

# MARINE GLYCOBIOLOGY Principles and Applications

edited by Se-Kwon Kim

## MARINE GLYCOBIOLOGY Principles and Applications



## MARINE GLYCOBIOLOGY Principles and Applications

edited by Se-Kwon Kim



CRC Press is an imprint of the Taylor & Francis Group, an **informa** business CRC Press Taylor & Francis Group 6000 Broken Sound Parkway NW, Suite 300 Boca Raton, FL 33487-2742

© 2017 by Taylor & Francis Group, LLC CRC Press is an imprint of Taylor & Francis Group, an Informa business

No claim to original U.S. Government works

Printed on acid-free paper Version Date: 20160622

International Standard Book Number-13: 978-1-4987-0961-3 (Hardback)

This book contains information obtained from authentic and highly regarded sources. Reasonable efforts have been made to publish reliable data and information, but the author and publisher cannot assume responsibility for the validity of all materials or the consequences of their use. The authors and publishers have attempted to trace the copyright holders of all material reproduced in this publication and apologize to copyright holders if permission to publish in this form has not been obtained. If any copyright material has not been acknowledged please write and let us know so we may rectify in any future reprint.

Except as permitted under U.S. Copyright Law, no part of this book may be reprinted, reproduced, transmitted, or utilized in any form by any electronic, mechanical, or other means, now known or hereafter invented, including photocopying, microfilming, and recording, or in any information storage or retrieval system, without written permission from the publishers.

For permission to photocopy or use material electronically from this work, please access www.copyright.com (http://www.copyright.com/) or contact the Copyright Clearance Center, Inc. (CCC), 222 Rosewood Drive, Danvers, MA 01923, 978-750-8400. CCC is a not-for-profit organization that provides licenses and registration for a variety of users. For organizations that have been granted a photocopy license by the CCC, a separate system of payment has been arranged.

Trademark Notice: Product or corporate names may be trademarks or registered trademarks, and are used only for identification and explanation without intent to infringe.

#### Library of Congress Cataloging-in-Publication Data

Names: Kim, Se-Kwon, editor. Title: Marine glycobiology : principles and applications / [edited by] Se-Kwon Kim. Description: Boca Raton : CRC Press/Taylor & Francis Group, 2017. | Includes bibliographical references and index. Identifiers: LCCN 2016010385 | ISBN 9781498709613 (hardback : alk. paper) Subjects: | MESH: Glycomics--methods | Aquatic Organisms | Glycoconjugates--pharmacology | Bioprocting--methods | Biotechnology--methods Classification: LCC QP702.G577 | NLM QH 91.8.B5 | DDC 572/.56--dc23 LC record available at http://lccn.loc.gov/2016010385

### Visit the Taylor & Francis Web site at http://www.taylorandfrancis.com

and the CRC Press Web site at http://www.crcpress.com

### Contents

Preface		ix
Acknowled	gments	X1
Contributor	rS	xv
Section I: I	Introduction to marine glycobiology	
Chapter 1	<b>Introduction to marine glycobiology</b> Se-Kwon Kim and Jayachandran Venkatesan	3
Chapter 2	<b>Glycoscience: The current state of the research</b>	7
Section II:	Marine glycoconjugates of reproduction and chemical communications	
Chapter 3	<b>Marine glycoconjugates in gamete physiology and fertilization</b> <i></i>	25
Chapter 4	<b>Heparin from marine mollusks: Occurrence, structure, and biological role</b> Nicola Volpi and Francesca Maccari	39
Section III:	: Marine glycans	
Chapter 5	<b>Bioactivity and mechanism of action of marine glycans</b> Visamsetti Amarendra and Ramachandran Sarojini Santhosh	51
Chapter 6	Marine glycans in relationship with probiotic microorganisms to improve human and animal health Van Duy Nguyen	67
Chapter 7	<b>Principle and biological properties of sulfated polysaccharides from seaweed</b> Jantana Praiboon, Anong Chirapart, and Nattanun Soisarp	85
Section IV:	Marine glycoproteins	
Chapter 8	<b>Biomedical benefits of algal glycoproteins</b> Hari Eko Irianto and Ariyanti Suhita Dewi	121
Chapter 9	Partial sequencing, structural characterization, and anticoagulant activity of heparan sulfate and sulfated chitosan from selected Indian marine mollusks Ramachandran Saravanan, Ramachandran Karthik, and Annian Shanmugam	129

Chapter 10	<b>Biomedical potential of natural glycoproteins with special reference to marine collagen</b>	145
Chapter 11	<b>Glycoproteins and detoxification in the marine environment</b>	161
Chapter 12	<b>Recent trends in bioprospecting of marine collagen</b> <i>Kirti and Samanta S. Khora</i>	169
Chapter 13	Marine fungi: Glycolipidomics	183
Section V:	Marine glycoenzymes	
Chapter 14	Sialyltransferases from marine environments: Preparation of sialyloligosaccharides and its application Takeshi Yamamoto	195
Section VI:	Marine carbohydrates	
Chapter 15	<b>Polysaccharides from marine sources and their pharmaceutical approaches</b>	209
Chapter 16	<b>Pharmaceutical importance of marine algal-derived carbohydrates</b>	227
Chapter 17	Marine bacterial exopolysaccharides: Functional diversity and prospects in environmental restoration	235
Chapter 18	Agar-abundant marine carbohydrate from seaweeds in Indonesia: Production, bioactivity, and utilization	255
Chapter 19	<b>Bioprospecting potential of marine natural polymers of chitin and chitosan</b>	263
Chapter 20	<b>Bioactivities of sulfated polysaccharide porphyran isolated from edible red alga</b> <i>Porphyra</i> <i>yezoensis</i> <i>Zedong Jiang and Tatsuya Oda</i>	279
Chapter 21	Marine polysaccharides as biostimulants of plant growth	293
Chapter 22	<b>Carbohydrates in drug discovery: Insights into sulfated marine polysaccharides</b>	311
Chapter 23	<b>Cyanobacterial extracellular polysaccharide sheath pigment, scytonemin: A novel</b> <b>multipurpose pharmacophore</b>	323
Chapter 24	<b>Biomedical application of carbohydrates from marine microbes</b>	339
Chapter 25	Antimicrobial properties of chitosan and chitosan derivatives	345

#### Contents

Chapter 26	Marine bacterial extracellular polymeric substances: Characteristics and applications
Chapter 27	<b>Brown algal polysaccharide: Alginate and its biotechnological perspectives</b>
Chapter 28	Mangroves: A potent source of polysaccharides 393   Narayanasamy Rajendran, Kandasamy Saravanakumar, and Kandasamy Kathiresan
Chapter 29	<b>It's all about the marine carbohydrates</b>
Section VII	: Bioinformatics of glycobiology
Chapter 30	Glycans predictive modeling using modern algorithms
Section VII	I: Biological role of glycoconjugates
Chapter 31	Glyco-conjugated bioactive compounds derived from brown algae and its biological applications
Section IX:	Glycoconjugates in biomedicine and biotechnology
Chapter 32	D- <b>Glucosamine contributes to cell membrane stability</b>
Chapter 33	<b>Structural glycobiology for lectin to promote advanced biomedical research</b>
Chapter 34	Chitin derivatives as functional foods
Chapter 35	Marine carbohydrates and their applications469Bishnu Pada Chatterjee and Partha Pratim Bose
Chapter 36	<b>Glycobiotechnology</b>
Chapter 37	Application of bacterial chitinase
Chapter 38	<b>Biological activity of marine sponge lectins</b>
Index	



### Preface

Marine glycobiology is the study of carbohydrate and carbohydrate with molecules (protein, lipids, enzymes, or small molecules). Glycoconjugates are glycan linked with other biological molecules. The study and research on marine glycobiology is less well-known. However, the recent development on analytical instruments and chemical characterizations increases the research on glycoconjugates. The knowledge gained of the exact chemical structure of marine glyconjugates increases its use in biological and biomedical applications.

This book contains 38 chapters under different sections.

- 1. Section I—Chapter 1 provides a general introduction to the topics covered in this book.
- 2. Section II—marine glycoconjugates of reproduction and chemical communications (Chapters 3 and 4) are described.
- 3. Section III—Bioactivity, principles, and applications of marine glycans (Chapters 5 through 7) are explored.
- 4. Section IV—marine glycoproteins (Chapters 8 through 13)—deals with biomedical benefits of algal glycoproteins, marine source collagen, and detoxification in the marine environment.
- 5. Section V—marine glycoenzymes (Chapter 14) discusses the sialyltransferases from the marine environment and their applications.
- Section VI—marine carbohydrates (Chapters 15 through 29)—discusses several marine carbohydrates and their glycobiology. Marine bacterial exopolysaccharides, agar, chitin and chitosan,

sulfated polysaccharides, carbohydrates from marine microbes, algal polysaccharides, and mangroves are discussed in detail. In addition, the use of these polysaccharides in pharmaceutical applications, plant growth, and various biological activities are also discussed.

- 7. Section VII and VIII—bioinformatics and biological role of glyconjugates (Chapters 30 and 31) describes glycan's predictive modeling using modern algorithms and glycoconjugated bioactivity compounds and biological applications.
- 8. Section IX—glycoconjugates as biomedicine (Chapters 32 through 38)—presents the application of glyconjugates in biomedicine and their biotechnological applications.

I express my sincere thanks to all the authors who have contributed toward this book. Their relentless effort was the result of their strong inclination towards scientific research, and great perseverance descended from their experience. I am grateful to the experts who have contributed to this book.

I hope that fundamental as well as applied contributions to this book might serve as potential research and development leads for the benefit of humankind. Marine glycobiology will be the excellent field in the future towards the enrichment of targeted marine glycans, which further sets up a suitable for further applications. This book would be a reference book for students in academic and industrial research.

> **Prof. Se-Kwon Kim** *Busan, South Korea*



### Acknowledgments

I thank CRC Press staff for their continuous encouragement and suggestions to get this wonderful compilation published. I also extend my sincere gratitude to all the contributors for providing their help, support, and advice to accomplish this task. Further, I thank Dr. Panchanathan Manivasagan and Dr. Jayachandran Venkatesan, who worked with me throughout the course of this book project. I strongly recommend this book for marine biotechnology and glycobiology researchers/students/industrialists and hope that it helps to enhance their understanding in this field.

> **Prof. Se-Kwon Kim** Pukyong National University Busan, South Korea



### Editor

**Se-Kwon Kim, PhD**, is a distinguished professor in the Department of Marine Bio Convergence Science and Technology and as director of Marine Bioprocess Research Center (MBPRC) at Pukyong National University, Busan, South Korea.

He received his MSc and PhD from Pukyong National University and conducted his postdoctoral studies in the Laboratory of Biochemical Engineering, University of Illinois, Urbana–Champaign, Illinois. Later, he became a visiting scientist at the Memorial University of Newfoundland and the University of British Colombia in Canada.

Dr. Kim served as president of the Korean Society of Chitin and Chitosan during 1986–1990 and the Korean Society of Marine Biotechnology during 2006– 2007. In recognition of his research, he won the best paper award from the American Oil Chemists' Society. In 2002, Dr. Kim was also the chairman for the 7th Asia-Pacific Chitin and Chitosan Symposium, which was held in South Korea in 2006. He was the chief editor of the journal *Korean Society of Fisheries and Aquatic Science* during 2008–2009. Also, he is a board member of the International Marine Biotechnology Association (IMBA) and the International Society of Nutraceuticals and Functional Food (ISNFF).

His major research interests are investigation and development of bioactive substances from marine resources. His immense experience in marine bioprocessing and mass production technologies for marine bio-industry is the key asset of holding majorly funded marine bio projects in Korea. Furthermore, he expended his research fields up to the development of bioactive materials from marine organisms for their applications in oriental medicine, cosmeceuticals, and nutraceuticals. To date, he has authored around 700 research papers and 70 books and holds 130 patents.



Imran Ahmad Chaplin School of Hospitality and Tourism Management Florida International University North Miami, Florida

**Bakrudeen Ali Ahmed** Department of Biotechnology Sri Shakthi Institute of Engineering and Technology Coimbatore, Tamil Nadu, India

Thipramalai Thankappan Ajithkumar National Bureau of Fish Genetic Resources Indian Council of Agricultural Research Lucknow, Uttar Pradesh, India

Abdul Shirin Alijani Institute of Biological Sciences and Department of Chemical engineering University of Malaya Kuala Lumpur, Malaysia

Visamsetti Amarendra School of Chemical and Biotechnology SASTRA University Thanjavur, Tamil Nadu, India

**Perumal Anantharaman** Faculty of Marine Sciences Annamalai University Chidambaram, Tamil Nadu, India

**Muthuvel Arumugam** Centre of Advanced Study in Marine Biology Annamalai University Chidambaram, Tamil Nadu, India

**Kazuo Azuma** Department of Veterinary Clinical Medicine Tottori University Tottori, Japan **Thangavel Balasubramanian** Centre of Advanced Study in Marine Biology Annamalai University Chidambaram, Tamil Nadu, India

**Partha Pratim Bose** Division of Molecular Medicine Bose Institute Kolkata, West Bengal, India

**Jaya Chakraborty** Department of Life Science National Institute of Technology Rourkela, Odisha, India

**Bishnu Pada Chatterjee** Department of Natural Science West Bengal University of Technology Kolkata, West Bengal, India

**Urmimala Chatterjee** Department of Natural Science West Bengal University of Technology Kolkata, West Bengal, India

**Jie Chen** School of Agriculture and Biology Shanghai Jiao Tong University Minhang, Shanghai, People's Republic of China

Anong Chirapart Department of Fishery Biology Kasetsart University Bangkok, Thailand

**Katarzyna Chojnacka** Department of Advanced Material Technologies Wrocław University of Technology Wrocław, Poland

#### Surajit Das

Department of Life Science National Institute of Technology Rourkela, Odisha, India

#### Hirak R. Dash

Department of Life Science National Institute of Technology Rourkela, Odisha, India

#### Ariyanti Suhita Dewi

Research and Development Centre for Marine and Fisheries Products Competitiveness and Biotechnology Ministry of Marine Affairs and Fisheries Jakarta, Indonesia

Elangovan Dilipan Centre of Advanced Study in Marine Biology Annamalai University Chidambaram, Tamil Nadu, India

Agnieszka Dmytryk Department of Advanced Material Technologies Wrocław University of Technology Wrocław, Poland

**Reza Farzinebrahimi** Institute of Biological Sciences University of Malaya Kuala Lumpur, Malaysia

Yuki Fujii Department of Pharmacy Nagasaki International University Nagasaki, Japan

Alessandra Gallo Department of Biology and Evolution of Marine Organisms Stazione Zoologica Anton Dohrn Naples, Italy

Arijit Gandhi Department of Quality Assurance Albert David Limited Kolkata, West Bengal, India

Katarzyna Godlewska Department of Advanced Material Technologies Wrocław University of Technology Wrocław, Poland

#### Claudia Mariana Gomez-Gutierrez

Department of Bioengineering University of Baja California Baja California, Mexico

#### Dharmalingam Gowdhaman

School of Chemical and Biotechnology SASTRA University Thanjavur, Tamil Nadu, India

Graciela Guerra-Rivas

Marine Pharmacology and Toxicology Laboratory University of Baja California Baja California, Mexico

#### Imtiaj Hasan

Department of Life and Environmental System Science Yokohama City University Yokohama, Japan and Department of Biochemistry and Molecular Biology University of Rajshahi Rajshahi, Bangladesh

#### Yoshihiko Hayashi

Department of Cariology Nagasaki University Nagasaki, Japan

#### Martha Á. Hjálmarsdóttir

Faculty of Medicine University of Iceland Reykjavik, Iceland

#### Masahiro Hosono

Institute of Molecular Biomembrane and Glycobiology Tohoku Medical and Pharmaceutical University Sendai, Japan

Shinsuke Ifuku Graduate School of Engineering Tottori University Tottori, Japan

**Kazunari Igawa** Department of Cariology Nagasaki University Nagasaki, Japan

**Takeshi Ikeda** Department of Cariology Nagasaki University Nagasaki, Japan

xvi

#### Dhinakarasamy Inbakandan

Centre for Ocean Research Sathyabama University Chennai, Tamil Nadu, India

**Giyatmi Irianto** Department of Food Technology Jakarta Sahid University Jakarta, Indonesia

#### Hari Eko Irianto

Center for Fisheries Research and Development Agency for Marine and Fisheries Research and Development Ministry Marine Affairs and Fisheries and Department of Food Technology Jakarta Sahid University Jakarta, Indonesia

**Sougata Jana** Department of Pharmaceutics Gupta College of Technological Sciences Asansol, West Bengal, India

#### Subrata Jana

Department of Chemistry V.E.C, Sarguja University Ambikapur, Chhattisgarh, India

**Bhavanath Jha** 

Division of Marine Biotechnology and Ecology Central Salt and Marine Chemicals Research Institute Bhavnagar, Gujarat, India

**Zedong Jiang** College of Food and Biological Engineering Jimei University Xiamen, Fujian, People's Republic of China

**Kei Kaida** Department of Oral Physiology Nagasaki University Nagasaki, Japan

Kannan Kamala Center for Environmental Nuclear Research SRM University Kattankulathur, Tamil Nadu, India

#### Robert A. Kanaly

Department of Life and Environmental System Science Yokohama City University Yokohama, Japan

#### Jeyakumar Kandasamy

Department of Chemistry Indian Institute of Technology—Banaras Hindu University Varanasi, Uttar Pradesh, India

Vinod K. Kannaujiya

Center of Advanced Study in Botany Banaras Hindu University Varanasi, Uttar Pradesh, India

#### Ramachandran Karthik

Department of Medical Biotechnology Chettinad Academy of Research and Education Chennai, Tamil Nadu, India

**Kandasamy Kathiresan** Faculty of Marine Sciences

Annamalai University Chidambaram, Tamil Nadu, India

Yasushi Kawakami School of Life and Environmental Science Azabu University Sagamihara, Japan

#### Sarkar M.A. Kawsar

Department of Chemistry University of Chittagong Chittagong, Bangladesh

Samanta S. Khora

Division of Medical Biotechnology VIT University Vellore, Tamil Nadu, India

**Se-Kwon Kim** Department of Marine-Bio Convergence Science and Marine Bioprocess Research Center Pukyong National University Busan, South Korea

#### Kirti

Division of Medical Biotechnology VIT University Vellore, Tamil Nadu, India

Yasuhiro Koide Department of Life and Environmental System Science Yokohama City University Yokohama, Japan

**Ozcan Konur** Department of Materials Engineering Yildirim Beyazit University Ankara, Turkey

**Himanshu Kumar** Department of Life Science National Institute of Technology, Rourkela Rourkela, Odisha, India

**Supriya Kumari** Department of Life Science National Institute of Technology, Rourkela Rourkela, Odisha, India

**Francesca Maccari** Department of Life Sciences University of Modena and Reggio Emilia Modena, Italy

**Neelam Mangwani** Department of Life Science National Institute of Technology, Rourkela Rourkela, Odisha, India

Thangapandi Marudhupandi Centre for Ocean Research Sathyabama University Chennai, Tamil Nadu, India

Már Másson School of Health Sciences University of Iceland Reykjavik, Iceland

Izabela Michalak Department of Advanced Material Technologies Wrocław University of Technology Wrocław, Poland

Avinash Mishra Division of Marine Biotechnology and Ecology Central Salt and Marine Chemicals Research Institute Bhavnagar, Gujarat, India **Van Duy Nguyen** Institute of Biotechnology and Environment Nha Trang University Nha Trang, Vietnam

Athapol Noomhorm The School of Environment, Resources and

Development Asian Institute of Technology Bangkok, Thailand

Tatsuya Oda Graduate School of Fisheries Science and

Environmental Studies Nagasaki University Nagasaki, Japan

**Yukiko Ogawa** Department of Pharmacy Nagasaki International University Sasebo, Japan

**Yoshiharu Okamoto** Department of Veterinary Clinical Medicine Tottori University Tottori, Japan

**Tomohiro Osaki** Department of Veterinary Clinical Medicine Tottori University Tottori, Japan

Yasuhiro Ozeki Department of Life and Environmental System Science Yokohama City University Yokohama, Japan

Ratih Pangestuti Research Center for Oceanography Indonesian Institute of Sciences Jakarta Utara, Republic of Indonesia

**Jainendra Pathak** Center of Advanced Study in Botany Banaras Hindu University Varanasi, Uttar Pradesh, India

Jantana Praiboon Department of Fishery Biology Kasetsart University Bangkok, Thailand

#### xviii

#### Radhika Rajasree Santha Ravindranath

Centre for Ocean Research Sathyabama University Chennai, Tamil Nadu, India

#### Narayanasamy Rajendran

Department of Zoology Government Arts College Chidambaram, Tamil Nadu, India

#### Sultana Rajia

Department of Life and Environmental System Science Yokohama City University Yokohama, Japan and

Department of Natural Science Varendra University Rajshahi, Bangladesh

#### Rajneesh

Center of Advanced Study in Botany Banaras Hindu University Varanasi, Uttar Pradesh, India

**Richa** Center of Advanced Study in Botany Banaras Hindu University Varanasi, Uttar Pradesh, India

#### Idris Mohamed Saeed Institute of Biological Sciences and Department of Chemical engineering University of Malaya Kuala Lumpur, Malaysia

**Priyanka Sahariah** School of Health Sciences University of Iceland Reykjavik, Iceland

**Hiroyuki Saimoto** Graduate School of Engineering Tottori University Tottori, Japan

#### Ramachandran Sarojini Santhosh Genetic Engineering Laboratory and School of Chemical and Biotechnology SASTRA University Thanjavur, Tamil Nadu, India

Kandasamy Saravanakumar School of Agriculture and Biology Shanghai Jiao Tong University Minhang, Shanghai, People's Republic of China

#### Ramachandran Saravanan

Department of Marine Pharmacology Chettinad Academy of Research and Education Chennai, Tamil Nadu, India and Centre of Advanced Study in Marine Biology Annamalai University Chidambaram, Tamil Nadu, India

#### Siswa Setyahadi

Center for Bioindustrial Technology Agency for the Assessment and Application of Technology Puspiptek, Serpong, Banten, Indonesia

#### Sabiah Shahul Hameed

Department of Chemistry Pondicherry University Pondicherry, Tamil Nadu, India

#### Annian Shanmugam

Centre of Advanced Study in Marine Biology Annamalai University Chidambaram, Tamil Nadu, India

#### **Vijay Kumar Singh**

Division of Marine Biotechnology and Ecology Central Salt and Marine Chemicals Research Institute Bhavnagar, Gujarat, India

**Rajeshwar P. Sinha** Center of Advanced Study in Marine Biology Banaras Hindu University Varanasi, Uttar Pradesh, India

#### Pitchiah Sivaperumal

Center for Environmental Nuclear Research SRM University Kattankulathur, Tamil Nadu, India

Nattanun Soisarp Department of Fishery Biology Kasetsart University Bangkok, Thailand

**Arun S. Sonker** Center of Advanced Study in Botany Banaras Hindu University Varanasi, Uttar Pradesh, India

**Vasuki Subramanian** Faculty of Marine Sciences Annamalai University Chidambaram, Tamil Nadu, India

Shigeki Sugawara Institute of Molecular Biomembrane and Glycobiology Tohoku Medical and Pharmaceutical University Sendai, Japan

#### Syamdidi

Research and Development Center for Marine and Fisheries Product Competitiveness and Biotechnology Agency for Marine and Fisheries Research and Development

Jakarta Pusat, Indonesia

#### Nallthambi Tamilkumar Varsha

School of Chemical and Biotechnology SASTRA University Thanjavur, Tamil Nadu, India

#### Anguchamy Veeruraj

Centre for Ocean Research Sathyabama University Chennai, Tamil Nadu, India and Centre of Advanced Study in Marine Biology Annamalai University Chidambaram, Tamil Nadu, India

#### Jayachandran Venkatesan

Department of Marine-Bio Convergence Science and Marine Bioprocess Research Center Pukyong National University Busan, South Korea **Nicola Volpi** Department of Life Sciences University of Modena and Reggio Emilia Modena, Italy

#### Radosław Wilk

Department of Advanced Material Technologies Wrocław University of Technology Wrocław, Poland

#### Shizuka Yamada

Department of Cariology Nagasaki University Nagasaki, Japan

#### Daiki Yamamoto

Department of Life and Environmental System Science Yokohama City University Yokohama, Japan

#### Takeshi Yamamoto

Tobacco Science Research Center Japan Tobacco Inc. Kanagawa, Japan

#### Kajiro Yanagiguchi

Department of Cariology Nagasaki University Nagasaki, Japan

#### Chuanjin Yu

School of Agriculture and Biology Shanghai Jiao Tong University Minhang, Shanghai, People's Republic of China

### chapter eighteen

# *Agar-abundant marine carbohydrate from seaweeds in Indonesia*

Production, bioactivity, and utilization

#### Syamdidi, Hari Eko Irianto and Giyatmi Irianto

#### Contents

18.1 Introduction	
18.2 Agar industries in Indonesia	
18.2.1 Agar source	
18.2.2 Seaweed harvesting	
18.2.3 Agar extraction	
18.2.4 Agar quality	
18.3 Bioactivity of agar	
18.3.1 Anti-inflammatory	
18.3.2 Contraception activity	
18.3.3 Antioxidant	
18.3.4 Gastrointestinal and cardivascular effects	
18.3.5 Antibiotic activity	
18.3.6 Antiviral activity	
18.4 Agar utilization	
18.5 Conclusions	
References	

#### 18.1 Introduction

Agar is one of the marine carbohydrates and, as a polysaccharide complex, obtained through bleaching and hot water extraction of agarocytes from the red alga Rhodophyceae. There are two genera of seaweeds used as agar sources for agar industries in Indonesia: Gelidium and Gracilaria. In general, Gelidium, Acanthopeltis, Ceramium, Pterocladia, and Gracilaria are predominant raw materials in agar production in the world. Agar consists of about 70% agarose and 30% agaropectin (Scott and Eagleson 1998). Agarose is a neutral gelling fraction, which consists of a linear polymer of alternating D-galactose and 3,6-anhydrogalactose units. Agaropectin is a non-gelling fraction, which consists of 1,3 glycosidically linked D-galactose units, some of which are sulfated at position 6. Chemical structures of agarose and agaropectin are shown in Figure 18.1.

Seaweed is abundant in Indonesia, of which around 555 species have been already identified. About 21 species have been utilized as raw material by seaweed processing industries (Aslan 1991). Tremendous development of seaweed farming during the past 5 years has brought about the situation in which the seaweed production of Indonesia (in volume) is higher than in the Philippines; the production volume in 2013 reached 9,298,474 tons (Ministry of Marine Affairs and Fisheries 2014). Most of them are mainly *Eucheuma* sp. and *Gracilaria* sp.

Red algae are the most popular seaweeds that are used for food, pharmaceutical, and other industries. From 17 genera of red algae, 34 species have already been used for various purposes. Moreover, 23 species are able to be cultured: 6 species from the genus *Eucheuma*, 3 species from *Gelidium*, 10 species from *Gracillaria*, and 4 species from *Hypnea*. The genus *Eucheuma*, *Gracilaria*, and *Gelidium* are commonly found and cultured in Indonesian waters. However, only the genera producing agar (agarophytes) and carrageenan (carrageenophytes) are commercially cultured to support the seaweed industry in Indonesia and also to fulfill worldwide demand (Kordi 2011).



*Figure 18.1* Structure of agarose (1,4)-3,6 anhydro L-galactose, (1,3) D-galactose, and agaropectin. (From Ramadhan, W., Utilization of agar powder as texturizer in guava (*Psidium guajava* L.) spread sheet and shelf-life prediction, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia, 2011. With permission.)

Many countries have developed methods of agar processing, but only two methods are widely used: the freeze-thaw method, and the pressing method. Indonesia is currently the largest agar producer in the world. Agar produced by Indonesian processing factories is not only used for domestic consumption but also export to several countries, for example, China, Japan, and European countries.

Several investigations have been conducted by researchers all over the world to reveal the health benefits of agar, the findings of which are expected to guide into new prospective utilization. Some research has shown that the bioprospecting substances of agar have the potential to alleviate health problems. Therefore, new value-added products based on those findings are expected to be developed in the future, and Indonesia is poised to play an important role due to the abundance resources of agar-producing seaweeds as raw material.

#### 18.2 Agar industries in Indonesia

World trade of agar in terms of raw material of agar and finished products shows an increasing trend. The world demand was estimated to be 10,000 tons/year of raw material and 3,500 tons/year of finished products (Directorate General of Aquaculture 2005). Indonesia can develop the agar industry in three directions: as a raw material supplier, an agar producer, or both. Agar production looks more strategic for Indonesia, because the industry can contribute to the welfare of people by providing them job opportunities.

#### Marine Glycobiology: Principles and Applications

Agar industry is growing rapidly in Indonesia. Production of agar in Indonesia started before the World War II. In 1930, the first agar factory was established in Kudus, Central Java. This was followed by others in Jakarta, Surabaya, and Makassar (Sulistyo 2002). PT Sinar Kencana, an agar factory in Surabaya, East Java, was founded in 1947. In 1955, there were five companies operating with a total production around 13.7 tons per annum (tpa). After four decades, 10 new agar companies were established with the total production reaching 108.7 tpa. Thus the number of agar producing companies became 15 in 1993 (Zatnika 1997) but currently only 11 companies are existing. One of them is the largest agar company in the world with total production of over 3000 tpa.

#### 18.2.1 Agar source

Red algae genera such as Gelidium, Gracilaria, Grateloupia, Halymenia, Hypnea, and Porphyra (Table 18.1) are used as raw material in agar production in Indonesia. They are naturally available throughout the Indonesian waters. Currently, the demand of Gracilaria has grown to supply raw material for agar processing because of the increasing demand of agar and for establishing new agar factories. Seaweed from the wild cannot fulfill raw material need of the agar industry, which has led to the exploration of another source of raw material, farmed seaweeds. Culturing Gracilaria was developed later on around the year 1980 to ensure continuous supply of raw material for the agar industry. Gracilaria is now widely cultured by the farmers along the coast, particularly in Sulawesi and Java. One of the biggest Gracilaria culture ponds is located in Tangerang, Banten Province.

#### 18.2.2 Seaweed harvesting

Seaweed is normally harvested after 45 days if cultured in the beach but after 60–75 days if cultured in ponds (Kordi 2011). The Directorate General of Aquaculture (2005) suggested that *Gracilaria* is harvested after 90 days of cultivation, and the next harvesting can be performed after 60 days. Harvesting should be performed at the right age to obtain the optimum yield of agar during processing. Harvesting age also affects the quality of the extracted agar, in which underage seaweed will result in a lower agar quality.

Harvested *Gracilaria* is then washed with fresh water to free it from unwanted materials including other seaweed species. Seaweeds are subsequently sun-dried by placing them on drying racks, mats, or floors. The drying step should not be delayed so as to avoid deterioration due to fermentation. Drying can take 3–4 days till achieving a moisture content of about 25% as required by the market. While waiting for marketing, dried Chapter eighteen: Agar-abundant marine carbohydrate from seaweeds in Indonesia

0
Location in Indonesia
Alor Islands, Tanimbar Islands, Maluku Islands
Scattered across Indonesian coast
Bengkulu, Lampung, South part of Java Island, West Nusa Tenggara Islands
Scattered across Indonesian coast
West Sumbawa, Sawu Island, South Sulawesi, Southeast Sulawesi
South Lampung, South Java Island, Southeast Celebes, South and Southeast Moluccas
Scattered across Indonesian coast
Scattered across Indonesian coast
Riau Islands, Bangka and Belitung Islands, Lampung
West and South Java Island, South Lampung, Seribu Islands
South and Southeast Celebes, Ambon and Seram Islands, Papua, East Nusa Tenggara, Lombok, Sumbawa and Halmahera
Riau Islands, Bali, Tawi-tawi
Riau Islands, Moluccas Islands
Scattered across Indonesian coast
Halmahera Island and Kei Island

Table 18.1 Seaweeds used as agar source in Indonesia

Source: Anggadiredja, J.T. et al., Seaweed: Culturing, Processing and Marketing Potential Fisheries Commodity, Penebar Swadaya, Jakarta, Indonesia, 2006.

seaweed is stored for a certain period. According to Rodarte et al. (2010), the agar content of *Gracilaria* from the tropics decreases in a few months because of hydrolisis. The hydrolysis in *Gracilaria* could be due to the presence of agarolytic bacteria, of which the most important is *Bacillus cereus*, and to the presence of the algae's own agarolytic enzyme. Storage of *Gracilaria cornea* for 2 years and *Gracilaria eucheumatoides* for 1 year resulted in a reduction of a gel strength of 17% and 35%, respectively.

#### 18.2.3 Agar extraction

In Indonesia, there are three types of agar products in the market: agar sheet, agar bar, and agar powder. Basically, the extraction methods used to make those products are similar.

Agar is extracted from *Gracilaria* by means of two steps of cooking using water, in which the ratio of dried seaweed and water is approximately 1:20. The first cooking is carried out with the dried seaweed and an agar/water ratio of 1:14 at 85°C–95°C and pH 6–7 for 2 h. The agar extract and seaweed pulp are separated using fabrics. The seaweed pulp obtained is boiled again for the second cooking with the dried seaweed and with a water ratio of 1:6 for 1 h. The agar extract is then added with 2%–3% KOH or KCl for gel formation (Directorate General of Aquaculture 2005). The gel undergoes further processing for producing agar sheet, agar bar, or agar powder by applying specific treatments for each product.

Mostly, farmed *Gracilaria* produces a soft-textured gel resulting in difficulties for further processing,

particularly for industries operated on a small scale. To improve the gel properties, the farmed *Gracilaria* is mixed with that harvested from the nature before agar extraction (Aji et al. 2003).

Some authors (Rao and Bekheet 1976; Chapman and Chapman 1980; Robello et al. 1997; Montano et al. 1999) have found that soaking the seaweed in an alkali solution can improve the gel strength of agar. The chemical structures of the agar precursor in seaweed and of the idealized agar after alkali treatment are shown in Figure 18.2. Kusuma et al. (2013) have shown that the concentration of NaOH affected the gel strength, sulfate content, ash content, moisture content, and yield



*Figure 18.2* Chemical structure of agar. (a) Idealized agar. (b) Agar precursor prior to extraction (From Distantina, S., et al., *Jurnal Rekayasa Proses*, 2, 11, 2008. With permission.)

of agar *G. verrucosa*. The higher the concentration of NaOH, the higher the gel strength, the ash content, the water content, and the yield, but the lower the sulfate levels. The best agar quality was obtained using 6% NaOH solution. Distantina et al. (2008) and Van et al. (2008) noted that soaking seaweeds in an alkali solution resulted in lower extraction rate and lower yield, but higher gel strength compared to soaking in an acid solution.

#### 18.2.4 Agar quality

The quality of Indonesian agar is regulated under National Standardization Board. This institution regulates the specification of agar for both safety and wholesomeness (Table 18.2). Most agar industries in Indonesia have no problem meeting the standards required by the Board. Moreover, they usually sell the products above requirement standard, especially for moisture content, which ranges from 10% to 15%. International standards

Table 18.2 Indonesian agar specification

Parameter	Unit	Requirement
Sensory		Min. 7 (1–9 scale)
Chemical quality		
Moisture content	%	Max. 22
Ash content <sup>b</sup>	%	Max. 6.5
Acid-insoluble ash <sup>b</sup>	%	Max. 0.5
Starch <sup>b</sup>	_	Negative
Gelatin and protein <sup>b</sup>	—	Negative
Microbial quality		
TPC	Colony/g	Max. 5000
Escherichia coli	APM/g	<3
Salmonella	Per 25 g	Negative
Yeast and moulds	Colony/g	Max. 300
Heavy metals <sup>b</sup>		
Arsenic (As)	mg/kg	Max. 3
Cadmium (Cd)	mg/kg	Max. 1
Mercury (Hg)	mg/kg	Max. 1
Lead (Pb)	mg/kg	Max. 3
Zinc (Zn)	mg/kg	Max. 40
Physic <sup>b</sup>		
Water absorption	—	Min. five times
Foreign insoluble matter	%	Max. 1
Particle size (pass 60 mesh size)	%	Min. 80

Source: National Standardization Board, SNI Agar Powder, BSN, Jakarta, Indonesia, 2014.

<sup>a</sup> For each sensory attribute.

<sup>b</sup> If only requested.

published by, for example, by the EU (European Union) and JECFA (The Joint FAO/WHO Expert Committee on Food Additives) are quite similar to the Indonesian standard. However, in industrial trading, moisture content, color, and odor are the most demanding parameters. Other parameters are conditional, depending on the buyer's requirements.

#### 18.3 Bioactivity of agar

Studies have been carried out to reveal the bioactivity of agar-producing seaweeds, not specifically agar itself. Some species of red algae were reported to exhibit a broad spectrum of biological activities. The bioactivity of the most common agar seaweeds in Indonesia—from genus *Gracilaria* and *Gelidium*—has been examined. Almeida et al. (2011) have excellently reviewed the bioactivity of *Gracilaria* from some studies that highlight the potential pharmaceutical uses, particularly in terms of toxic, cytotoxic, spermicidal, anti-implantation, antibacterial, antiviral, antifungal, antiprotozoa, antihypertensive, antioxidant, anti-inflammatory, analgesic, and spasmolytic effects in the gastrointestinal tract.

#### 18.3.1 Anti-inflammatory

Anti-inflammatory activities have been reported for a sulfated polysaccharide fraction from *Gracilaria caudate* (Chavez et al. 2013), a galactan from *Gelidium crinale* (Sousa et al. 2013), and polysaccharide fractions from *G. verrucosa* (Yoshizawa et al. 1996). Sajiki (1997) reported that *G. verrucosa*, *G. asiatica*, *G. lichenoides* and other species contain PGE2, which produce physiological effects including hyperthermia, hypotension, smooth-muscle dilatation, hyperalgesia, and gastric secretion inhibition (Minghetti and Levi 1998).

#### 18.3.2 *Contraception activity*

A methanol/methylene chloride (1:1) extract from *G. corticata* exhibited post-coital contraceptive activity due to enhanced pre-implantation without any marked side effects when introduced orally to female rats. This shows that red marine algae are a potential source for post-coital contraceptive drugs. Extracts from *G. edulis* demonstrated 100% inhibition of sperm motility, and this effect was related to the disruption of the plasma membrane by spermicidal compounds (Almeida et al. 2011).

#### 18.3.3 Antioxidant

The antioxidant activity of *Gracilaria* is related to the anti-inflammatory effects. Swantara and Parwata (2011) found that *G. coronopifolia* has 90.27% antioxidant activity consisting of 1-nonadecane, hexadecanoic acid,

9-octadecanoic acid, cholest-4,6-dien-3-ol, cholest-5en-3 $\beta$ -ol (cholesterol), and cholest-4-en-3-one. Murugan and Iyer (2012) noted that *G. edulis* possesses antioxidant activity.

#### 18.3.4 Gastrointestinal and cardivascular effects

Aqueous extract from dried *G. verrucosa* or fresh *G. chorda* at a dose of 0.5 mg per animal was able to control gastrointestinal disorders in mice, the active compounds being zeaxanthin, antheraxanthin, carotenoids, pyrimidine 2-amino-4-carboxy, non-alkaloid nitrogen heterocycle, steroids, 5- $\alpha$ -poriferastane, 3- $\beta$ -6- $\alpha$ -diol poriferastane, 5- $\alpha$ -3- $\beta$ -6- $\beta$ -diol, and gigatinine. Meanwhile, a 90% ethanol extract from *G. edulis* showed diuretic activity, and an aqueous extract from *G. lichenoides* administered intravenously showed antihypertensive effect in rats (Almeida et al. 2011).

#### 18.3.5 Antibiotic activity

*Gracilaria corticata* were found to be active against gram-positive cultures of *Bacillus* (Bhakuni and Rawat 2005). The authors also found that an extract of *Gelidium cartilagineum* and *Chondrus crispus* were active against influenza B and mumps virus. This activity has been attributed to the presence of a galactan unit in the polysaccharides, agar, and carrageenan present in both species.

Another genus of *Gracilaria*—*Gracilaria* Greville contains a gelatinous nontoxic colloidal carbohydrate in the cell wall and intercellular spaces of the algae and has wide use in the preparation of food, ice creams, jellies, soups, bacteriological samples, and cosmetics (Gosh et al. 2012; Kerton et al. 2013). They are sources of important bioactive metabolites with antibiotic activity (Smit 2004).

A chloroform extract of G. edulis (Gmelin) Silva was found to have antibacterial activity against some bacterial strains, in which the isolated steroids (carotenoids,  $\beta$ -cryptoxanthin, and  $\beta$ -carotene) and carbohydrates are suspected as the active compounds. An ethanol extract from G. debilis showed antibacterial activity against Staphylococcus aureus. A 95% ethanol extract from whole dried G. cervicornisalgae was active against S. aureus at a concentration of 5.0 mg/mL. A methanol extract from fresh G. corticata was active against Bacillus subtilis, B. megaterium, S. aureus, and Streptococcus viridans. Ethanol extracts from G. domigensis and G. sjoestedii showed antibacterial activity against E. coli and S. aureus. Ethanol extracts from G. debilis, G. domingensis, and G. sjoestedti were active against Candida albicans. An ethanol extract from G. domigensis was active against Mycobacterium smegmatis and Neurospora crassa. G. domigensis has as chemical

constituents polysaccharide CT-1, palmitic acid, and steroids (stigmasterol, sitosterol, campesterol, cholest-7-en-3-β-ol, and brassicasterol) (Almeida et al. 2011).

#### 18.3.6 Antiviral activity

Extracts from *G. bursa-pastoris* and *Gracilaria* sp. were inactive against the herpes simplex 1 virus (HSV) and the human immunodeficiency virus (HIV) when evaluated in cell cultures. Granin BP and citrullinylarginine proteins were isolated from these extracts. A methanol extract from dried *G. pacifica* at a concentration of 200.0 µg/mL was active against Sindbis virus, but was not effective against HSV when tested at a concentration of 400 µg/mL. Extracts and compounds obtained from *Gracilaria* sp. with anti-HIV activity are also active against other retroviruses such as HSV (Almeida et al. 2011).

#### 18.4 Agar utilization

The many uses of agar and agarose are related to the formation of thermoreversible gels at low concentration in water with large hysteresis. The physicochemical and rheological properties of these algal polysaccharides are linked to their chemical structure (Lahaye and Rochas 1991). Agar has been in use as a source of food in some Asian countries for a long time. Most of agar utilization is for food (~80%–90%) and the rest is used for biotechnological applications (FAO 2003; Armisen and Galatas 2009). Based on their types, agar was divided into natural agar and industrial agar. Natural agar was produced by traditional method while the industrial agar using freezing-defreezing techniques by artificial freezing, or accelerated synaeresis through pressure (Armisen 1995) (Table 18.3).

Agar is used as a thickening agent and also to improve the texture of processed food such as jellies, dairy products, fruit pastilles, chewing gum, canned meats, soups, confectionery, and baked goods and icings, as well as frozen and salted fish (Armisen and Galatas 2009). Its neutral taste and high fiber content make agar superior to other hydrocolloid additives. These properties have led to broadening the application of agar to many food products. Agar is generally safe for human consumption. According to the U.S. Food and Drug Administration (FDA), agar is classified as GRAS (Generally Recognized as Safe), as an E406 additive by the European Commission, and is registered as 9002-18-0 in the Register Service of the Chemical Abstracts (Armisen and Galatas 2009).

In future, agar is expected to be widely used in biotechnology application because of its benefits. About 10% of the production is classified as too low for a GRAS product. Agar consists of agarose, which is a

	Agar type applications	Type of seaweed
Natural agar	"Strip"	Produced mostly with <i>Gelidium</i> by traditional methods
	"Square": accustomed only in Far East traditional kitchen	
Industrial agar	Food-grade agar used for industrial food production	Gelidium, Gracilaria, Gracilariopsis, Pterocladia, Ahnfeltia, Gelidiella
	Pharmacological agar	Gelidium
	Clonic plants production grade	Gelidium or Pterocladia
	Bacteriological grade used for bacteriological culture media formulation	Gelidium or Pterocladia
	Purified agar used in biochemistry and in culture media for very difficult bacteria	Gelidium

Table 18.3 Agar grades, their application and plant origins

Source: Armisen, R., J. Appl. Phycol., 7, 231, 1995. With permission.

neutral polysaccharide, and agaropectin, which is polysaccharide sulfate. As a gelling agent, agar is widely used in the food, pharmaceutical, and cosmetics industries. Agarose is widely used in the field of biotechnology, both as a culture medium and an electrophoresis medium. Pure agarose is one of main ingredients of microbial and plant culture media (Istini et al. 2001).

The possible use of agar in food application is related to its properties: its gel-forming capability and its unique, reversible gelling performance. In the food industry, agar mostly used as gelling agent for bread, jelly, candies, dairy products, and ice cream. The food industry has been growing fast during the last decades in line with the increasing world population. Food trend nowadays is changing to healthy products that are low in fat but high in fiber. Agar contains high fiber, which can be used in food fortification to increase health benefit due to its indigestibility by the human metabolic system. Compared to carrageenan, agar is composed of soluble fibers since its content is above 94%, which is higher than that of carrageenan (67%-80%) (Armisen and Galatas 2009). Murata and Nakazoe (2001) found that agar consumption led to a decrease in the concentration of blood glucose and caused an anti-aggregation effect on red blood cells. In the beverage industry, agar is used in beer, coffee, and wine purifying process and also as an emulsifier for chocolate products. Agar with a concentration 0.1%-1% can be used as an emulsifier for some products such as yoghurt, cheese, jelly, and bakery products.

Agar consists of two fractions: agarose and agaropectin (Armisen and Galatas 1987). Agarose has low sulfate content and hence mostly used for biotechnology application (Wahyuni 2003). Agarose is used as an ingredient of making gels for electrophoresis of protein isolation and purification. In addition, agarose is used in chromatography columns, which has been commercialized production with brand Sepharose (Pharmacia) and Bio-Gel A (Bio-Rad). Agarose also has been widely used in up to 250,000 Da for particle separation as well as for virus, protein, and chromosome separation (Wahyuni 2003).

#### 18.5 Conclusions

Indonesia has abundance resources for marine carbohydrate production, especially red algae as a source of agar. However, the existing utilization of those resources is mainly for the production of conventional food products, namely agar powder. Recent exploration has revealed other benefits of agar, which is actually not only to fulfill human consumption needs but also to overcome some health problems based on the bioactive substances contained in agar-producing seaweeds. A new use for agar by taking advantage of its bioactive property is to generate products with significantly higher added value instead of producing conventional products. It is expected that the high added value can be realized by all stakeholders involved in the agar industry, including seaweed farmers whose role is often neglected. This way, the goals of the Indonesian government of improving the national welfare and alleviating poverty through seaweed development can be achieved as planned.

#### References

Aji, N., Ariyani, F., and Suryaningrum, T.D. 2003. Technology of seaweed utilization. Research Center for Marine and Fisheries Product Processing and Socioeconomic: Jakarta, Indonesia (in Indonesian).

- Almeida, C.L.F., Falcão, H., Lima, G.R.M., Montenegro, C.A, Lira, N.S., and Athayde-Filho, P.F. 2011. Bioactivities from marine algae of the genus *Gracilaria*. *International Journal of Molecular Science*, 12: 4550–4573.
- Anggadiredja, J.T., Zatnika, A., Purwoto, H., and Istini, S. 2006. Seaweed: Culturing, Processing and Marketing Potential Fisheries Commodity. Penebar Swadaya: Jakarta, Indonesia (in Indonesian).
- Armisen, R.1995. Worldwide use and importance of Gracilaria. Journal of Applied Phycology, 7: 231–243.
- Armisen, R. and Galatas, F. 1987. Production, properties and uses of agar. In: *Production and Utilization of Products from Commercial Seaweeds*, McHugh, D.J. (ed.). FAO Fisheries Technical Paper. FAO: Rome, Italy.
- Armisen, R. and Galatas, F. 2009. Agar. In: Handbook of Hydrocolloids, Williams, P.A. and Phillips, G.O. (eds.). Woodhead Publishing: Cambridge, U.K., pp. 807–828.
- Aslan, M.L. 1991. *Seaweed Cultures*. Kanisius Publishing, Yogyakarta, Indonesia, pp. 11–34 (in Indonesian).
- Bhakuni, D.S. and Rawat, D.S. 2005. *Bioactive Marine Natural Products.* Anamaya Publishers: New Delhi, India.
- Chapman, V.J. and Chapman, C.J. 1980. Seaweed and Their Uses, 3rd edn. Chapman and Hall Ltd.: London, U.K., pp. 148–193.
- Chaves Lde, S., Nicolau, L.A., Silva, R.O., Barros, F.C., Freitas, A.L., Aragao, K.S., Ribeiro Rde, A., Souza, M.H., Barbosa, A.L., and Medeiros, J.V. 2013. Anti-inflammatory and antinociceptive effects in mice of a sulfated polysaccharide fraction extracted from the marine red algae *Gracilaria caudata*. *Immunopharmacology and Immunotoxicology*, 35: 93–100.
- Directorate General of Aquaculture. 2005. Seaweed profiles of Indonesia. Directorate of Aquaculture, Ministry for Marine Affairs and Fisheries: Jakarta, Indonesia (in Indonesian).
- Distantina, S., Anggraeni, D.R., and Fitri, L.E. 2008. Effects of concentration and kind of soaking solution on extraction rate and gel properties of agar from *Gracilaria verrucosa*. *Jurnal Rekayasa Proses*, 2(1): 11–16 (in Indonesian).
- FAO. 2003. A guide to the seaweed industry. FAO Fisheries Technical Paper 441. Retrieved September 24, 2014 from http://www.fao.org/docrep/006/y4765e/y4765e00.htm# Contents.
- Ghosh, R., Banerjee, K., and Mitra, A. 2012. Eco-biochemical studies of common seaweeds in the lower Gangetic Delta. In: *Handbook of Marine Macroalgae: Biotechnology* and Applied Phycology, Se-Kwon, K. (ed.), 1st edn. John Wiley & Sons, Ltd.: New Delhi, India, pp. 45–57.
- Istini, S., Abraham, S., and Zatnika, A. 2001. Purification process of agar from *Gracilaria* sp. *Jurnal Sains dan Teknologi Indonesia*, 3(9): 89–93 (in Indonesian).
- Kerton, F.M., Liu, Y., Omaria, K.W., and Hawboldt, K. 2013. Green chemistry and the ocean-based biorefinery. *Green Chemistry*, 15: 860–871.
- Kordi, M.G.H. 2011. *The Secret of Seaweed Culture in Bay and Pond*. Lily Publisher: Jakarta, Indonesia (in Indonesian).
- Kusuma, W.I., Santosa, G.W., and Pramesti, R. 2013. Pengaruh Konsentrasi NaOH yang Berbeda Terhadaap Mutu Agar Rumput Laut Gracilaria verrucosa. Journal of Marine Research, 2(2): 120–129 (in Indonesian).

- Lahaye, M. and Rochas, C. 1991. Chemical structure and physico-chemical properties of agar. *Hydrobiologia*, 221: 137–148.
- Minghetti, L. and Levi, G. 1998. Microglia as effector cells in brain damage and repair: Focus on prostanoids and nitric oxide. *Progress in Neurobiology*, 54: 99–125.
- Ministry of Marine Affairs and Fisheries. 2014. Marine and fisheries statistics 2013. Center of Data Statistics and Information: Jakarta, Indonesia (in Indonesian).
- Montano, N.E., Villanueva, R.D., and Romero, J.B. 1999. Chemical characteristic and gelling properties of agar from two Philippine *Glacilaria* spp. (Glacilariales, Rhodophyta), *Journal of Applied Phycology*, 11: 27–34.
- Murata, M. and Nakazoe, J. 2001. Production and use of marine algae in Japan. Japan Agriculture Research Quarterly, 35: 281–290.
- Murugan, K. and Iyer, V.V. 2012. Antioxidant and antiproliferative activities of marine algae, *Gracilaria edulis* and *Enteromorpha lingulata*, from Chennai Coast. *International Journal of Cancer Research*, 8: 15–26.
- National Standardization Board. 2014. SNI Agar Powder. BSN, Jakarta, Indonesia (in Indonesian).
- Ramadhan, W. 2011. Utilization of agar powder as texturizer in guava (*Psidium guajava* L.) spread sheet and shelflife prediction. Faculty of Fisheries and Marine Science, Bogor Agricultural University: Bogor, Indonesia (in Indonesian).
- Rao, A.V. and Bekheet, I.A. 1976. Preparation of agar—Agar from the red seaweed *Pterocladia capillacea* of the Coast of Alexandria, Egypt. *Applied Environmental Microbiology*, 32(4): 479–482.
- Robello, J., Ohno, M., Ukeda, H., and Sawamura, M. 1997. Agar quality of commercial agarophytes from different geographical origins: 1. Physical and rheological properties. *Journal of Applied Phycology*, 8: 517–521.
- Rodarte, M.A.V., Carmona, G.H., Montesinos, Y.E.R., Higuera, D.L.A., Rodríguez, R.R., and Álvarez, J.I.M. 2010. Seasonal variation of agar from *Gracilaria vermiculophylla*, effect of alkali treatment time, and stability of its Colagar. *Journal of Applied Phycology*, 22: 753–759.
- Sajiki, J. 1997. Effect of acetic acid treatment on the concentrations of arachidonic acid and prostaglandin E2 in the red algae, *Gracilaria asiatica* and *G. rhodocaudata*. *Fish Science*, 63: 128–131.
- Scott, T. and Eagleson, M. 1988. *Concise Encyclopedia: Biochemistry*, 2nd edn. Walter de Gruyter: New York p. 18.
- Smit, A.J. 2004. Medicinal and pharmaceutical uses of seaweed natural products: A review. *Journal of Applied Phycology*, 16: 245–262.
- Sousa, A.A., Benevides, N.M., de Freitas Pires, A., Fiuza, F.P., Queiroz, M.G., Morais, T.M., Pereira, M.G., and Assreuy, A.M. 2013. A report of a galactan from marine alga *Gelidium crinale* with in vivo anti-inflammatory and antinociceptive effects. *Fundamental and Clinical Pharmacology*, 27: 173–180.
- Sulistijo. 2002. Research on seaweed culture in Indonesia. Paper presented at the Speech on Research Professor Inauguration. LIPI: Jakarta, Indonesia (in Indonesian).

- Swantara, I.M.D and Parwata, I.M.O.A. 2011. Study on Antioxidant Compound of Seaweed from Bali Coast. The excellent research University of Udayana, Denpasar, Indonesia, pp. 89–96 (in Indonesian).
- Van, T.T.T., Ly, B.M., Buu, N.Q., and Kinh, C.D. 2008. Structural characterization of agar extracted from six red seaweed species growing in the coast of Vietnam. *Asean Journal* on Science and Technology for Development, 25(2): 395–403.
- Wahyuni, M. 2003. Biotechnology in Fisheries Product Processing. Institut Pertanian Bogor: Bogor, Indonesia (in Indonesian).
- Yoshizawa, Y., Tsunehiro, J., Nomura, K., Itoh, M., Fukui, F., Ametani, A., and Kaminogawa, S. 1996. In vivo macrophage-stimulation activity of the enzyme-degraded water-soluble polysaccharide fraction from a marine alga (*Gracilaria verrucosa*). *Bioscience Biotechnology Biochemistry*, 60: 1667–1671.
- Zatnika A. 1997. *The Profile of Indonesian Seaweed Industry.* Seaweed team of BPPT, BPPT: Jakarta, Indonesia (in Indonesian).