *R. sphaeroides* (a) strain 2.4.1, (b) strain GA, and (c) strain G1C

Furthermore, amount of bchl and cars in late log phase of *R. sphaeroides* GA are decreased compared to initi log phase instead of increased like the other strains. General absorption patterns of both spectra are highly similar. I is proposed that the decrease of bchl and cars is the result of degradation. During the fractionation process o sucrose column by Holt[9], products of degradation were observed. Moreover, deliberate photo-oxidation was carrie out by them to study the consequences on bchl. After complete fractionation and spectral analysis, they had reveale that the bchl oxidized to produce an unstable fraction, which has similar absorption pattern with bchl but in a relative low amount[10]. Here, it is predicted that photo-oxidation reaction occurred during the incubation of bacteri cultures and resulting the decrease in amount of bchl. Over exposure to light during incubation of bacterial cultur will speed up the degradation process of pigments. It is predicted that the pigment from *R. sphaeroides* GA is les stable compared to the other two strains.

3.3. Determination of pigment ratio

To determine pigment ratio present in initial log and late log phase, crude pigments were extracted fro individual cells harvested at different growth phases as described. Pigments isolated from the individual strains we analyzed by HPLC (Figure 3).

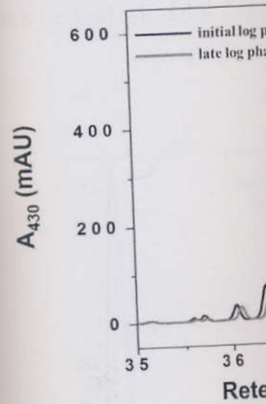


Fig. 3. Chromatogram of ca (c) *R.sphaeroides* G

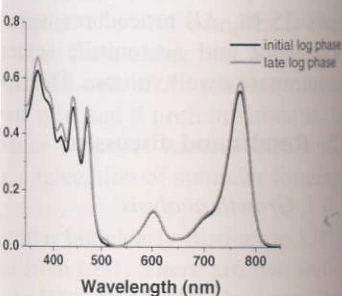
Table 1. Ratio of carotenoids, de

R. sphaeroides strains
2.4.1
GA
G1C

The general pattern o composition of caroteno min, 38.62 min). Peak *R. sphaeroides* 2.4.1 an phase, although slight s pigment peaks. Small According to the HPLC log phase, except in *R*

o be converted into succinyl-CoA
ng in formation of malate before
shorter lag phase before growth
ugh EM-CoA cycle.

nent was extracted from bacterial
y using solvent acetone:methanol
le pigments from three strains of
70 nm, which represents the bchl
to 500 nm.
ws an evident that the quantity of
se to late log phase[8]. However, it
ffect occurred during the bacterial
duce the effectiveness of the light
educed when the effectiveness of
smic membrane synthesized and



GA, and (c) strain G1C

are decreased compared to initial
both spectra are highly similar. It
ring the fractionation process on
erate photo-oxidation was carried
pectral analysis, they had revealed
rption pattern with bchl but in a
during the incubation of bacterial
ng incubation of bacterial culture
t from *R. sphaeroides* GA is less

e pigments were extracted from
d from the individual strains were

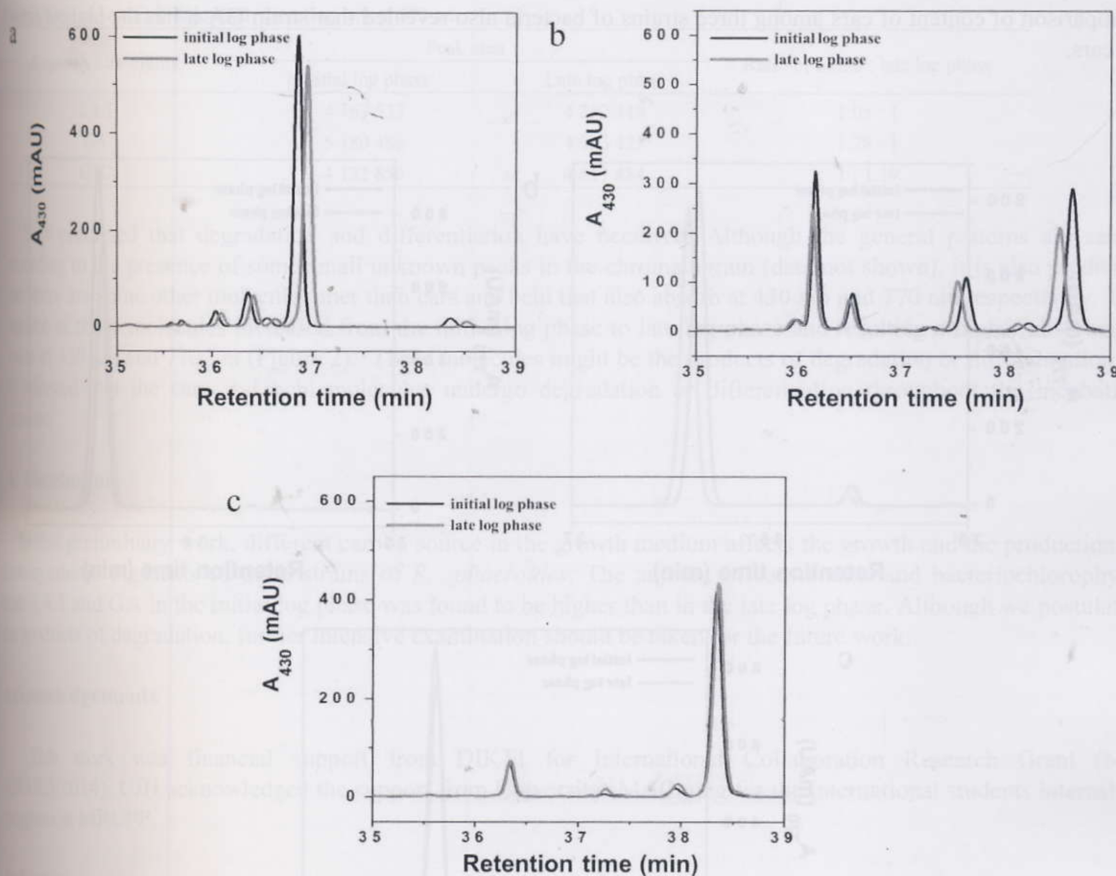


Fig. 3. Chromatogram of carotenoid extracted from 0.1 g of bacterial membrane of (a) *R. sphaeroides* 2.4.1, (b) *R. sphaeroides* GA and (c) *R.sphaeroides* G1C using acetone : methanol (7:3 v/v). Black line represents initial log phase, red line represents late log phase

Table 1. Ratio of carotenoids, detection was at 430 nm.

R. sphaeroides strains	Peak area		Ratio of initial : late log phase
	Initial log phase	Late log phase	
2.4.1	3 423 707	3 142 245	1.09 : 1
GA	4 697 636	3 589 859	1.39 : 1
G1C	3 025 434	3 265 852	1 : 1.08

The general pattern of peaks between initial log phase and late log phase are same, which indicates that the main composition of carotenoid has no changes. *R. sphaeroides* GA has three dominant peaks of cars (36.15 min, 37.6 min, 38.62 min). Peak appeared at 36.36 min, 36.51 min and 36.33 min represent bchl. In the other hand, *R. sphaeroides* 2.4.1 and G1C have one dominant peaks which appeared at 36.58 min and 38.34 min in initial log phase, although slight shift was obtained. However, absorption spectra of each peaks were used for identification of pigment peaks. Small peaks were not included due to unclear absorption spectra and extremely low intensity. According to the HPLC analysis data, amount of cars slightly decreased in all strains of *R. sphaeroides* during late log phase, except in *R. sphaeroides* G1C (Table 1). In addition, among all three strains of *R. sphaeroides*, the

comparison of content of cars among three strains of bacteria also revealed that strain GA it has the highest amou of cars.

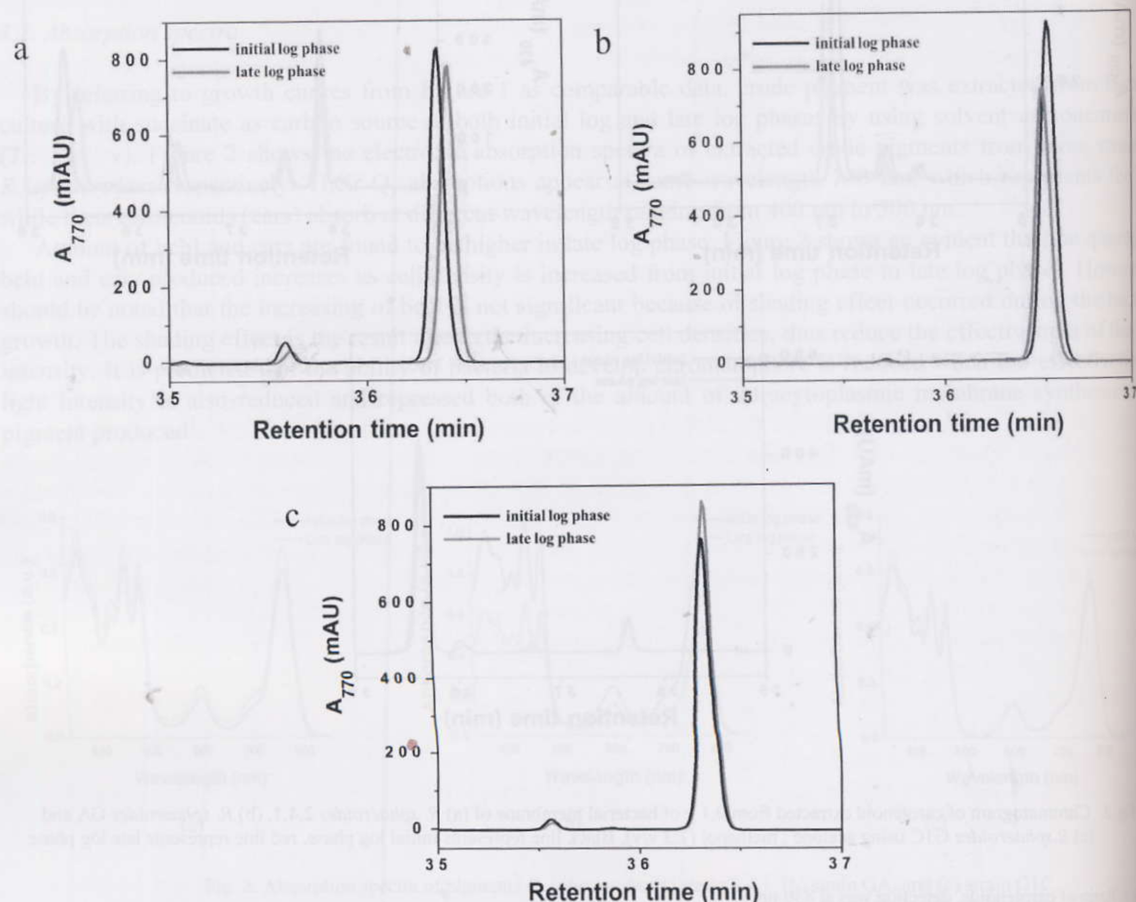


Fig. 4. Chromatogram of bacteriochlorohylls extracted from 0.1 g of bacterial membrane of (a) *R. sphaeroides* 2.4.1, (b) *R. sphaeroides* GA and (c) *R.sphaeroides* G1C using acetone : methanol (7:3 v/v). Black line represents initial log phase, red line represents late log phase

Bchl peaks of R. sphaeroides 2.4.1, R. sphaeroides GA and R. sphaeroides G1C appeard at 36.36 min, 36.51 m and 36.33 min, respectively. The ratio of bchl of all strains also have the same general patterns as of carotenoids both growth phases. There is slight increase of bchl in *R sphaeroides* G1C along with an increase in cell density late log phase. *R. sphaeroides* G1C has the highest amount of bchl at late log phase compared to other strains, wh at initial log phase *R. sphaeroides* GA has the highest amount. However, there is a decrease of bchl content *R. sphaeroides* GA and 2.4.1 and which is most likely due to photo-oxidation[8], however, further study is required determine the exact degradation ratio of pigment.

Table 2. Ratio of bacter

R. sphaeroides st
2.4.1
GA
G1C

It is predicted according to the pr that there are some amount of those m value at 430 nm an is believed that th process.

4. Conclusion

In this prelimina photosynthetic pig from 2.4.1 and GA the products of deg

Acknowledgement

This work w 0263/E5/2014). CJ program at MRCPF

References

1. Patricia JK, Samu 1988;52(1):50–69 .
2. Richard JC, Andrew in vivo membranes.
3. Paul AL, Pamela S structural subunit *anoxygenicphotosyn*
4. Bose SK. *Bacterial*
5. Manuel Z, Fransisc reversed phase C_8 c
6. Kathrin S, Rèmi P, AM1 during growth
7. Saheed I, Daniel R 2013;7:89–104.
8. Alexei AY, Jesus *Rhodobacter sphae* availability. *J Bacte*
9. Holt AS, Jacobs EE 722.
10. WS Kim. Complete

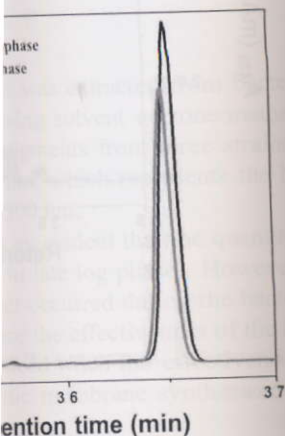


Table 2. Ratio of bacteriochlorophyll, detection was at 770 nm.

R. sphaeroides strains	Peak area		Ratio of initial : late log phase
	Initial log phase	Late log phase	
2.4.1	4 467 533	4 252 118	1.05 : 1
GA	5 180 486	4 053 425	1.28 : 1
GIC	4 122 859	4 611 884	1 : 1.19

It is predicted that degradation and differentiation have occurred. Although the general patterns are same, according to the presence of some small unknown peaks in the chromatogram (data not shown), it is also predicted that there are some other molecule other than cars and bchl that also absorb at 430 nm and 770 nm respectively. The amount of those molecules increased from the initial log phase to late log phase and resulting a higher absorbance value at 430 nm and 770 nm (Figure 2). These molecules might be the products of degradation or differentiation. It is believed that the cars and bchl molecules undergo degradation or differentiation throughout the incubation process.

4. Conclusion

In this preliminary work, different carbon source in the growth medium affects the growth and the production of photosynthetic pigments of three strains of *R. sphaeroides*. The amount of carotenoids and bacteriochlorophylls from 2.4.1 and GA in the initial log phase was found to be higher than in the late log phase. Although we postulated the products of degradation, further intensive examination should be taken for the future work.

Acknowledgements

This work was financial support from DIKTI for International Collaboration Research Grant (No. 0263/E5/2014). CJH acknowledged the support from Universitas Ma Chung for the international students internship program at MRCPP.

References

1. Patricia JK, Samuel K. Molecular genetics of photosynthetic membrane biosynthesis in *Rhodobacter sphaeroides*. *Microbial Rev* 1988;52(1):50–69 .
2. Richard JC, Andrew G, Jürgen K. The architecture and function of the light–harvesting apparatus of purple bacteria :from single molecules to in vivo membranes. *Quarterly Reviews of Biophysics* 2006;39:227–324.
3. Paul AL, Pamela SP. Structure–function relationships in core light–harvesting complexes (LH1) as determined by characterization of the structural subunit and by reconstitution experiments. In: Robert EB, Michael TM, Carl EB, editors. *Advances in photosynthesis, anoxygenicphotosynthetic bacteria*. Dordrecth: Kluwer Academic Publishers; 1995. p. 437–471.
4. Bose SK. *Bacterial photosynthesis*. Ohio: Antioch Press; 1963.
5. Manuel Z, Fransisco R, José LG. Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed phase C_8 column and pyridinecontaining mobile phases. *Mar Ecol Prog Ser* 2000;195:29–45.
6. Katrhin S, Rèmi P, Patrick K, et al. The ethylmalonyl–CoA pathway is used in place of the glyoxylate cycle by *Methylobacterium extorquens* AM1 during growth on acetate. *J Biol Chem* 2012;287(1):757–766.
7. Saheed I, Daniel RN, Timothy J. Global insights into energetic and metabolic networks in *Rhodobacter sphaeroides*. *BMC Systems Biology* 2013;7:89–104.
8. Alexei AY, Jesus ME, Samuel K. Differential carotenoid composition of the B875 and B800–850 photosynthetic antenna complexes in *Rhodobacter sphaeroides* 2.4.1: involvement of spheroidene and spheroidenone in adaptation to changes in light intensity and oxygen availability. *J Bacterio* 1996;178(20):5877–5883.
9. Holt AS, Jacobs EE. Spectroscopy of plant pigments. II. methyl bacteriochlorophyllsides and bacteriochlorophyll. *Am J Botany* 1954;41:718–722.
10. WS Kim. Complete fractionation of bacteriochlorophyll and its degradation products. *Biochimica Biophys Acta* 1966;112:392–402.

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Procedia Chemistry 14 (2015) 101 – 107

Procedia Chemistry

2nd Humboldt Kolleg in conjunction with International Conference on Natural Sciences, HK-ICONS 2014

Preliminary Evaluation of the Pigments Content from *Rhodobacter sphaeroides* at Stages during Photosynthetic Growth

Chan Jia Hui[a], Monika Nur Utami Prihastyanti[b], Tatas Hardo Panintingjati Brotosudarmo[b]*

[a]*Department of Cell and Molecular Biology, Faculty of Biotechnology and Science Biomolecule, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

[b]*Ma Chung Research Center for Photosynthetic Pigments, Villa Puncak Tidar N-01, Malang 65151, Indonesia*

Abstract

Under photosynthetic growth, purple non–sulfur photosynthetic bacterium *Rhodobacter sphaeroides* can use a diverse array of substrates for the source of carbon donor. Substrates such as acetate and succinate are most commonly used to study energetic and metabolic networks, especially in the production and consummation of NADPH during the citric acid cycle (TCA cycle) and ethylmalonyl–CoA pathway, respectively. Although the utilization of both substrate, the bacterium will grow at different growth rate and this also influence the biosynthesis of photosynthetic pigments as important components for overall photosynthesis. For this study, *Rhodobacter sphaeroides* strain 2.4.1, GA and G1C have been grown in acetate and succinate. Here, preliminary results on the evaluation the pigment ratio at different stages of the growth is reported, especially on the growth in succinate substrate.

© 2015 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Peer-review under responsibility of the Scientific Committee of HK-ICONS 2014

Keywords: Bacteriochlorophylls; carotenoids; pigment ratio; *Rhodobacter sphaeroides*.

Nomenclature

LH	light–harvesting complexes	**EM–CoA**	ethylmalonyl–Coenzyme A
RC–LH	core complexes	**Cars**	carotenoids
Bchl	bacteriochlorophylls	**NADPH**	nicotinamide adenine dinucleotide phosphate

* Corresponding author. Tel.: +62 821 4149 0052; fax: +62 341 550 175.
E-mail address: tatas.brotosudarmo@machung.ac.id

1876-6196 © 2015 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Peer-review under responsibility of the Scientific Committee of HK-ICONS 2014
doi:10.1016/j.proche.2015.03.015

1. Introduction

R. sphaeroides is a purple photosynthetic bacterium, which can grow under several condition, such as aerobic and anaerobic respiration, fermentation, and also anoxygenic photosynthesis[1]. Several group of carbon sources have been determined to be the most utilized carbon in the growth of *Rhodobacter sphaeroides*. D–glucose is one of the carbon sources used by *R. sphaeroides* to support the growth due to the ability to produce NADPH through assistance from glucose–6–phosphate dehydrogenase enzyme based on glycolysis pathway. Other example of carbon source is acetate which requires anabolic process to convert acetate into ethylmalonyl–CoA in Em–CoA pathway. This process also involves the consumption of NADPH . Thus, it takes time to increase optical density of the cells. The third group of carbon source is succinate which is able to enter TCA cycle and make the cells have a rapid growth as compared to cells grown in acetate[1]. On the other hand, *R. sphaeroides* is able to produce high amount of fatty acids, photopigments and other components of the photosynthetic apparatus[2].

Under anaerobic condition, cytoplasmic membrane of *R. sphaeroides* undergoes changes where invagination process occurred and developing intracytoplasmic membrane system, which is also known as chromatophore. The intracytoplasmic membrane is physically continuous with cytoplasmic membrane but both of them performing the different functions and structures. In these resulting specialized domains, containing the pigments and proteins that are essential for light reaction of photosynthesis. Basically, chromatophore is light–reflecting organelles that can be found in the membrane of photosynthetic microorganisms, which can absorb light energy by using light–absorbing antenna complexes. For some organisms, there are two types of antenna complexes in the chromatophore, which are known as light harvesting complex 1 (LH1) and 2 (LH2)[3]. In the last 25 years, the antenna system from this group of bacteria has been studied extensively and perhaps the best-analyzed antenna complex is LH2.

R. sphaeroides contains two antenna complexes which absorb at different wavelengths. The LH1 complex is core antenna complex, which exists as an integral membrane pigment-protein complex. They form a ring that physically surrounding the reaction center, presenting RC–LH1 complex. From the intact bacterial cell of *R. sphaeroides*, it exhibits an intense band at ~875 nm. A single subunit of LH1 is consisting of a pair of α and β protein subunits. It also contains two bacteriochlorophyll (bchl) molecules which have an absorbance value at around 820 nm[4]. However, there is a shifting of absorbance band to ~875 nm which representing the aggregation of subunits forming the intact LH1, these are known as B875 pigments

LH2 integral membrane antenna complex in purple photosynthetic bacteria is similar but clearly distinct to LH1. Bacteriochlorophyll pigments in LH2 exhibit spectrally different compared to those from LH1. There are two well–separated absorption bands which centered at around 800 nm and 850 nm, referring to B800 and B850 pigments. For B800 pigments, the bacteriochlorophyll exist in monomeric form and forming a ring that parallel to the plane of the membrane that the complex embedded in, while for the B850 pigments, each subunit consists of two bacteriochlorophylls, in the form of arrangement of coupled dimer.

2. Materials and methods

2.1. Isolation of bacterial membrane

Three different strains of *Rhodobacter sphaeroides*, namely *R. sphaeroides* 2.4.1, Ga, and G1C were incubated at room temperature, 2 500 lux (1 lux = 1 lm · m^{-2}) under anaerobic condition in four rectangular bottles respectively which containing succinate medium[4] with sodium succinate ($C_4H_4Na_2O_4$) or sodium acetate (CH_3COONa) as carbon source. The bacterial cultures which have achieved initial log phase and late log phase were harvested respectively by centrifugation at 6 000 rpm (60 rpm = 1 hertz) for 10 min at 4 °C after 3 d to 4 d and 5 d to 7 d after inoculation. The harvested bacterial cells were resuspended well with 20 mM of Tris HCl (pH 8.0) buffer, and disrupted by using a sonicator for 10 min. Then, the bacterial membrane which is in the pellet form of each strain of *R. sphaeroides* was collected by carrying out centrifugation at 11 500 rpm for 15 min at 4 °C.

2.2. Pigment extraction

For pigment extraction, 0.1 g of bacterial membranes was taken. Rapid extraction was carried out in this experiment. Solvent used to extract pigment is acetone-methanol which was prepared in 7 : 3 (v / v) proportions. Three mL of solvent was added to bacterial membrane sample and vortex for 2 min in a conical tube. Extraction process was carried out at room temperature under green light condition.

2.3. Determination of pigment ratio

The electronic absorbance spectra of the pigment component were recorded, at room temperature, by using a Spectrophotometer UV-Vis-NIR (UV1700–Shimadzu). Sample was prepared in 1 : 10 ratio (0.5 ml of sample diluted with 4.5 ml of solvent). The crude pigments extracted from both initial and late log phases of *R. sphaeroides* were subjected to HPLC analysis.

Pigment ratio was determined using HPLC equipped with photodiode array detector. Analytical separations were carried out by using a Waters Symmetry C_8 COLUMN (150 mm × 4.6 mm, 3.5 µm particle size, 100 Å pore size) as the stationary phase. While for the mobile phases, eluent A was a mixture of methanol:acetonitrile: queous pyridine solution (0.25 M pyridine) (50 : 25 : 25 v / v / v) while eluent B was mixture of methanol:acetonitrile:acetone (20 : 60 : 20 v / v / v). Organic solvent that was employed to prepare mobile phases were HPLC–grade. The aqueous pyridine solution (0.25 M) was prepared by 10 mL of acetic acid and 20 mL of pyridine were added to 900 mL of milli–Q (Millipore) water in a 1 L flask and mixed by using a magnetic stirrer. Acetic acid was then added dropwise until the pH was 5.0. The mixture was diluted to 100 mL with distilled water to achieve final pyridine concentration of 0.25 M. All procedures were carried out in a fume hood. The pyridine solution was filtered after mixing with methanol and acetonitrile (eluent A). Different gradient profiles were adjusted for minimizing differences of equipment dwell volume. The flow rate was fixed at 1 mL · min^{-1} [5].

3. Results and discussion

3. 1. Growth analysis

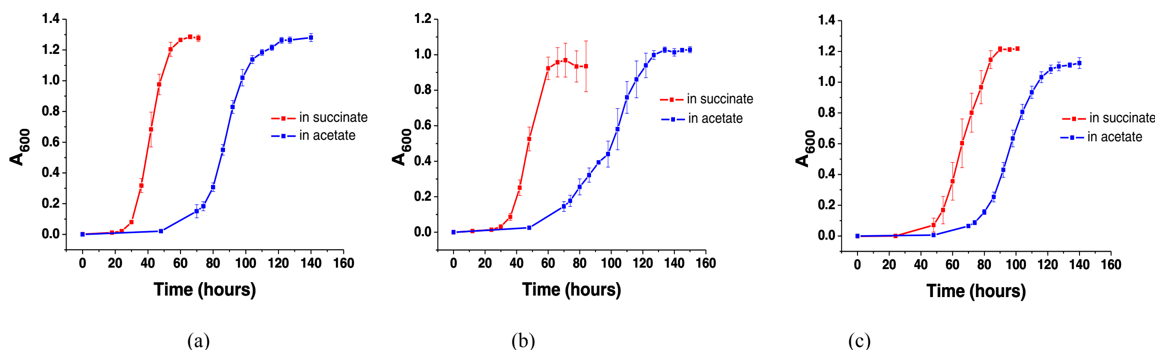


Fig.1. Growth curves of *R. sphaeroides* strain (a) 2.4.1., (b) GA, (c) G1C with succinate and acetate as carbon sources

In order to extract pigment from bacteria of different growth phases, growth analysis was carried out to generate comparable growth curves for both carbon sources. Acetate and succinate are carbon sources that capable of supporting photosynthetic growth of *R. sphaeroides* along with some differences on metabolic activity. Growth of all three strains of *R. sphaeroides* when using acetate as carbon source, were significantly impaired (Figure 1) compared to those which were cultured with succinate as carbon source. A long lag phase was exhibited by every strain before log phase started. It is because growth of *R. sphaeroides* on acetate occurred via the ethylmalonyl-CoA

(EM-CoA) pathway. In this metabolic pathway, longer time is needed for acetate to be converted into succinyl-CoA and condensed with glyoxylate, which is the EM-CoA pathway product[6,7], resulting in formation of malate before entering tricarboxylic cycle (TCA cycle). It should be noted, however, there is shorter lag phase before growth commenced. Succinate is able to directly enter the TCA cycle without passing through EM-CoA cycle.

3.2. Absorption spectra

By referring to growth curves from Figure 1 as comparable data, crude pigment was extracted from bacterial culture with succinate as carbon source at both initial log and late log phases by using solvent acetone:methanol (7 : 3 v / v). Figure 2 shows the electronic absorption spectra of extracted crude pigments from three strains of *R. sphaeroides* respectively. Their Q_y absorptions appear at same wavelength 770 nm, which represents the bchl while their carotenoids (cars) absorb at different wavelength ranging from 400 nm to 500 nm.

Amount of bchl and cars are found to be higher in late log phase. Figure 2 shows an evident that the quantity of bchl and cars produced increases as cell density is increased from initial log phase to late log phase[8]. However, it should be noted that the increasing of bchl is not significant because of shading effect occurred during the bacterial growth. The shading effect is the result due to the increasing cell densities, thus reduce the effectiveness of the light intensity. It is predicted that the ability of bacteria to develop chromatophore is reduced when the effectiveness of light intensity is also reduced and repressed both of the amount of intracytoplasmic membrane synthesized and pigment produced[1].

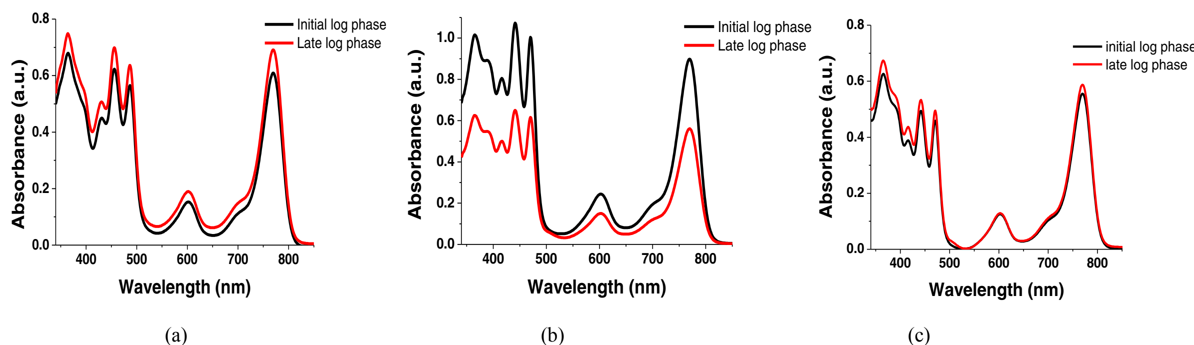


Fig. 2. Absorption spectra of pigments *R. sphaeroides* (a) strain 2.4.1, (b) strain GA, and (c) strain G1C

Furthermore, amount of bchl and cars in late log phase of *R. sphaeroides* GA are decreased compared to initial log phase instead of increased like the other strains. General absorption patterns of both spectra are highly similar. It is proposed that the decrease of bchl and cars is the result of degradation. During the fractionation process on sucrose column by Holt[9], products of degradation were observed. Moreover, deliberate photo-oxidation was carried out by them to study the consequences on bchl. After complete fractionation and spectral analysis, they had revealed that the bchl oxidized to produce an unstable fraction, which has similar absorption pattern with bchl but in a relative low amount[10]. Here, it is predicted that photo-oxidation reaction occurred during the incubation of bacterial cultures and resulting the decrease in amount of bchl. Over exposure to light during incubation of bacterial culture will speed up the degradation process of pigments. It is predicted that the pigment from *R. sphaeroides* GA is less stable compared to the other two strains.

3.3. Determination of pigment ratio

To determine pigment ratio present in initial log and late log phase, crude pigments were extracted from individual cells harvested at different growth phases as described. Pigments isolated from the individual strains were analyzed by HPLC (Figure 3).

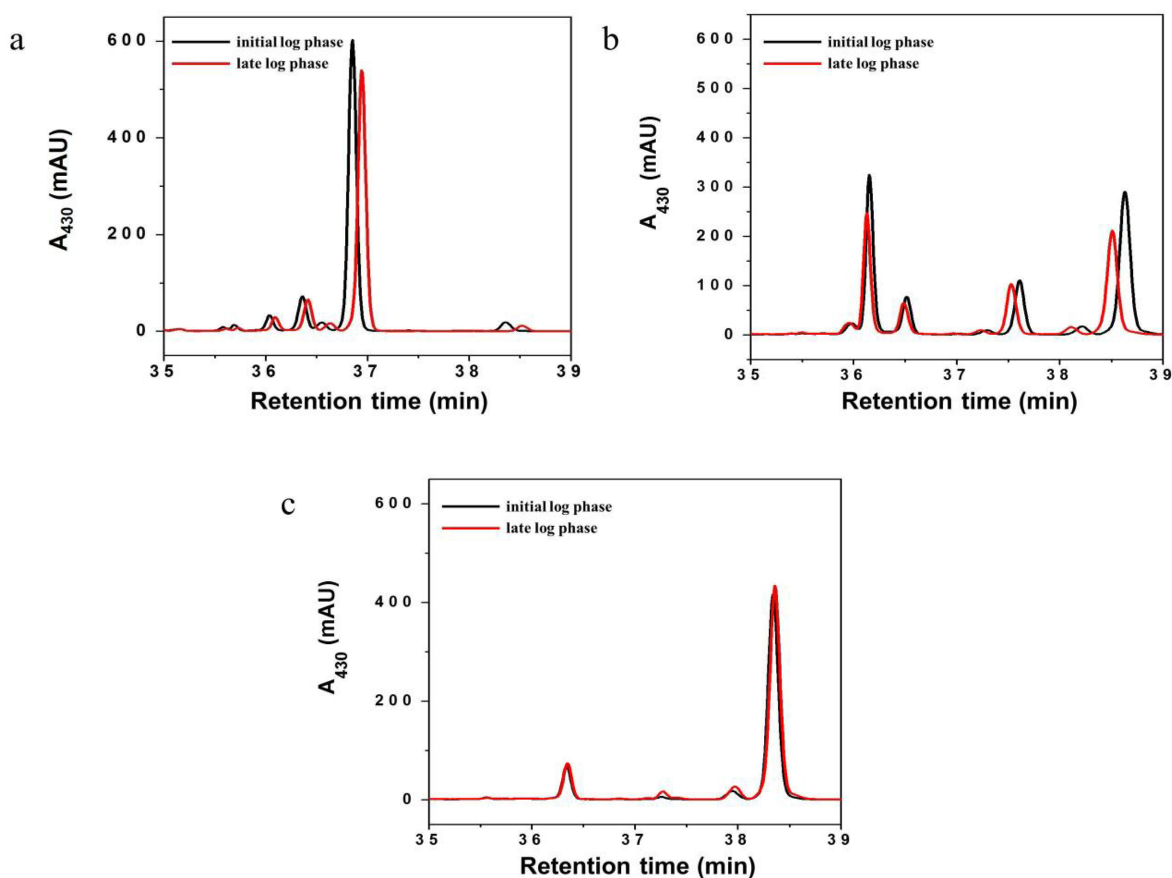


Fig. 3. Chromatogram of carotenoid extracted from 0.1 g of bacterial membrane of (a) *R. sphaeroides* 2.4.1, (b) *R. sphaeroides* GA and (c) *R.sphaeroides* G1C using acetone : methanol (7:3 v/v). Black line represents initial log phase, red line represents late log phase

Table 1. Ratio of carotenoids, detection was at 430 nm.

R. sphaeroides strains	Peak area		Ratio of initial : late log phase
	Initial log phase	Late log phase	
2.4.1	3 423 707	3 142 245	1.09 : 1
GA	4 697 636	3 589 859	1.39 : 1
G1C	3 025 434	3 265 852	1 : 1.08

The general pattern of peaks between initial log phase and late log phase are same, which indicates that the main composition of carotenoid has no changes. *R. sphaeroides* GA has three dominant peaks of cars (36.15 min, 37.6 min, 38.62 min). Peak appeared at 36.36 min, 36.51 min and 36.33 min represent bchl. In the other hand, *R. sphaeroides* 2.4.1 and G1C have one dominant peaks which appeared at 36.58 min and 38.34 min in initial log phase, although slight shift was obtained. However, absorption spectra of each peaks were used for identification of pigment peaks. Small peaks were not included due to unclear absorption spectra and extremely low intensity. According to the HPLC analysis data, amount of cars slightly decreased in all strains of *R. sphaeroides* during late log phase, except in *R. sphaeroides* G1C (Table 1). In addition, among all three strains of *R. sphaeroides*, the

comparison of content of cars among three strains of bacteria also revealed that strain GA it has the highest amount of cars.

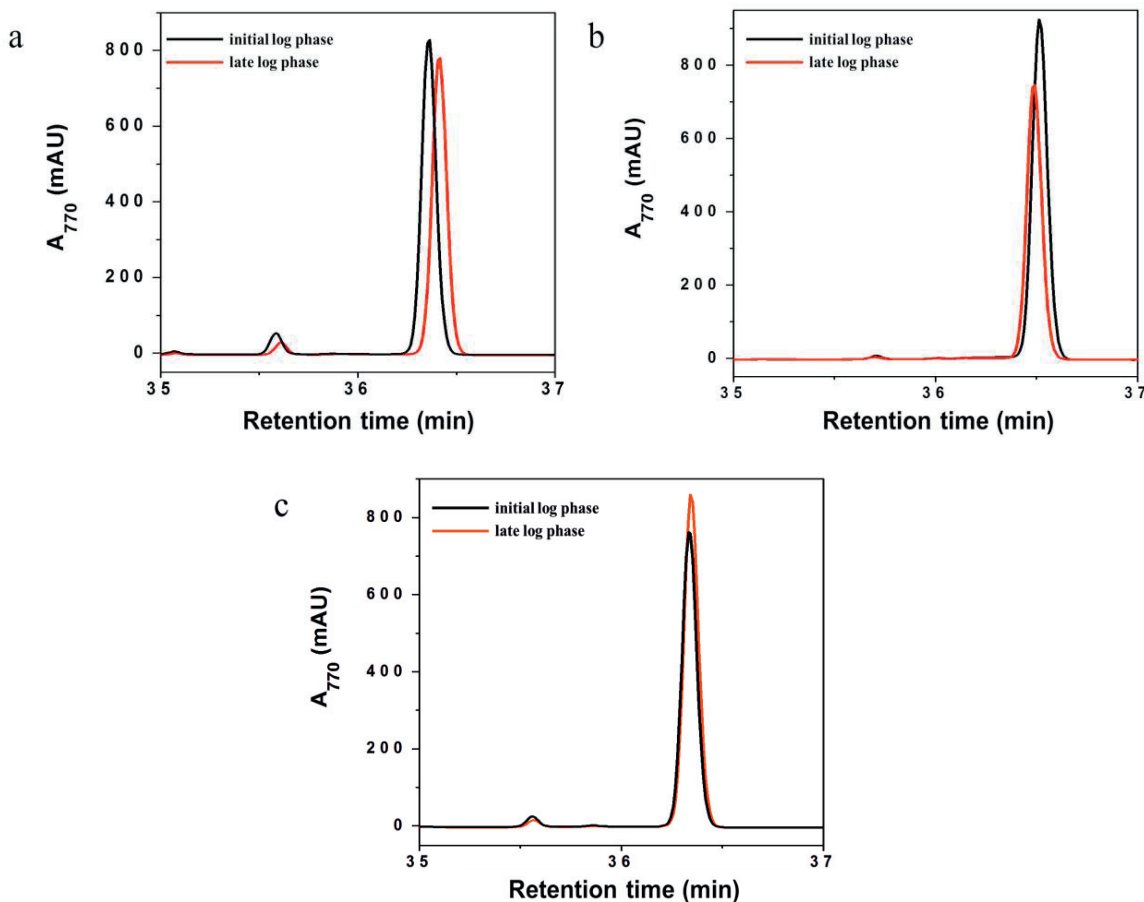


Fig. 4. Chromatogram of bacteriochlorohylls extracted from 0.1 g of bacterial membrane of (a) *R. sphaeroides* 2.4.1, (b) *R. sphaeroides* GA and (c) *R.sphaeroides* G1C using acetone : methanol (7:3 v/v). Black line represents initial log phase, red line represents late log phase

Bchl peaks of R. sphaeroides 2.4.1, R. sphaeroides GA and R. sphaeroides G1C appeard at 36.36 min, 36.51 min and 36.33 min, respectively. The ratio of bchl of all strains also have the same general patterns as of carotenoids for both growth phases. There is slight increase of bchl in *R sphaeroides* G1C along with an increase in cell density in late log phase. *R. sphaeroides* G1C has the highest amount of bchl at late log phase compared to other strains, while at initial log phase *R. sphaeroides* GA has the highest amount. However, there is a decrease of bchl content in *R. sphaeroides* GA and 2.4.1 and which is most likely due to photo-oxidation[8], however, further study is required to determine the exact degradation ratio of pigment.

Table 2. Ratio of bacteriochlorophyll, detection was at 770 nm.

R. sphaeroides strains	Peak area		Ratio of initial : late log phase
	Initial log phase	Late log phase	
2.4.1	4 467 533	4 252 118	1.05 : 1
GA	5 180 486	4 053 425	1.28 : 1
G1C	4 122 859	4 611 884	1 : 1.19

It is predicted that degradation and differentiation have occurred. Although the general patterns are same, according to the presence of some small unknown peaks in the chromatogram (data not shown), it is also predicted that there are some other molecule other than cars and bchl that also absorb at 430 nm and 770 nm respectively. The amount of those molecules increased from the initial log phase to late log phase and resulting a higher absorbance value at 430 nm and 770 nm (Figure 2). These molecules might be the products of degradation or differentiation. It is believed that the cars and bchl molecules undergo degradation or differentiation throughout the incubation process.

4. Conclusion

In this preliminary work, different carbon source in the growth medium affects the growth and the production of photosynthetic pigments of three strains of *R. sphaeroides*. The amount of carotenoids and bacteriochlorophylls from 2.4.1 and GA in the initial log phase was found to be higher than in the late log phase. Although we postulated the products of degradation, further intensive examination should be taken for the future work.

Acknowledgements

This work was financial support from DIKTI for International Collaboration Research Grant (No. 0263/E5/2014). CJH acknowledged the support from Universitas Ma Chung for the international students internship program at MRCPP.

References

1. Patricia JK, Samuel K. Molecular genetics of photosynthetic membrane biosynthesis in *Rhodobacter sphaeroides*. *Microbial Rev* 1988;52(1):50–69 .
2. Richard JC, Andrew G, Jürgen K. The architecture and function of the light–harvesting apparatus of purple bacteria :from single molecules to in vivo membranes. *Quarterly Reviews of Biophysics* 2006;39:227–324.
3. Paul AL, Pamela SP. Structure–function relationships in core light–harvesting complexes (LH1) as determined by characterization of the structural subunit and by reconstitution experiments. In: Robert EB, Michael TM, Carl EB, editors. *Advances in photosynthesis, anoxygenicphotosynthetic bacteria*. Dordrecth: Kluwer Academic Publishers; 1995. p. 437–471.
4. Bose SK. *Bacterial photosynthesis*. Ohio: Antioch Press; 1963.
5. Manuel Z, Fransisco R, José LG. Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed phase C_8 column and pyridinecontaining mobile phases. *Mar Ecol Prog Ser* 2000;195:29–45.
6. Katrhin S, Rèmi P, Patrick K, et al. The ethylmalonyl–CoA pathway is used in place of the glyoxylate cycle by *Methylobacterium extorquens* AM1 during growth on acetate. *J Biol Chem* 2012;287(1):757–766.
7. Saheed I, Daniel RN, Timothy J. Global insights into energetic and metabolic networks in *Rhodobacter sphaeroides*. *BMC Systems Biology* 2013;**7**:89–104.
8. Alexei AY, Jesus ME, Samuel K. Differential carotenoid composition of the B875 and B800–850 photosynthetic antenna complexes in *Rhodobacter sphaeroides* 2.4.1: involvement of spheroidene and spheroidenone in adaptation to changes in light intensity and oxygen availability. *J Bacterio* 1996;178(20):5877–5883.
9. Holt AS, Jacobs EE. Spectroscopy of plant pigments. II. methyl bacteriochlorophyllsides and bacteriochlorophyll. *Am J Botany* 1954;41:718–722.
10. WS Kim. Complete fractionation of bacteriochlorophyll and its degradation products. *Biochimica Biophys Acta* 1966;112:392–402.