3rd NPSEA

# Conference Program & Extended Abstract

## The Natural Pigments Conference for South-East Asia
## In Conjunction with: LCMS WORKSHOP from SHIMADZU

# RESEARCH AND DEVELOPMENT OF PIGMENT-BASED INNOVATION AND TECHNOLOGY


**22-23 AUGUST 2016**

**The Core R&D Center, Universitas Ma Chung**

**Malang, East Java, Indonesia**

**Organized by:**

Universitas Ma Chung

SHIMADZU Excellence in Science

**Partners:**

HP2I Himpunan Peneliti Pigmen Indonesia

Ikatan Apoteker Indonesia IAI

HKI Himpunan Kimia Indonesia

IGN

**Supported by:**

RISTEKDIKTI

DAAD Deutscher Akademischer Austausch Dienst

PT DITEK JAYA Analytical & Measuring Instruments

its

**np-sea.machung.ac.id**

# PREFACE



The 3rd Natural Pigment Conference for South-East Asia (NP-SEA) Secretariat Office
Ma Chung Research Center for Photosynthetic Pigments (MRCPP)
Universitas Ma Chung, Villa Puncak Tidar N01, Malang 65151 East Java, INDONESIA
Tel./Fax +62 341 550 171/175, E-mail: mrcpp@machung.ac.id

# Opening Remark from The Chairman of The 3th-NPSEA

Dear researchers and friends,

On behalf of the organizing committee, I would like to give you a warm welcome in the 3rd Natural Pigments Conference for South-East Asia (NP-SEA) 2016.

Natural pigments are the most obvious and eye-catching substances that can be found in flowers, leaves, bird feather, algae, photosynthetic bacteria, and many more. These pigments have been used as bountiful colorants for food, cosmetics, and textiles and very close connected to the culture of South-East Asia. If we look closely, pigments such as chlorophylls and carotenoids play importance role as key pigments, which capture radiant of energy from the Sun in the process called photosynthesis – a process that convert solar energy into fuels. In agriculture, the natural pigments are important photosensors and indicators for health status. There are many applications that have been revealed through the study of structure and function of natural pigments.

We are looking forward of your active participation during the Natural Pigment Conference for South-East Asia to present your works, to raise questions, and triggers discussion on the recent research and development of pigment-based innovation and technology. We are inviting high profile scientists and practitioner in the industry as the keynote and plenary speakers. We wish that their presence would be a great encouragement and motivation for students and young researchers in the South-East Asia to take part in the research and development of natural pigments.

We are very happy to have 125 participants including the keynote speaker, plenary speakers, invited speakers, poster speakers, students and other participants from Germany, Switzerland, France, US, the Philippines, Singapore, and Indonesia. In this event, we would like to extend our acknowledgement to our partners, who support us financially, i.e. Kemenristekdikti, DAAD, ITS Scientific as well as PT Ditek Jaya and Shimadzu (Asia Pacific) Pte Ltd. We thanks to the Indonesian Society Pigment Researchers (Himpunan Peneliti Pigmen Indonesia, HP2I) for cooperation as steering committee, the Indonesia Pharmacist Association (Ikatan Apoteker Indonesia, IAI) for certifying this event with 6 credit points, the Indonesian Chemical Society (Himpunan Kimia Indonesia, HKI) and Indonesian-German Network (IGN) for disseminating this event to their members. We thanks also to Universitas Ma Chung for having this venue with superb facilities and supports from the staffs.

I do hope that you will find your time here enjoyable and a source of many insights that will help to advance the understanding of natural pigments and to encourage the collaborations and friendship, scientific exchange, the development of joint interests and project that are of scientific and economic importance in order to exploit the natural pigments and their importance in the most aspect of living, e.g. food and health, fashion, agriculture and advanced technologies.

Thank you very much and please enjoy this event.

Yours sincerely,

Tatas H.P. Brotosudarmo, Dipl.Chem., Ph.D

Chair of the NP-SEA 2016



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# Opening Remark from The Rector of Universitas Ma Chung

Dear Friends, participants of the Natural Pigments Conference for South East Asia 2016.

Welcome to Universitas Ma Chung, welcome to Malang, a beautiful city in Indonesia. We are happy that natural pigments experts from several countries, Indonesia, German, Switzerland, USA, France, the Philippines and Singapore gathered together here to share their knowledge and research results. We hold the NP Conference South East Asia this year in Universitas Ma Chung, Malang. It is not by chance that the international conference is holding here, because Universitas Ma Chung has an expertise in Natural Pigments which is institutionally embodied as Ma Chung Research Center for Photosynthetic Pigments (MRCPP). MRCPP is not only supported by Universitas Ma Chung but also by Indonesian government by recognizing MRCPP as one of the national scientific center of excellence.

Indonesia is one of the countries with a rich biodiversity, and consequently rich of natural pigments source. However, natural pigments industry in Indonesia and South East Asia is still lag behind the developed countries, whereas it is expected that the demands of natural pigments in various industries will increasing in the future. Therefore the research on natural pigments is one of the important research field. Indonesian government is also promoting the dissemination of research results to be applied in industry, not only finish in scientific publication.

Many kind of natural pigments in Indonesia and their properties are still unknown, they are remains to be investigated to provide benefit for human welfare. The international research cooperation in natural pigments will accelerate the rate of discovery and innovation in applying the knowledge for human welfare. Therefore, this conference is an important conference not only for the pigments research society but also for other research field and industry.

Besides the conference I hope the participants can also enjoy the natural beauty of Malang and its historic heritage.

I hope you enjoy staying in Malang, obtain a great benefit from the conference, and develop cooperation framework with other conference participants.

I wish to thank the speakers, poster presenters, students, and other attenders for attending the conference also partners who supported the conference.

Malang, August 18, 2016

Rector of Universitas Ma Chung



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1. Ferry F. Karwur, Ph.D. (Satya Wacana Christian University, Indonesia)
2. Prof. Dr. Hideki Hashimoto (Kwansei Gakuin University, Japan)
3. Prof. Dr. Hugo Scheer (Ludwig Maximilians University, Germany)
4. Leenawaty Limantara, M.Sc., Ph.D. (Universitas Pembangunan Jaya, Indonesia)
5. Prof. Dr. Ocky Karna Radjasa (Diponegoro University, Indonesia)
6. Tatas H.P. Brotosudarmo, Dipl.Chem., Ph.D. (Universitas Ma Chung, Indonesia)
7. Prof. Dr. Yuzo Shioi (Universitas Ma Chung, Indonesia)

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**GENERAL SCHEDULE**
**THE 3rd NATURAL PIGMENTS CONFERENCE FOR SOUTH-EAST ASIA (NP-SEA) 2016**
**August 22, R&D Center Universitas Ma Chung**

| Monday, August 22, 2014 | | |
|---|---|---|
| **The 3rd Natural Pigments Conference for South-East Asia (NP-SEA) 2016** | | |
| **Time** | **Program** | **Room** |
| 07:00 – 08:00 | Registration | R&D Center 1st floor |
| 08:00 – 08:10 | Opening Remark by Conference Chairman by Tatas H. P. Brotosudarmo, Ph.D. | R&D Center Hall 6th floor |
| 08:10 – 08:45 | Keynote Speaker : ***[Chlorophylls: From Photosynthesis to Photodynamic Therapy]*** by Prof. Dr. Hugo Scheer (Moderator : Leenawaty Limantara, Ph.D.) | R&D Center Hall 6th floor |
| 08:45 – 09:10 | Plenary Speaker : ***[Chlorophyll Breakdown During Leaf Senescence: A Novel Role for TIC55 as a Hydroxylase of Phyllobilins, the Products of Chlorophyll Breakdown]*** by Prof. Stefan Hörtensteiner (Moderator : Leenawaty Limantara, Ph.D.) | R&D Center Hall 6th floor |
| 09:10 – 09:35 | Plenary Speaker : ***[The Untapped Richness of Pigment-producing Marine Organisms and Their Associants]*** by Prof. Ocky Karna Radjasa (Moderator : Leenawaty Limantara, Ph.D.) | R&D Center Hall 6th floor |
| 09:35 – 10:00 | Plenary Speaker : ***[Marine Fungal Pigments Diversity and Potential Use]*** by Dr. Kustiariyah Tarman (Moderator : Leenawaty Limantara, Ph.D.) | R&D Center Hall 6th floor |
| 10:00 – 10:15 | Coffee break | R&D Center Hall 4th floor |
| 10:15 – 10:50 | Keynote Speaker : ***[Potential Market of Pigments in Daily Life: Food, Health and Fashion in Indonesia]*** by Ir. Thomas Darmawan (Moderator : Ferry F. Karwur, Ph.D.) | R&D Center Hall 6th floor |
| 10:50 – 11:15 | Plenary Speaker : ***[Review on the Metabolites of Monascus]*** by Dr. Philippe J. Blanc (Moderator : Ferry F. Karwur, Ph.D.) | R&D Center Hall 6th floor |
| 11:15 – 11:40 | Plenary Speaker : ***[Nexera UC, New Concept On-pine SFE-SFC-MS: Principles and Applications of SFE-SFC-MS/MS]*** by Dr. Xing Jie (Moderator : Ferry F. Karwur, Ph.D.) | R&D Center Hall 6th floor |
| 11:40 – 12:05 | Plenary Speaker : by Prof. Sherry A. Tanumihardjo, Ph.D. (Moderator : Ferry F. Karwur, Ph.D.) | R&D Center Hall 6th floor |
| 12:05 – 13:30 | Lunch | R&D Center Hall 4th floor |



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| Time | Program | Room |
|---|---|---|
| 13:30 – 14:45 | Invited speaker – Session 1 | R&D Center 6th floor (Class A) |
| | | R&D Center 6th floor (Class B) |
| 14:45 – 15:45 | Coffee Break<br>Poster Session | R&D Center Hall 4th floor |
| 15:45 – 16:45 | Invited speaker – Session 2 | R&D Center 6th floor (Class A) |
| | | R&D Center 6th floor (Class B) |
| 16:45 – 17:15 | 3rd NP-SEA Awards for Best Poster and Closing Remark | R&D Center Hall 6th floor |
| 17:15 – 18:00 | Preparation for Gala dinner | - |
| 18:00 – 20:00 | Gala dinner | Balai Pertiwi |

**Tuesday, August 23, 2014**

**Shimadzu Advance Liquid Chromatography Mass Spectrometry (LCMS/MS) Technologies and Applications Workshop in Junction with The Natural Pigments Conference for South-East Asia**

| Time | Program | Room |
|---|---|---|
| 07:00 – 8:00 | Registration | MRCPP R&D Center Hall 3rd floor |
| 08:00 – 09:00 | Session 1 : Introduction of LCMS/MS and Application | |
| 09:00 – 12:00 | Session 2 : Technical Workshop (I) | |
| 12:00 – 13:00 | Lunch | |
| 13:00 – 16:00 | Session 3 : Technical Workshop (II) | |
| 16:00 – 16:15 | Clossing | |



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## Schedule of Oral Presentation

| Oral Presentation – Session 1 | | | |
|---|---|---|---|
| **Meeting Hall at 6th Floor of The R&D Center (Class A)**<br>**Moderator: Dr. Ir. Edia Rahayuningsih, M.S.** | | | |
| **Time** | **Authors** | **Title** | **Code** |
| 13:30-13:45 | Delianis Pringgenies, Riyanda Idris, Muhammad Zainudin | The Antioxidant Activity of Carotenoid Pigments in the Bacterial Symbionts of Seagrass *Syringodium isoetifolium* | ON-01 |
| 13:45-14:00 | Victor Aprilyanto, Andrea Putri Subroto, Chris Darmawan, Reno Tryono, Condro Utomo, and Tony Liwang | In Vitro Selection of single guide RNA for Effective Cleavage of Exon-3 VIRESCENS Gene in Oil Palm Using CRISPR/Cas9 System | ON-02 |
| 14:00-14:15 | Abdullah Muzi Marpaung, Nuri Andarwulan, Purwiyatno Hariyadi and Didah Nur Faridah | The Color Stability of Butterfly Pea (*Clitoria ternatea* L.) Petal Extract at pH 6 to 8 are Highly Uncertain | ON-03 |
| 14:15-14:30 | Mohammad Junus | Algae Cells Density in Various Planting Period and Liquid Sludge Biogas Unit Proportion | ON-04 |
| 14:30-14:45 | Uun Yanuhar | The Involvement Fragment Pigment Protein (FPP) Microalga *Nanochloropsis oculata* of Response Heat Shock Protein 70 (HSP70) of Infection Nervous Necrotic Viral (NNV) on Grouper | ON-05 |
| **Room at 6th floor of The R&D Center (Class B)**<br>**Moderator: Prof. Erlinda A. Vasquez** | | | |
| 13:30-13:45 | Windu Merdekawati | The Uniqueness of Seaweed Pigments | ON-06 |
| 13:45-14:00 | Ermiziar, T., Saragih, R., Hanum, L. | Natural Pigment from Red Colour Melinjo Peels | ON-07 |
| 14:00-14:15 | Pujiyanto, Muhammad Iqbal Prawira Atmadja and Dadan Rohdiana | Theaflavin, Natural Pigment on Black Tea and Its Pharmacological Activities | ON-08 |
| 14:15-14:30 | Failisnur, Sofyan and Anwar Kasim | Dyeing of Cotton Fabric with Natural Dye from Gambier (*Uncaria gambir* Roxb.) | ON-09 |
| 14:30-14:45 | Defri Yona and Park Mi Ok | Seasonal variation of phycoerythrin chromophores of *Synechococcus* spp. in the East Sea, Korea | ON-10 |



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| Oral Presentation – Session 2 | | | |
|---|---|---|---|
| **Meeting Hall at 6th floor of The R&D Center (Class A)**<br>**Moderator: Mr. Victor Aprilyanto** | | | |
| 15:45-16:00 | Darda Efendi, H. Muthmainnah, T.S. Arzam, I. H. Sumiasih, R. Poerwanto, and Y.A. Purwanto, A. Agusta, and S. Yuliarni | Degradation of Chlorophyll and formation of β-cryptoxanthin and ß-citraurin in Citrus Degreening | ON-11 |
| 16:00-16:15 | Edia Rahayuningsih | The Sustainable Economic Development through Research, Production, and Application of Natural Dye | ON-12 |
| 16:15-16:30 | Delicia Yunita Rahman, Dwi Susilaningsih and Marc J.E.C. van der Maarel | Heterotrophic growth of LIPI13-AD014 for Phycocyanin Production | ON-13 |
| 16:30-16:45 | Muh. Thoyib, Catur Harsito, Suyitno, Syamsul Hadi | Simple Procedure for Reducing Cratering Defect of Water-Based Paint Using *Caesalpinia Sappan* Dye | ON-14 |
| **Room at 6th floor of The R&D Center (Class B)**<br>**Moderator: Dr. Dadan Rohdiana** | | | |
| 15:45-16:00 | Anna Yuliana, Marlia Singgih Wibowo, Elin Julianti | Toxicity Level of Monascus Pigments Using Ecosar Program | ON-15 |
| 16:00-16:15 | Erlinda A. Vasquez,Candelario L.Calibo, Ronnel M. Godoy and Lady Fatima G. Palermo | Alteration of the Chlorophyll Content in Phytoplasma-Infected Cassava | ON-16 |
| 16:15-16:30 | Rika Wahyuningtyas & Uun Yanuhar | The Expression of MHC Class 1 in *Cyprinus carpio* Infected Koi Herpes Virus through Induction of Crude Protein from Macroalgae *Halimeda* sp | ON-17 |
| 16:30-16:45 | Mada Triandala Sibero, Kustiariyah Tarman, Rita Sahara | Exploration of Red Pigment from Coastal Endophyte Fungi Isolated from *Hydnophytum formicarum* | ON-18 |



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| Poster Presentation<br>Room at 4th floor of The R&D Center<br>14:45 – 15:45 | | | |
|---|---|---|---|
| **No** | **Authors** | **Title** | **Code** |
| 1 | Elfi Anis Saati, Sita Ayu Pangesti, Sri Winarsih and Moch. Wachid | Co-pigmentation Anthocyanins of Rose Pigment (varieties of Batu Local) with Catechin from Black Tea and Green Tea Extracts | PN-01 |
| 2 | Andreas Lucky Effendy, Rollando | In silico screening study of potent human breast cancer drug from natural pigments | PN-02 |
| 3 | Antonius Herry Cahyana, Kam Natania and Hong Fu Sheng | Study on Antioxidant Activity, Binding Capacity and Stability of Curcumin-Functionalized $Fe_3O_4$ Magnetic Nanoparticles | PN-03 |
| 4 | Ayda Krisnawati and M. Muchlish Adie | Consistency of Biomass Production from Several Soybean Genotypes in Various Agro Ecology of Indonesia | PN-04 |
| 5 | Diah Mustika Lukitasari, Rosita Dwi Chandra, Heriyanto, Renny Indrawati | Stability and Antioxidant Activity of Microencapsulated Pigment from Red Spinach (*Amaranthus tricolor*) for Food Colourants | PN-05 |
| 6 | Elin Julianti, Laida Neti Mulyani,Marlia Singgih Wibowo, Susanti | Comparison Different Extraction method of C-Phycocianin, a Phycobiliprotein from Dry Biomass of *Spirullina platensis* | PN-06 |
| 7 | Ervika Rahayu NH, Dini Ariani, Miftakhussolikhah, Maharani P.E., Yudi P | The Effect of Yellow Natural Color from Turmeric on Physical and Sensory Properties of Arenga Starch-*Colocasia Esculanta* L. Noodle | PN-07 |
| 8 | Giacinta Mutiara Beta Maharani, Filiana Santoso, and Abdullah Muzi Marpaung | Stability Improvement of Anthocyanin from Various Local Plants using Metal Complexation | PN-08 |
| 9 | Kam Natania, Antonius Herry Cahyana, Melanie Cornelia dan Edison Sutiyono | Microencapsulation of Soursop (*Annona muricata* Linn.) Leaf Tea Extract Using Natural Mucilages | PN-09 |



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| Poster Presentation<br>Room at 4th floor of The R&D Center<br>14:45 – 15:45 | | | |
|---|---|---|---|
| **No** | **Authors** | **Title** | **Code** |
| 10 | M. Muchlish Adie and Ayda Krisnawati | Identification and Clustering Soybean Genotypes with High Biomass Production as a Source of Renewable Energy | PN-10 |
| 11 | Melanie Cornelia and Oktafielia Putri | Application of Goji Berry Fruit (*Lycium barbarum* L.) extract as Food colorant in Dried Noodle | PN-11 |
| 12 | Miftakhussolikhah, Dini Ariani, Ervika RNH, Azkia Nastiti, Yudi Pranoto | Effect of Additional Suji Leaves and Turmeric Extract on Physicochemical Characteristic and Antioxidant Activity of Arenga-Canna Noodle | PN-12 |
| 13 | Selfina Gala, Dhaniar Rulandri Widoretno, Delita Kunhermanti, Lailatul Qadariyah, Sumarno and Mahfud | Microwave-assisted Extraction of Natural Dyes from Jackfruit Wood Waste (*Artocarpus heterophyllus* Lamk) | PN-13 |
| 14 | Renny Indrawati, Gita, Kristine, Melissa, Yuyun Yuniati, Leenawaty Limantara | How extensive does the artificial dye color our food? | PN-14 |
| 15 | Swanty Rahmazania Mustika and Abdullah Muzi Marpaung M.P | Color properties and Stabilizing Effect of Metal ion on Blue Anthocyanin Color from Buni (*Antidesma bunius*) Fruit | PN-15 |
| 16 | Rosita Dwi Chandra, Renny Indrawati, Mario Sent Anugrah, Jodiawan, Ricky Santoso, Tatas H. P. Brotosudarmo, Leenawaty Limantara | Uncovering the Availability of Products Enriched with Vitamin A in Local Supermarket | PN-16 |



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| Poster Presentation<br>Room at 4th floor of The R&D Center<br>14:45 – 15:45 | | | |
|---|---|---|---|
| **No** | **Authors** | **Title** | **Code** |
| 17 | Yudi Purnomo, Fajar Audra Pratama, Nur Rohman | Hepatoprotector and Anti-Hemolysis Activity of Tommato (*Lycopersicon pimpinellifolium*) Juices In Rats Induced Alum | PN-17 |
| 18 | Endang Kusdiyantini, Iffan Alif, Salma Fuadiyah, Dyah Wulandari, Anto Budiharjo | Identification of Red-Pigmented Thermophile Bacteria Isolated from Gedong Songo Hot Spring, Semarang – Central Jawa | PN-18 |
| 19 | Setiyono, E., Pringgenies., Heriyanto, Prihastyanti, M.N.U, Shioi, Y., Brotosudarmo, T.H.P | Carotenoid Analysis from *Erythrobacter flavus* Symbiont of *Acropora nasuta* | PN-19 |
| 20 | Husnatain, I.D., Salim, K.P., Heriyanto, Purwantiningrum, I., Harijono, Limantara, L. | Effect of Dried Fruit Processing on Lycopene Content and Pigment Composition of Tommato (*Lycopensicum esculentum* var Marta) | PN-20 |
| 21 | Wibowo, A.A., Elim, P.E., Heriyanto, Prihastyanti, M.N.U, Shioi, Y., Brotosudarmo, T.H.P | Effect of Drying Treatments on the Concentration of Fucoxanthin and Chlorophyll *a* and Pigment composition of Three *Sargassum* Species | PN-21 |
| 22 | Yuyun Yuniati, Renny Indrawati, Jovine, Tantiana, Wynona | Tracing the antioxidant-rich products in local groceries: naturalness, biofunctionality, and price | PN-22 |
| 23 | Yuyun Yuniati, Juliana, Lidwina Angelica Soetantijo, and Ratna Yulianti Wijaya, Renny Indrawati | Preparation of Antioxidant Drinks from Mulberry *Morus nigra* L. | PN-23 |



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# Analysis of Carotenoids from *Erythrobacter flavus* Isolated from Soft-Coral *Acropora nasuta*

Edi Setiyono[a], Delianis Pringgenies[a], Heriyanto[b], Monika N.U. Prihastyanti[b], Yuzo Shioi[b], Leenawaty Limantara[c], and Tatas H.P. Brotosudarmo[b]*

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**Abstract**

Separation and composition of carotenoids from *Erythrobacter flavus* are reported. *E. flavus* is a yellow aerobic marine bacterium. It was isolated from soft-coral *Acropora nasuta*. *E. flavus* was cultured in Shioi medium at 28°C for 3 days. The cells of *E. flavus* from each growth phase were extracted with a mixture of methanol and acetone (7:3, $^{v}/_{v}$). The crude pigment extract was injected into a reverse-phase of high performance liquid chromatography using C8 column. The results showed that *E. flavus* contain of 18 carotenoids pigments with 3 dominant carotenoids eluted at 18.7, 20.5, and 21.1 min. Zeaxanthin (at 28.3 min) and β-carotene (at 37.3 min) were minor carotenoids and the identification refers to their spectral, chromatographic and mass properties. The area of peak 1 and peak 2 increased 46% and 735%, respectively from 15 hour to 90 hour of culture, whereas the area of peak 3 did not change in each growth phase.

*Keywords:* β-carotene, carotenoids, *Erythrobacter flavus*, high-performance liquid chromatography, co-chromatography, zeaxanthin
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## 1. Introduction

Carotenoids, found in plants, animal, and microorganism (bacterium and microalgae), play a critical role in the photosynthetic process to collect light energy in the visible region and to protect against photooxidation [1]. In addition, carotenoids have been reported to have significant value to support human health, i.e. antioxidant, anticancer, antiobesity [4].. Cars are consisted of 40-carbon atom to form 8-isoprena and have yellow, orange, and red colour [2,3]. *E. flavus* is a yellow aerobic marine bacterium. It was isolated from soft-coral *Acropora nasuta* [5]. This study was aimed to separate and identify cars from *E. flavus* and to determine the Cars composition from its growth phases by reverse phase-high performance liquid chromatography (RP-HPLC).

## 2. Methodology

### *2.1. Cells culture*

The cells were grown in Shioi liquid medium [6]. The culture was incubated by shaking (100 rpm) at 28°C under the dark condition. The cells were harvested after each growth phase, i.e. 15, 22, 46 and 90 hour, by centrifugation at 15,880 g, 4°C for 10 min. The cells were collected and then stored at -30°C until used.

### *2.2. Cells extraction*

The cells (0.1 g) were homogenized in a mixture of methanol and acetone (7:3, $^{v}/_{v}$; 1 mL) by vortexing for 3 times (1 min vortex, 1 min on ice) and then lysed by sonication. Sonication process was carried out at a pulse mode with 60% amplitude and 10-s on/30-s off for 10 min (QSonica, Newtown, US). The crude pigment extract was separated by a centrifuge at 8,000 g for 2 min. The extract was dried by $N_2$ gas and stored at -30°C until used.

### *2.3. Separation, identification and composition of cars*

The cars of *E. flavus* were separated by a RP-HPLC using $C_8$ column (150 x 4.6 mm; Water) according to the method of Zapata *et al.* [7]. Elution gradient of 2 solvents, i.e. solvent A (methanol:acetonenitril:pyridine solution (0.25 M) = 50:25:25 (v/v/v)) and solvent B (methanol:acetonenitril:acetone = 20:60:20 (v/v/v)) was performed at the flow rate of 1 ml/min with the temperature of column oven at 30°C. Chromatographic, spectral and mass properties were used for identification of Cars. Co-chromatography with the standard pigments and the saponification

process were done to support the identification. The content of Cars for each growth phase was determined from the peak area of the dominant Cars detected at 450 nm.

### 3. Results and discussion

Cars separation of crude pigment extract from *E. flavus* is shown in Figure 1. At least 18 Cars have been well separated by RP-HPLC with three dominant Cars appeared at retention time ($t_R$) of 18.7 (peak 1), 20.5 (peak 2), and 21.1 (peak 3) min. Most of the Cars of this marine bacterium have a similar of maximum absorption wavelength ($\lambda_{max}$) at around 451-453 nm. This similarity of $\lambda_{max}$ indicates that Cars of *E. flavus* have the same number of conjugated double bonds in their chromophore. Juliadiningtyas *et al* (2016) reported most of the Cars of this bacterium have similar core chemical structure.

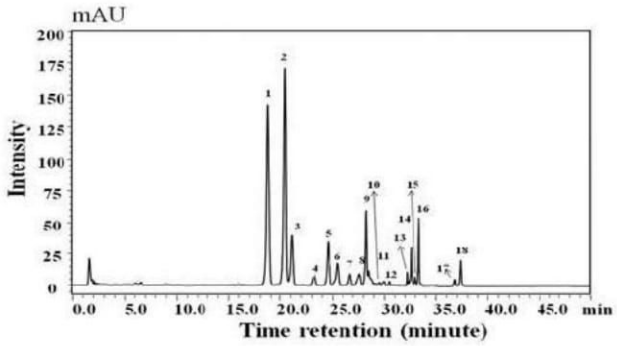


Figure 1. HPLC Chromatogram of crude pigment extract from *E. flavus* detected at 450 nm. The number of peak is described in the text.

Co-chromatography results with zeaxanthin and β-carotene standards suggested peak 9 at 28.3 min and peak 18 at 37.3 min were identified as zeaxanthin and β-carotene, respectively. Mass spectra of these Cars confirm the identification. In addition, the $\lambda_{max}$ of zeaxanthin (at 453) and β-carotene (at 452 nm) is in agreement with the values in Zapata *et al* [7]. Zeaxanthin is one of baterial Cars produced by several marine bacteria, such as *Staphylococcus aureus, Vibrio psychroerythrus, Streptomyces* sp., and *Hahella chejuensis* [9]. Peak 1 and peak 3 were identified as the esterified Car according to the HPLC result of saponificated pigment extract. These peaks decreased and on other hand other peak increased as a free Car compared to the result of unsaponificated sample. The other dominant Car (peak 2) was a free pigment due to no effect on the saponification treatment.

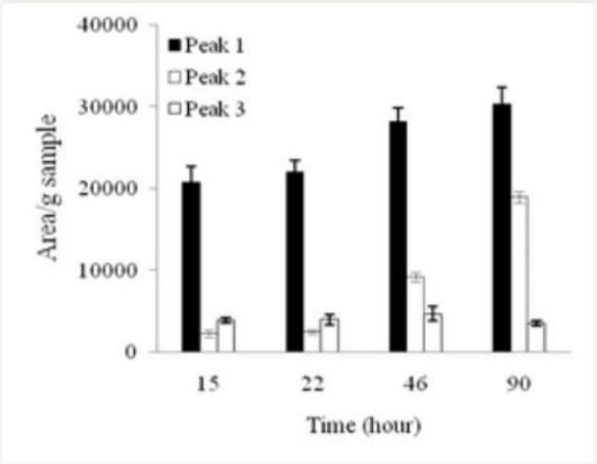


Figure 2. The Cars concentration (area per 1 g of the cell) of three dominant Cars in every growth phase

In this study Cars biosynthesis of *E. flavus* was determined from the peak area of the dominant Cars. The result of Cars composition from peak 1, 2, 3 at each growth phase is shown in Figure 2. The growth phase of *E. flavus* culture was classified into three phases, such as lag phase (0-15 hour), exponential phase (22-46 hour), and stationary phase (90 hour). The total area (per 1 g of the cells) of peak 1 increased 46% from 15 hour to 90 hour, whereas peak 2 accumulated 735% and it was higher than the peak 1. The total area of peak 3 was relatively same in each growth phase. The cars biosynthesis of *E. flavus* was continuously occurred to the peak 1 and peak 2, whereas peak 3 did not change. The cars composition from purple bacterium was influenced by species, age of the cell, and culture condition [10]. Taylor *et al* reported total Cars lineary increased with the curve of growth in *Streptocoocus faecium* [11].

### 4. Conclusion

At least 18 Cars have been separated by RP-HPLC from the crude pigment extract of *E. flavus*. Zeaxanthin and β-carotene were identified as the minor Cars, while the three dominant Cars were eluted in front of the zeaxanthin peak and have the same $\lambda_{max}$ as those minor Cars. Cars at peak 1 and peak 2 were extensively biosynthesized by *E. flavus*.

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