Springer Handbook of Marine Biotechnology

Kim
Editor

Springer

Springer Handbook of Marine Biotechnology

Springer Handbooks provide a concise compilation of approved key information on methods of research, general principles, and functional relationships in physical and applied sciences. The world's leading experts in the fields of physics and engineering will be assigned by one or several renowned editors to write the chapters comprising each volume. The content is selected by these experts from Springer sources (books, journals, online content) and other systematic and approved recent publications of scientific and technical information.

The volumes are designed to be useful as readable desk reference book to give a fast and comprehensive overview and easy retrieval of essential reliable key information, including tables, graphs, and bibliographies. References to extensive sources are provided.

Springer Handbook of Marine Biotechnology

Se-Kwon Kim (Ed.)

With 580 Figures and 181 Tables

Springer

Editor
Se-Kwon Kim
Pukyong National University
Department of Marine-Bio. Convergence Science
Specialized Graduate School Science & Technology Convergence
Marine Bioprocess Research Center
Busan, Korea

ISBN: 978-3-642-53970-1 e-ISBN: 978-3-642-53971-8
DOI 10.1007/978-3-642-53971-8
Springer Dordrecht Heidelberg London New York

Library of Congress Control Number: 2014956694

Production and typesetting: le-tex publishing services GmbH, Leipzig
Coordination Management: V. Hamm, Dr. W. Skolaut, Heidelberg
Typography and layout: schreiberVIS, Seeheim
Illustrations: Hippmann GbR, Schwarzenbruck
Cover design: eStudio Calamar Steinen, Barcelona
Cover production: WMXDesign GmbH, Heidelberg
Printing and binding: Printer Trento s.r.l., Trento

Printed on acid free paper

Springer is part of Springer Science+Business Media (www.springer.com)

List of Authors

Toshihiro Akaike
Tokyo Institute of Technology
Graduate School of Bioscience and Biotechnology
4259 Nagatsuta-cho, Midori-ku
226-8501, Yokohama, Japan
e-mail: *akaike@fais.or.jp*

Visamsetti Amarendra
SASTRA University
School of Chemical and Biotechnology
Thirumalaisamudram
613401, Thanjavur, India
e-mail: *amarendravissamsetty@yahoo.co.in*

Mugdha Apte
University of Pune
Institute of Bioinformatics and Biotechnology
Ganeshkhind Road
411007, Pune, India
e-mail: *mapte@unipune.ac.in*

Elena M. Balboa
University of Vigo
Department of Chemical Engineering
As Lagoas s/n
32004, Ourense, Spain
e-mail: *elenamba@uvigo.es*

Colin J. Barrow
Deakin University
Centre for Chemistry and Biotechnology, LES
75 Pigdons Road, Waurn Ponds
Victoria, 3217, Australia
e-mail: *cbarrow@deakin.edu.au*

Begoña Ben-Gigirey
Ministry of Health
EU Reference Laboratory for Marine Biotoxins
Fonte das Abelleiras
36310, Vigo, Spain
e-mail: *bbeng@msssi.es*

Francesca Beolchini
Polytechnic University of the Marche
Department of Life and Environmental Sciences
Via Brecce Bianche
60131, Ancona, Italy
e-mail: *f.beolchini@univpm.it*

A. Mir Bilal
YeungNam University
Department of Biotechnology
214-1 Dae dong
Gyeongsan, 712-749, Korea
e-mail: *biobilal14@ynu.ac.kr*

Nathan J. Bott
RMIT University
School of Applied Sciences
Bundoora, 3754, Australia
e-mail: *nathan.bott@rmit.edu.au*

Vincent Boursicot
Ifremer, Atlantic Center
Marine Resources and Biotechnology
Cedex 03
44311, Nantes, France
e-mail: *vboursicot@gmail.com*

Thiago Bruce
Faculdade de Tecnologia e Ciências (FTC)
Department of Bioenergy
Av. Luís Vianna Filho
8812, Salvador, Brazil
e-mail: *thbruce@gmail.com*

Dumitru Bulgariu
University Alexandru Ioan Cuza of Iasi
Department of Geology
Carol I, No. 20A
700506, Iasi, Romania
e-mail: *dbulgariu@yahoo.com*

Laura Bulgariu
Technical University Gheorghe Asachi of Iasi
Environmental Engineering and Management
D. Mangeron, No. 71A
700050, Iasi, Romania
e-mail: *lbulg@tuiasi.ro*

Ana G. Cabado
ANFACO-CECOPESCA
Food Quality and Safety
Col Univ 16
36310, Vigo-Po, Spain
e-mail: *agcabado@anfaco.es*

Ricardo Calado
University of Aveiro
Campus Universitário de Santiago
Department of Biology
3810-193, Aveiro, Portugal
e-mail: *rjcalado@ua.pt*

Ana P. Carvalho
Universidade Católica Portuguesa
Escola Superior de Biotecnologia
Rua Dr. António Bernardino de Almeida
4200-072, Porto, Portugal
e-mail: *ana.p.santos.carvalho@gmail.com*

Hyung J. Cha
Pohang University of Science and Technology
Department of Chemical Engineering
Pohang, 790-784, Korea
e-mail: *hjcha@postech.ac.kr*

Pranjal Chandra
Amity University Uttar Pradesh
Amity Institute of Biotechnology
Sector 125
Noida, India
e-mail: *pranjalmicro13@gmail.com*

María José Chapela
ANFACO-CECOPESCA
Research & Development
Col Univ 16
36310, Vigo-Po, Spain
e-mail: *mjchapela@anfaco.es*

Jyh-Yih Chen
Academia Sinica
Institute of Cellular and Organismic Biology,
Marine Research Station
Dahuen Road
Ilan, Taiwan
e-mail: *zoocjy@gate.sinica.edu.tw*

Maria J. Chen
University of Connecticut
Department of Molecular and Cell Biology
91 N. Eagleville Rd.
Storrs, CT, 06269, USA
e-mail: *maria.chen@uconn.edu*

Mark H.-C. Chen
National Taiwan University College of Medicine
Department of Orthopedics
No. 1, Jjen Ai. Road Section 1
10002, Taipei, Taiwan
e-mail: *blackfishgenome@me.com*

Thomas T. Chen
University of Connecticut
Department of Molecular and Cell Biology
91 N. Eagleville Rd.
Storrs, CT, 06269, USA
e-mail: *thomas.chen@uconn.edu*

Hogyun Cheong
Pohang University of Science and Technology
Department of Chemical Engineering
Pohang, 790-784, Korea
e-mail: *hcheong2@postech.ac.kr*

Pinwen P. Chiou
Academia Sinica
Institute of Cellular and Organismic Biology,
Marine Research Station
23-10 Dawun Road
26242, Yilan, Taiwan
e-mail: *ppchiou@gate.sinica.edu.tw*

Chong-Su Cho
Seoul National University
Research Institute for Agriculture and Life Sciences
Seoul, 151-921, Korea
e-mail: *chocs@snu.ac.kr*

MyoungLae Cho
Korea Institute of Ocean Science and Technology
(KIOST)
East Sea Research Institute
48 Haeyang Gwahak-gil
Uljin, Gyeongbuk, 767-813, Korea
e-mail: *mlcho@kiost.ac.kr*

Bong-Hyuk Choi
Pohang University of Science and Technology
Department of Chemical Engineering
Pohang, 790-784, Korea
e-mail: *bbongs@postech.ac.kr*

Inho Choi
YeungNam University
Department of Biotechnology
214-1 Dae dong
Gyeongsan, 712-749, Korea
e-mail: *inhochoi@ynu.ac.kr*

Yun-Jaie Choi
Seoul National University
Department of Agricultural Biotechnology
Gwanak-ro
Seoul, 151-921, Korea
e-mail: *cyjcow@snu.ac.kr*

Sylvia Colliec-Jouault
Ifremer, Atlantic Center
Marine Resources and Biotechnology
Cedex 03
44311, Nantes, France
e-mail: *Sylvia.Colliec.Jouault@ifremer.fr*

Enma Conde
GLECEX Global and Ecofriendly
Natural Extracts, S.L.
Edificio CEI
Parque Tecnológico de Galicia, s/n
32900, San Cibrao das Viñas, Spain
e-mail: *econde@glecex.com*

Jana Čopíková
Institute of Chemical Technology in Prague
Department of Carbohydrates and Cereals
Technicka 5
166 28, Prague, Czech Republic
e-mail: *copikovj@vscht.cz*

Vernon E. Coyne
University of Cape Town
Molecular and Cell Biology Department
7700, Rondebosch, South Africa
e-mail: *vernon.coyne@uct.ac.za*

Jing Dai
Auburn University
Mechanical Department
Auburn, AL 36849, USA
e-mail: *JZD0011@auburn.edu*

Christine Delbarre-Ladrat
Ifremer, Atlantic Center
Marine Resources and Biotechnology
Cedex 03
44311, Nantes, France
e-mail: *Christine.Delbarre.Ladrat@ifremer.fr*

Hebe M. Dionisi
Patagonian National Research Center
(CENPAT-CONICET)
Environmental Microbiology Laboratory
Bvd. Brown 2915
Puerto Madryn, Chubut, Argentina
e-mail: *hdionisi@cenpat.edu.ar*

Alan D. W. Dobson
University College Cork
Marine Biotechnology Centre,
Environmental Research Institute
Cork, Ireland
e-mail: *a.dobson@ucc.ie*

Herminia Domínguez
University of Vigo
Department of Chemical Engineering
As Lagoas s/n
32004, Ourense, Spain
e-mail: *herminia@uvigo.es*

Armando C. Duarte
University of Aveiro
Department of Chemistry
3810-193, Aveiro, Portugal
e-mail: *aduarte@ua.pt*

Sherif S. Ebada
Ain-Shams University
Faculty of Pharmacy,
Department of Pharmacognosy and
Phytochemistry
Cairo, Egypt
e-mail: *sherif_elsayed@pharma.asu.edu.eg*

Don A. S. Elvitigala
Jeju National University
Department of Marine Life Science
Jeju, 690-756, Korea
e-mail: *anushka@jejunu.ac.kr*

Astria D. Ferrão-Gonzales
Universidade do Estado da Bahia-UNEB
Departamento de Ciências da Vida
Rua Silveira Martins, 2555
BA 41150-000, Salvador, Brazil
e-mail: *agonzales@uneb.br*

Burkhardt Flemer
University College Cork
Alimentary Pharmabiotic Centre
College Road
Cork, Ireland
e-mail: *bflemer@gmail.com*

Ana C. Freitas
University of Aveiro
Department of Chemistry
3810-193, Aveiro, Portugal
e-mail: *afreitas@ua.pt*

Alejandro Garrido-Maestu
ANFACO-CECOPESCA
Microbiology and Bioassays Laboratory
Col Univ 16
36310, Vigo-Po, Spain
e-mail: *a.garrido@anfaco.es*

Ille C. Gebeshuber
Universiti Kebangsaan Malaysia
Institute of Microengineering and
Nanoelectronics
43600, Bangi, Malaysia
and
Vienna University of Technology
Institute of Applied Physics
Wiedner Hauptstrasse 8-10/134
1040, Vienna, Austria
e-mail: *gebeshuber@iap.tuwien.ac.at*

Thandapani Gomathi
D.K.M. College for Women
Department of Chemistry
Sainathapuram
63200, Vellore, India
e-mail: *chemist.goms@gmail.com*

Ana M. Gomes
Universidade Católica Portuguesa
CBQF – Centro de Biotecnologia e Química Fina
Rua Dr. António Bernardino de Almeida
4200-072, Porto, Portugal
e-mail: *amgomes@porto.ucp.pt*

Hong-Yi Gong
National Taiwan Ocean University
Department of Aquaculture
No.2, Beining Road
202, Keelung City, Taiwan
e-mail: *hygong@mail.ntou.edu.tw*

Virginia González
ANFACO-CECOPESCA
Food Quality and Safety
Col Univ 16
36310, Vigo-Po, Spain
e-mail: *virginia@anfaco.es*

Tanja Grkovic
Griffith University
Eskitis Institute for Drug Discovery
Brisbane Innovation Park, Don Young Road
Nathan, QLD 4111, Australia
e-mail: *t.grkovic@griffith.edu.au*

Adarsha Gupta
Deakin University
Centre for Chemistry and Biotechnology, LES
75 Pigdons Road, Waurn Ponds
Victoria, 3217, Australia
e-mail: *agupt@deakin.edu.au*

Soumya Haldar
CSIR-Central Salt & Marine Chemicals Research
Institute
Discipline of Marine Biotechnology & Ecology
Gijubhai Badheka Marg
364002, Bhavnagar, India
e-mail: *shaldar@csmcri.org*

Morgan Hamon
Auburn University
Department of Mechanical Engineering
275 Wilmore Labs
Auburn, AL 36849, USA

S.W.A. Himaya
Pukyong National University
Marine Bio-Process Research Center
Busan, 608-737, Korea
e-mail: *himayaswa@yahoo.com*

Jong W. Hong
Auburn University
Department of Mechanical Engineering
275 Wilmore Labs
Auburn, AL 36849, USA
e-mail: *hongjon@auburn.edu*

Stephen A. Jackson
University College Cork
Department of Microbiology
Lee Road
Cork, Ireland
e-mail: *sjackson@ucc.ie*

Sachin Jambovane
Pacific Northwest National Laboratory
Environmental Molecular Sciences Laboratory
902 Battelle Boulevard
Richland, WA, 99352, USA

Venkateswara Rao Janapala
CSIR-Indian Institute of Chemical Technology
Biology Division
Tarnaka
500607, Hyderabad, India
e-mail: *jv@iict.res.in*

You-Jin Jeon
Jeju National University
Department of Marine Life Science
Jeju, 690-756, Korea
e-mail: *youjinj@jejunu.ac.kr*

Murugaiyan Kalaiselvam
Annamalai University
Centre of Advanced Study in Marine Biology,
Faculty of Marine Sciences
608502, Parangipettai, Tamil Nadu, India
e-mail: *kalaifms@gmail.com*

Vipin C. Kalia
CSIR-Institute of Genomics and Integrative Biology
Microbial Biotechnology and Genomics
110007, Delhi, India
e-mail: *vckalia@igib.res.in*

Valliappan Karuppiah
Shanghai Jiao Tong University
School of Life Sciences and Biotechnology
800 Dongchuan Road
200240, Shanghai, China
e-mail: *vkactinobacteria@gmail.com*

Jonathan Kennedy
University College Cork
Marine Biotechnology Centre, Environmental Research Institute
Cork, Ireland
e-mail: *jonathan.kennedy@ucc.ie*

Mohammad Kazem Khalesi
Sari University of Agricultural Sciences and Natural Resources (SANRU)
Department of Fishery Sciences
Sea Road
Sari, Iran
e-mail: *m.khalesi@sanru.ac.ir*

Samanta S. Khora
Vellore Institute of Technology University
School of Biosciences and Technology
632014, Vellore, India
e-mail: *sskhora@vit.ac.in*

Bum J. Kim
Pohang University of Science and Technology
Department of Chemical Engineering
Pohang, 790-784, Korea
e-mail: *yougio@postech.ac.kr*

Chang S. Kim
Pohang University of Science and Technology
Department of Chemical Engineering
Pohang, 790-784, Korea
e-mail: *redcskim@postech.ac.kr*

Se-Kwon Kim
Pukyong National University
Department of Marine-Bio. Convergence Science
Specialized Graduate School Science & Technology
Convergence Marine Bioprocess Research Center
Yongdang Campus, 365, Sinseon-ro, Nam-gu
Busan, 608-739, Korea
e-mail: *sknkim@pknu.ac.kr*

Woo J. Kim
The Catholic University of Korea
Research Center for Biopharmaceutical Lead Molecule (GRRC)
43 Jibong-ro, Wonmi-gu
Bucheon, 420-743, Korea
e-mail: *wj0504@catholic.ac.kr*

Ozcan Konur
Yildirim Beyazit University, Faculty of Engineering and Natural Sciences
Department of Materials Engineering
Cankiri Caddesi, Cicek Sokak No.3
Ankara, Turkey
e-mail: *okonur@hotmail.com*

Ameeta R. Kumar
University of Pune
Institute of Bioinformatics and Biotechnology
Ganeshkhind Road
411007, Pune, India
e-mail: *ameeta@unipune.ac.in*

Prasun Kumar
CSIR-Institute of Genomics and Integrative Biology
Microbial Biotechnology and Genomics
110007, Delhi, India
e-mail: *prasun.kumar@igib.in*

Frithjof C. Küpper
University of Aberdeen
School of Biological Sciences
Main Street
Newburgh, AB41 6AA, UK
e-mail: *fkuepper@abdn.ac.uk*

D. İpek Kurtböke
University of the Sunshine Coast
Faculty of Science, Health, Education and Engineering
Maroochydore DC, QLD 4558, Australia
e-mail: *ikurtbok@usc.edu.au*

Miguel Leal
University of Aveiro
Campus Universitário de Santiago Department of Biology
3810-193, Aveiro, Portugal
e-mail: *miguelcleal@gmail.com*

Smita S. Lele
Institute of Chemical Technology (Formerly UDCT/UICT)
Food Engineering and Technology
Nathalal Parekh Marg
400019, Mumbai, India
e-mail: *ss.lele@ictmumbai.edu.in*

Zhiyong Li
Shanghai Jiao Tong University
School of life Sciences and Biotechnology
800 Dongchuan Road, Minhang District
200240, Shanghai, China
e-mail: *zyli@sjtu.edu.cn*

Yue Liang
Tokyo University of Agriculture and Technology
Division of Biotechnology and Life Science, Institute of Engineering
2-24-16, Naka-cho, Koganei
184-8588, Tokyo, Japan
e-mail: *y_liang@cc.tuat.ac.jp*

Seonghye Lim
Hanwha Chemical Co.
Biologics Business
Daejeon, 305-804, Korea
e-mail: *seonghye.lim@hanwah.com*

Chun-Mean Lin
University of Connecticut
Department of Molecular and Cell Biology
91 N. Eagleville Rd.
Storrs, CT, 06269, USA
e-mail: *chun-mean.lin@uconn.edu*

Jay H. Lo
University of Connecticut
Department of Molecular and Cell Biology
91 N. Eagleville Rd.
Storrs, CT, 06269, USA
e-mail: *hung-chieh.lo@uconn.edu*

Lus Lopez
Universidad Autónoma de Baja California (UABC)
Facultad de Ciencias Marinas, Laboratorio de Nutrición Acuícola
Ensenada, B.C., Mexico
e-mail: *llopez@uabc.edu.mx*

Mariana Lozada
Patagonian National Research Center (CENPAT-CONICET)
Environmental Microbiology Laboratory
Bvd. Brown 2915
Puerto Madryn, Chubut, Argentina
e-mail: *lozada@cenpat.edu.ar*

Jenn-Kan Lu
National Taiwan Ocean University
Department of Aquaculture
2, PeiNing Rd
202, Keelung City, Taiwan
e-mail: *F0008@mail.ntou.edu.tw*

Gian M. Luna
National Research Council of Italy
Institute of Marine Sciences (ISMAR – CNR)
30122, Venice, Italy
e-mail: *gianmarco.luna@ve.ismar.cnr.it*

Sushila Maharjan
Seoul National University
Agricultural Biotechnology
Silim-9-dong, Gwanak-gu
Seoul, 151-921, Korea
e-mail: *ruma_sushila@snu.ac.kr*

Panchanathan Manivasagan
Pukyong National University
Department of Marine-Bio Convergence Science
45, Yongso-ro, Nam-gu
608-737, Busan, Korea
e-mail: *manimaribtech@gmail.com*

Pravat Manjari Mishra
CSIR-Institute of Minerals & Materials Technology
Department of Bioresources Engineering
Acharya Vihar
751013, Bhubaneswar, India
e-mail: *pravatmanjari@immt.res.in*

Lekha Menon Margassery
University College Cork
Marine Biotechnology Centre,
Environmental Research Institute
Cork, Ireland
e-mail: *lekha513@gmail.com*

Aníbal Martínez
ANFACO-CECOPESCA
Food Quality and Safety
Col Univ 16
36310, Vigo-Po, Spain
e-mail: *anibal@anfaco.es*

Yuta Matsumura
Kao Corp.
2606 Ichikai
321-3497, Haga, Japan
e-mail: *matsumura.yuuta@kao.co.jp*

Tadashi Matsunaga
Tokyo University of Agriculture and Technology
2-24-16, Naka-cho, Koganei
183-8588, Tokyo, Japan
e-mail: *tmatsuna@cc.tuat.ac.jp*

André Luiz Meleiro Porto
University of São Paulo
Institute of Chemistry of São Carlos, Department of Physical Chemistry
Av. João Dagnone, 1100
13563-970, São Carlos, Brazil
e-mail: *almporto@iqsc.usp.br*

V. Venugopal Menon
Bhabha Atomic Research Center
Food Technology Division
Mumbai, India
e-mail: *vvenugopalmenon@gmail.com*

Hideaki Miyashita
Kyoto University
Graduate School of Global Environmental Studies,
Graduate School of Human and Environmental Studies
Yoshida-nihonmatsu-cho, Sakyo-ku
606-8501, Kyoto, Japan
e-mail: *miyashita.hideaki.6v@kyoto-u.ac.jp*

Kalpana H. Mody
CSIR-Central Salt & Marine Chemicals Research Institute
Discipline of Marine Biotechnology & Ecology
Gijubhai Badheka Marg
364002, Bhavnagar, India
e-mail: *khmody@csmcri.org*

Pallavi Mohite
University of Pune
Institute of Bioinformatics and Biotechnology
Ganeshkhind Road
411007, Pune, India
e-mail: *pmohite@unipune.ac.in*

Eduardo Morales-Guerrero
Centro de Investigacion Cientifica y de Educacion Superior de Ensenada (CICESE)
Department of Marine Biotechnology
Km 107 Carretera
22860, Tijuana-Ensenada, Mexico
e-mail: *edmorale@cicese.mx*

Tetsushi Mori
Waseda University, Center for Advanced Biomedical Sciences (TWIns)
International Center for Science and Engineering Programs (ICSEP),
Faculty of Science and Engineering
2-2, Wakamatsu-cho, Shinjuku-ku
162-8480, Tokyo, Japan
e-mail: *moritets@aoni.waseda.jp*

John P. Morrissey
University College Cork
Department of Microbiology
Western Road
Cork, Ireland
e-mail: *j.morrissey@ucc.ie*

Kai Muffler
University of Kaiserslautern
Institute of Bioprocess Engineering
Gottlieb-Daimler-Straße 49
67663, Kaiserslautern, Germany
e-mail: *muffler@rhrk.uni-kl.de*

Joydeep Mukherjee
Jadavpur University
School of Environmental Studies
700032, Kolkata, India
e-mail: *joydeep_envstu@school.jdvu.ac.in*

Werner E.G. Müller
University Medical Center of the Johannes Gutenberg University Mainz
Institute for Physiological Chemistry
Duesbergweg 6
55128, Mainz, Germany
e-mail: *wmueller@uni-mainz.de*

Masaki Muto
Tokyo University of Agriculture and Technology
Division of Biotechnology and Life Science
2-24-16 Naka-cho
Koganei, Japan
e-mail: *m_muto@cc.tuat.ac.jp*

Yutaka Nakashimada
Hiroshima University
Department of Molecular Biotechnology
1-3-1 Kagamiyama
739-8530, Hiroshima, Japan
e-mail: *nyutaka@hiroshima-u.ac.jp*

Leonel Ochoa
Centro de Innovación Biotecnológica AC (CENBIOTEC)
22860, Ensenada, B.C., Mexico
e-mail: *Leonel.ochoa@gmail.com*

Tatsuya Oda
Nagasaki University
Division of Biochemistry, Faculty of Fisheries
1-14 Bunkyo-machi
852-8521, Nagasaki, Japan
e-mail: *t-oda@nagasaki-u.ac.jp*

Jorge Olmos Soto
Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE),
Molecular Microbiology Laboratory,
Departement of Marine Biotechnology
Ensenada, B.C., Mexico
e-mail: *jolmos@cicese.mx*

Fergal O'Gara
University College Cork
Marine Biotechnology Centre
Cork, Ireland
e-mail: *f.ogara@ucc.ie*

Niall O'Leary
University College Cork
Department of Microbiology
Cork, Ireland
e-mail: *n.oleary@ucc.ie*

Prasanna K. Panda
CSIR-Institute of Minerals & Materials Technology
Department of Bioresources Engineering
Acharya Vihar
751013, Bhubaneswar, India
e-mail: *pkpanda@immt.res.in*

Shunmughiah T. K. Pandian
Alagappa University
Department of Biotechnology
Science Campus
630003, Karaikudi, India
e-mail: *sk_pandian@rediffmail.com*

Ratih Pangestuti
Indonesian Institute of Sciences
Research Center for Oceanography
Jl. Pasir Putih 1 Ancol Timur
14430, Jakarta Utara, Indonesia
e-mail: *pangesturth@yahoo.com*

José de Jesús Paniagua-Michel
Centro de Investigacion Cientifica y de Educacion Superior de Ensenada (CICESE)
Department of Marine Biotechnology
Km 107 Carretera
22860, Tijuana-Ensenada, Mexico

Teresa Panteleitchouk
Instituto Piaget
Estrada do Alto do Gaio, Galifonge
3515-776, Lordosa, Viseu, Portugal
e-mail: *teresa.p@viseu.ipiaget.pt*

Yong Il Park
The Catholic University of Korea
Department of Biotechnology and The Research Centre for Biopharmaceutical Lead Molecule (GRRC)
Gyeonggi-do 420-743
Bucheon, Korea
e-mail: *yongil382@catholic.ac.kr*

Chiara Pennesi
Polytechnic University of the Marche
Department of Life and Environmental Sciences
Via Brecce Bianche
60131, Ancona, Italy
e-mail: *c.pennesi@univpm.it*

Leonel Pereira
University of Coimbra
IMAR, Department of Life Sciences
Calçada Martim de Freitas
3001-401, Coimbra, Portugal
e-mail: *leonel.pereira@uc.pt*

Lorena Pérez-Armada
University of Vigo
Department of Chemical Engineering
As Lagoas s/n
32004, Ourense, Spain
e-mail: *lorenaperezarmada@gmail.com*

Peter Proksch
Heinrich-Heine University
Institute of Pharmaceutical Biology and Biotechology
Universitätsstr. 1
40225, Düsseldorf, Germany
e-mail: *proksch@uni-duesseldorf.de*

Munish Puri
Deakin University
Centre for Chemistry and Biotechnology
76 Pigdons Road
Geelong, 3217, Australia
e-mail: *Munish.puri@deakin.edu.au*

Ronald J. Quinn
Griffith University
Eskitis Institute for Drug Discovery
Brisbane Innovation Park, Don Young Road
Nathan, QLD 4111, Australia
e-mail: *r.quinn@griffith.edu.au*

Venugopal Rajanbabu
Tamil Nadu Agricultural University
Centre for Plant Molecular Biology and Bioinformatics
641003, Coimbatore, India
e-mail: *vrajanbabu@hotmail.com*

Julieta Rangel de Oliveira
Federal University of Pernambuco
Departamento de Química Fundamental
Av. Jornalista Anibal Fernandes s/n, Cidade Universitária
Recife, Brazil
e-mail: *julietaquimica@yahoo.com.br*

Mirna H. Reagli Seleghim
Federal University of São Carlos
Ecology and Evolutive Biology (DEBE)
Via Washington Luiz, Km 235
13565-905, São Carlos, Brazil
e-mail: *seleghim@uol.com.br*

Fabio Rindi
Università Politecnica delle Marche (DISVA-UNIVPM)
Dipartimento di Scienze della Vita e dell'Ambiente
Via Brecce Bianche
60131, Ancona, Italy
e-mail: *f.rindi@univpm.it*

Joana Rocha
University of Aveiro
Campus Universitário de Santiago Department of Biology
3810-193, Aveiro, Portugal
e-mail: *joanacmrocha@gmail.com*

Dina Rodrigues
University of Aveiro
Department of Chemistry
3810-193, Aveiro, Portugal
e-mail: *dinarodrigues@ua.pt*

Gregory L. Rorrer
Oregon State University
School of Chemical, Biological, and
Environmental Engineering
Corvallis, OR, 97331, USA
e-mail: *gregory.rorrer@oregonstate.edu*

Debashis Roy
Jadavpur University
Department of Chemical Engineering
700032, Kolkata, India
e-mail: *deebie_roy@yahoo.com*

Constantin Rusu
University Alexandru Ioan Cuza of Iasi
Department of Geography
Carol I, No. 20A
700506, Iasi, Romania
e-mail: *c.v.rusu@yahoo.com*

H. Mir Sajjad
Yamagata University
Chemistry and Chemical Engineering
4-3-16 Jonan,Yonezawa, Yamagata
992-851, Yonezawa, Japan
e-mail: *sajjad.h.mir@gmail.com*

Kalpa W. Samarakoon
Industrial Technology Institute (CISIR)
Herbal Technology Division
No: 363, Bauddhaloka Mawatha
Colombo, Sri Lanka
e-mail: *kalpa.samarakoon@gmail.com*

Barindra Sana
p53 Laboratory
Agency for Science, Technology and Research
8A Biomedical Grove, #06-04/05
Neuros-Immunos
138648, Singapore
e-mail: *bsana@p53Lab.a-star.edu.sg*

Ramachandran S. Santhosh
SASTRA University
School of Chemical and Biotechnology
Thirumalaisamudram
613401, Thanjavur, India
e-mail: *rs_santhosh@yahoo.co.in*

Tomoo Sawabe
Hokkaido University
Faculty of Fisheries Sciences
3-1-1 Minato-cho
041-8611, Hakodate, Japan
e-mail: *sawabe@fish.hokudai.ac.jp*

Heinz C. Schröder
University Medical Center of the Johannes
Gutenberg University Mainz
Institute for Physiological Chemistry
Duesbergweg 6
55128, Mainz, Germany
e-mail: *hschroed@uni-mainz.de*

Jeong H. Seo
Yeungnam University
School of Chemical Engineering
Gyeongsan, 712-749, Korea
e-mail: *jhseo78@yu.ac.kr*

Prince Sharma
Panjab University
Department of Microbiology
160014, Chandigarh, India
e-mail: *princess@pu.ac.in*

Yoon-Bo Shim
Pusan National University
Bio-Physio Sensor Technology, Chemistry
Department
63 beongil Daehakro
Busan, 609-735, Korea
e-mail: *ybshim@pusan.ac.kr*

Katsuhiko Shimizu
Tottori University
Organization for Regional Industrial Academic
Cooperation
4-101 Koyamacho Minami
680-8550, Tottori, Japan
e-mail: *kshimizu@cjrd.tottori-u.ac.jp*

Bijay Singh
Seoul National University
Department of Animal Cell Biotechnology
Daehak-dong, Gwanak-gu
Seoul, 151-921, Korea
e-mail: *singhbijay@hotmail.com*

Kannan Sivakumar
Annamalai University
Centre of Advanced Study in Marine Biology,
Faculty of Marine Sciences
608 502, Parangipettai, India
e-mail: *oceanactino@gmail.com*

M. Luisa Soto
IES Lauro Olmo
Calle de Calabagueiros
32005, O Barco, Spain
e-mail: *marisa-sotoalvarez@edu.xunta.es*

Ayinampudi Sree
CSIR-Institute of Minerals & Materials Technology
Department of Bioresources Engineering
Lenin Road, Nandula Peta
522201, Tenali, India
e-mail: *asree18@yahoo.in*

Ananya Srivastava
Deen Dayal Upadhaya Gorakhpur University
Department of Chemistry
Gorakhpur Street
273009, Gorakhpur, India
e-mail: *ananya.gkp@gmail.com*

Arti Srivastava
Amity University Uttar Pradesh
Amity Institute of Biotechnology
Sector 125
Noida, India
e-mail: *asrivastava@amity.edu*

Ashutosh Srivastava
Amity University Uttar Pradesh
1. Amity Institute of Marine Science and
Technology
Sector 125
Noida, India
e-mail: *asrivastava4@amity.edu*

Jeeva Subbiah
Konkuk University
Department of Infectious Disease
Gwangjin-gu
Seoul, Korea
e-mail: *jeevabio@gmail.com*

Parappurath Narayanan Sudha
D.K.M. College for Women
Department of Chemistry
Sainathapuram
63200, Vellore, India
e-mail: *parsu8@yahoo.com*

Andriy Synytsya
Institute of Chemical Technology in Prague
Department of Carbohydrates and Cereals
Technicka 5
166 28, Prague, Czech Republic
e-mail: *sinicaa@vscht.cz*

Haruko Takeyama
Waseda University
Department of Life Science and Medical
Bio-Science
2-2, Wakamatsu-cho, Shinjuku-ku
162-8480, Tokyo, Japan
e-mail: *haruko-takeyama@waseda.jp*

Tsuyoshi Tanaka
Tokyo University of Agriculture and Technology
Division of Biotechnology and Life Science,
Institute of Engineering
2-24-16, Naka-cho, Koganei
184-8588, Tokyo, Japan
e-mail: *tsuyo@cc.tuat.ac.jp*

Fabiano Thompson
Federal University of Rio de Janeiro
Department of Marine Biology
Ilha do Fundão, CCS, Brazil
e-mail: *fabiano.thompson@biologia.ufrj.br*

Tamilselvi Thyagarajan
Deakin University
Centre for Chemistry and Biotechnology, LES
75 Pigdons Road, Waurn Ponds
Victoria, 3217, Australia
e-mail: *tthyagar@deakin.edu.au*

Cecilia Totti
Università Politecnica delle Marche
(DISVA-UNIVPM)
Dipartimento di Scienze della Vita e dell'Ambiente
Via Brecce Bianche
60131, Ancona, Italy
e-mail: *c.totti@univpm.it*

Antonio Trincone
Instituto di Chimica Biomolecolare
Consiglio Nazionale delle Ricerche
Via Campi Flegrei 34
80078, Pozzuoli, Naples, Italy
e-mail: *antonio.trincone@icb.cnr.it*

Roland Ulber
University of Kaiserslautern
Institute of Bioprocess Engineering
Gottlieb-Daimler-Straße 49
67663, Kaiserslautern, Germany
e-mail: *ulber@mv.uni-kl.de*

Jayachandran Venkatesan
Pukyong National University
Department of Marine-Bio Convergence Science
Busan, 608-737, Korea
e-mail: *venkatjchem@pknu.ac.kr*

Juan M. Vieites
ANFACO-CECOPESCA
General Management
Col Univ 16
36310, Vigo-Po, Spain
e-mail: *direccion@anfaco.es*

Xiaohong Wang
University Medical Center of the Johannes Gutenberg University Mainz
Institute for Physiological Chemistry
Duesbergweg 6
55128, Mainz, Germany
e-mail: *wang013@uni-mainz.de*

Jen-Leih Wu
Academia Sinica
Institute of Cellular and Organismic Biology
128 Academia Road
Taipei, Taiwan
e-mail: *jk.Lu@msa.hinet.net*

Michiaki Yamashita
National Research Institute of Fisheries Science
Food Safety Assessment Research Group
Huku-ura 2-12-4 Fukuura, Kanazawa-ku
236-8648, Yokohama, Japan
e-mail: *mic@affrc.go.jp*

Yumiko Yamashita
National Research Institute of Fisheries Science
Food Safety Assessment Research Group
Huku-ura 2-12-4 Fukuura, Kanazawa-ku
236-8648, Yokohama, Japan
e-mail: *ymk0125@affrc.go.jp*

Byeongseon Yang
Pohang University of Science and Technology
Department of Chemical Engineering
Pohang, 790-784, Korea
e-mail: *yangbs0520@postech.ac.kr*

Charles Yarish
University of Connecticut
Department of Ecology & Evolutionary Biology
1 University Place
Storrs, CT, 06901-2315, USA
e-mail: *charles.yarish@uconn.edu*

Tomoko Yoshino
Tokyo University of Agriculture and Technology
Division of Biotechnology and Life Science,
Institute of Engineering
2-24-16, Naka-cho, Koganei
184-8588, Tokyo, Japan
e-mail: *y-tomoko@cc.tuat.ac.jp*

SangGuan You
Gangneung-Wonju National University
Department of Marine Food Science and Technology
7, Jukheon-gil
Gangneung-si, 210-702, Korea
e-mail: *umyousg@gwnu.ac.kr*

Smita Zinjarde
University of Pune
Institute of Bioinformatics and Biotechnology
Ganeshkhind Road
411007, Pune, India
e-mail: *smita@unipune.ac.in*

Contents

Part B Tools and Methods in Marine Biotechnology

Part C Marine Genomics

Part D Marine Algal Biotechnology

Part E Marine Microbiology and Biotechnology

List of Abbreviations

Symbols

β-HB	β-hydroxy butyrate
γ-PGA	γ-polyglutamic acid
ω-3	Omega-3 fatty acid
^{1}H NMR	proton nuclear magnetic resonance
(HPLC–DAD)	high pressure liquid chromatography with diode array detection
L-DOPA	L-3,4-dihydroxyphenylalanine
1-D	one-dimensional
12-DS	12-doxylstearic acid
19HF	19′-hexanoyloxy-fucoxanthin
2-D	two-dimensional
2D-DIGE	two-dimensional difference in-gel electrophoresis
3-PGA	3-phosphoglyceric acid
4-DSC	four-disulfide core
8-HPETE	(8*R*)-hydroperoxyeicosa-5,9,11,14-tetraenoic acid

A

A549	human alveolar epithelial cells
A549	human lung adenocarcinoma
AA	arachidonic acid
aa	amino acid
AAPB	aerobic anoxygenic photosynthetic bacteria
AAPH	2,2′-azobis(2-amidino-propane) dihydrochloride
AAR	acyl–acyl carrier protein reductase
ABAP	2,2′-azo-bis-2-amidinopropane
ABC	ATP-binding cassette
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AC	alternating current
ACCase	acetyl-CoA carboxylase
ACE	angiotensin-I-converting enzyme
ACE	angiotensin-converting enzyme
AChE	acetylcholinesterase
ACP	acyl carrier protein
ACP	acyl–acyl carrier protein
ADBAC	alkyldimethylbenzylammonium chloride
ADHD	attention-deficit hyperactivity disorder
ADO	aldehyde-deformylating oxygenase
AF	antifouling
AfDD	acriflavine direct detection
AFLP	amplified fragment length polymorphism
AFM	atomic force microscope
AGC	arginine-graft-chitosan
AgNP	silver (argentum) nanoparticle
AGS	gastric adenocarcinoma
AGS	human gastric cancer cell
AHA	α-hydroxy acids
AHL	acyl-homoserine lactone
AHRE	aromatic hydrocarbon response element
AIDS	acquired immunodeficiency syndrome
AKG	α-ketoglutarate
Akt	nuclear phosphatidylinositol 3,4,5-triphosphate phosphotidyinositol 3-kinase
AL	airlift
ALA	α-linolenic acid
ALBR	airlift bioreactor
ALCL	anaplastic large cell lymphoma
AMD	acid mine drainage
AMIS	anaerobic methane incubation system
amoA	α-subunit
amoA	ammonia-oxidation
AmoB	ammonia mono-oxygenase membrane bound subunit β
AMOP	aquatic microbial oxygenic photoautotroph
AMP	antimicrobial peptide
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMS	air membrane surface
anammox	anaerobic ammonium-oxidizing
AnAPB	anaerobic anoxygenic photosynthetic bacteria
AnMBR	anaerobic membrane bioreactor
ANME	anaerobic methanotrophic archaea
anti-MRSA	anti-methicillin-resistant *Staphylococcus aureus*
AOA	ammonia-oxidizing archaea
AOB	ammonia oxidizing bioreactor
AOM	alginate oligosaccharide mixture
AOM	anaerobic oxidation of methane
APTT	activated partial thrombinplastin time
aPTT	activated partial thromboplastin time

ARA	arachidonic acid
Ara-A	arabinofuranosyl adenine or adenine arabinoside
Ara-A	vidarabine
Ara-C	cytarabine
Ara-C	arebinosyl cytosine
ARfD	acute reference doses
ARP	ankyrin repeat protein
ASAP	automated simultaneous analysis phylogenetics
ASC	acid-soluble collagen
ASP	amnesic shellfish poisoning
ASW	artificial seawater
AT	acyltransferase
ATCC	American Type Culture Collection
ATN	autotrophic nitrifier
ATP	adenosine triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
AttM	lactonase of *A. tumefaciens*
Au-CNT	gold-coated carbon nanotubes
AuNP	gold (aurum) nanoparticle
AZA	azaspiracid
AZP	azaspiracid shellfish poisoning

B

BAC	bacterial artificial chromosome
BALB/c	albino, laboratory-bred strain of the house mouse
BaP	benzo[*a*]pyrene
BAPD	bis(*p*-aminophenoxy)-dimethylsilane
BC	bubble column
BCBR	bubble column bioreactor
BChl	bacteriochlorophyll
BD	biofilm density
BDL	beyond detectable limit
BDS	bunodosine
BE	bioemulsifer
BF	biofouling
BFR	biofilm reactor
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
BLAST	basic local alignment search tool
BMNV	baculoviral midgut gland necrosis virus
BMP	bone morphogenetic protein
BMSC	bone marrow stomal cell
BNR	biological nitrate removal
BP	*Baculoviruspenaei*
BPA	bisphenol A
BPR	back pressure regulator
BPS	back-pressure-system
BS	biosurfactant
Bs	*Bacillus subtilis*
BSA	bovine serum albumin
BSD	blasticidin
BSP	sialoprotein
BTX	brevetoxin
BTX	square wave generator
bvPLA	bee venom, phospholipase AZ

C

C-CTX	Caribbean-CTX
C-g-PEI	chitosan-graft-PEI
C-PE	C-phycoerythrin
C12-TE	*Cinnamomum camphora*
C14-TE	*Umbellularia californica*
Ca-SP	calcium spirulan
CA4	combretastatin
Ca9-22	human gingival carcinoma
CAF	chlorophyll autofluorescence
CAMERA	community cyber infrastructure for advanced marine microbial ecology research and analysis
cAMP	cyclic adenosine monophosphate
Campto	camptothecin
CARD	catalyzed reporter deposition
CARMA	characterizing short read metagenome
CAS	CRISPR-associated
CAS	chemical abstracts service
CAS	chrome azurol S
CAT	chloramphenicol acetyltransferase
CC	column chromatography
CCCP	carbonylcyanide *m*-chlorophenylhydrazone
CCF	conico-cylindrical flask
CCI	chronic constriction injury
CCRF-CEM	human T-cell lymphoblast-like cell line
CCRF-CEM	human Caucasian acute lymphoblastic leukaemia
CD	compact disc
CD	circular dichroism
CD	cluster of differentiation
Cd	cadmium
cDNA	copy DNA
cDNA	complementary DNA
CdO	cadmium oxide
CdS	cadmium sulfide
CEB	cell entrapping bead

CEME	cecropin-medilitin hybrid peptide
CES	collagen and elastin sponge
CF	cystic fibrosis
CFE	cell-free extracts
CFP	ciguatera fish poisoning
Cg-Def	oyster defensin
CGC	cerebellar granule cell
CGN	cerebellar granule neuron
CGRP	calcitonin gene-related peptide
CGS	chitosan-graft-spermine
CH	carbohydrate
CHA	carbonated hydroxyapatite
CharProtDB	Characterized Protein Database
ChAT	choline acetyltransferase
CHD	coronary heart disease
CHL	chlorophyll
CHO	carbohydrate
CHO	Chinese hamster ovary
ChOx	cholesterol oxidase
CI	cyclic imines
CI	cyclic imine
CL	chemiluminescence
CLP	chitosan-linked-PEI
CMC	critical micelle concentration
CMFRI	Central Marine Fisheries Research Institute
CML	chronic myeloid leukemia
CMV	cytomegalovirus mosaic virus
CNN	cloud condensation nuclei
CNS	central nervous system (CNS)
CNS	central nervous system
CO_2-TR	CO_2 transfer rate
CoA	coenzyme A
COC	cyclic olefin co-polymer
COC1	human ovarian adenocarcinoma cisplatin-sensitive cell
COG	Clusters of Orthologous Group
COG0610	restriction enzymes, type I helicase
COG1715	restriction endonuclease
CoML	Census of Marine Life
ConA	concanvalin A
COS	chitooligosaccharide
COS	chitosan oligosaccharides
COS	chitosan chito-oligosaccharide
COSY	correlation spectroscopy
COX	cyclooxygenase
COX-2	cyclooxygenase-2
CPBR	column-type PBR
CPCD	chitosan-graft-PEI-β-cyclodextrin
CPG	consumer packaged good
CPP	cell-penetrating peptide
CPS	capsular polysaccharide
CPU	central processing unit
CR	complement receptor
Cr	chromium
CRISPR	clustered regularly interspersed short palindromic repeat
CRISPR	clustered regularly interspaced short palindromic repeat
CS	chondroitin sulfate
CS-F	Fucosylated CS
CSIR	Council of Scientific Industrial Research
CSMCRI	Central Salt and Marine Chemicals Research Institute
CSP	cell surface protein
CT	computer tomography
CT	collection tank
CTT	conventional thermal treatment
CTX	ciguatoxin
CTX	cholera toxin
CV	cardiovascular
CV	check valve
CyDyes	cyanine dyes
Cys	cysteine
Cytosar-U1	Cytarabine

D

DA	domoic acid
DAE	Diethylaminoethyl
DAG	diacylglycerol
DAN	diaminonaphthalene
DAP	dorsal arm plate
DB	digestion basin
DBMIB	2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethyl urea
DD	degree of deacetylation
DD	decadienal
DEAE	diethylaminoethyl cellulose
delta-DOPA	decarboxy (E)-alpha,beta-dehydro-3,4-dihydroxyphenylalanine
DENV	dengue virus
DF1	fraction 1
DGGE	denaturing gradient gel electrophoresis
DH	dehydratase
DHA	docosahexaenoic acid
DIG	digoxigenin 11-dUTP
DIN	dissolved inorganic nitrogen
DIPS	diffusion induced phase separation
DLD	dihydrolipoamide dehydrogenase

DLS	dynamic light scattering
DMAPP	dimethylallyl pyrophosphate
DMF	dimethylformamide
DMPO	5,5-dimethyl-1-pyrroline N-oxide
DMPO-OH	DMPO-hydroxyl
DMRB	dissimilatory metal reducing bacteria
DMSO	dimethyl sulfoxide
DMXBA	3-(2,4-dimethoxybenzylidene)-anabaseine
DN-BPR	denitrifying biological phosphorus removal
DNA	deoxyribonucleic acid
DNAML	DNA maximum likelihood
DNaseI	deoxyribonuclease
DO	dissolved oxygen
DOC	dissolved organic carbon
DON	dissolved organic nitrogen
DOPA	dihydroxy-L-phenylalanine
DOTUR	defining operational taxonomic units and estimating species richness
DOX	doxorubicin
DP	degree of polymerization
DPPH	1,1-diphenyl-2-picrylhydrazyl
DPPH	2,2-diphenyl-1-picrylhydrazyl
DS	dermatan sulfate
dsDNA	double-stranded DNA
DSLSS	disodium lauryl sulfosuccinate
DSP	diarrhetic shellfish poisoning
DspB	Dispersin B
dsRNA	double-stranded RNA
DTP	diethylenetriamine pentaacetic acid
DTX	dinophysis toxin
DVChl	divinyl-chlorophyll
DVD	digital video disc
DXP	1-deoxy-xylulose-5-phosphate

E

E7389	eribulin mesylate
EBPR	enhanced biological phosphorus removal
ECD	electronic circular dichroism
ECM	extracellular matrix
ECP	extracellular product
ED	electrodialysis
EDC	endocrine disrupting compound
eDNA	environmental DNA
EDP	Entner–Doudoroff pathway
EDS	energy dispersive spectra
EDS	energy dispersive spectroscopy
EDTA	ethylenediaminetetraacetic acid
EDX	energy dispersive X-ray spectroscopy
ee	enantiomeric excess
EF	Erlenmeyer flask
EFC	efficiency of feed conversion
EFSA	European Food Safety Authority
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
EMEA	European Medicines Agency
EMEA	European Agency for the Evaluation of Medicinal Products
EMP	Embden–Meyerhof pathway
EMP	Earth Microbiome Project
EPA	environmental protection agency
EPA	eicosapentaenoic acid
epi-DA	epi-domoic acid
EPRE	electrophile response element
EPS	extracellular polysaccharide
EPS	exopolysaccharide
ER	enoyl reductase
ESCRT	endosomal sorting complexes required for transport
ESI	electrospray ionization
ESI-MS	electrospray ionization mass spectrometry
ESR	electron spin resonance
ESSV	extended surface shaken vessel
EST	express sequence tag
ET-743	ecteinascidins
EtOAc	ethyl acetate
ETS	external transcribed spacer
EU	European Union

F

FA	formaldehyde
FA	fatty acid
FACS	fluorescence activated cell sorting
FAEE	fatty acid-ethyl-ester
FAME	fatty acid methyl ester
FAO	Food and Agriculture Organization
FAP	flat alveolar panel
FAP-PBR	flat alveolar panel PBR
FBBR	fluidized bed bioreactor
FcεRI	Fc region of immunoglobulin E
FCA	ferrous ion chelating ability
FCB	free-cell photobioreactor
FCC	face centred cubic
FCF	food conversion factor

FCR	food conversion ratio
FDA	Food and Drug Administration
FD&C	Food, Drug, and Cosmetic Act
FETAX	frog embryo teratogenesis assay-xenopus
FF	functional feed
FISH	fluorescence in situ hybridization
FM	fish meal
FO	fiber optic probe
FOS	fucooligosaccharide
FOSHU	food for specified health uses
FOSMID	F1 origin-based cosmid vector
FPH	fish protein hydrolyzate
FPT	farnesyl protein transferase
FP&LA	Fair Packaging and Labeling Act
FR	fouling-release
FRAP	ferric reducing antioxidant power
FRAP	ferric ion reducing antioxidant power
FRET	fluorescent resonance energy
FRET	fluorescence resonance energy transfer
FTIR	Fourier transform infrared spectroscopy
FXR	farnesoid X-activated receptor

G

G	guluronate
G	guluronic acid
G-CSF	granulocyte colony-stimulating factor
g.b.f.	glass-ball filter
G3–G9	trimer to nonamer of G
GA	glutaraldehyde
GABA	γ-amino-butyric acid
GAG	glycosaminoglycan
GAO	glycogen accumulating organism
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GAV	gill-associated virus
GB	glove box
GC	galactosylated chitosan
GC-FID	gas chromatography-flame ionization detector
GC-MS	gas chromatography-mass spectrometry
GC-TOF-MS	chromatography-time-of-flight mass spectrometry
GCL	glutamate cysteine ligase
GCS	GC-g-spermine
Gd	gadolinium
gDNA	genomic DNA
GDSL	amino acid motif specific to the esterases
GDSLS	amino acid residues considered as an important motif in the EstHE1 esterase
GEBA	Genomic Encyclopedia of Bacteria and Archaea
GERD	gastroesophageal reflux disease
GFP	expression of signal molecule
GFP	green fluorescent protein
GG	homopolymeric blocks in alginates
GH	glycoside hydrolase
GH	growth hormone
GHF	glycoside hydrolase family
GHG	greenhouse gas
GI	gastrointestinal
GI_{50}	growth inhibition
GLA	γ-linolenic acid
GlcNAc	*N*-acetylglucosamine
GLDC	glutamate decarboxylase
Glu	glutamic acid
Glu-Plg	glutamic plasminogen
Glu-tag	octaglutamic acid-tag
GM	alternating blocks in alginates
GM	genetically modified
GM-CSF	granulocyte-macrophage colony-stimulating factor
GMO	genetically modified organism
GMP	good manufacturing practices
GNO	hydrated gallium nitrate
GOD	glucose oxidase
GOLD	Genomes OnLine Database
GoM	Gulf of Mannar
GoMBR	Gulf of Mannar Marine Biosphere Reserve
GORD	gastroesophageal reflux disease
GOS	Global Ocean Sampling
GPC	gel permeation chromatography
GPCS	galactosylated PEG-chitosan-graft-spermine
GPDH	glycerol-3-phosphate dehydrogenase
GPx	glutathione peroxidase
GRAS	generally recognized as safe
GRPSp	gastrin-releasing peptide, Scylla paramamosain
GSH	glutathione
GST	genome sequence tag
GT	glycosyltransferase
GTA	glycidyl trimethyl ammonium
GulA	α-D-guluronic acid
GWP	green wall panel
GWP-PBR	green wall panel PBR
GYM	gymnodimine
GYM	glucose yeast extract and malt

H

HA	hydroxyapatite
HA	hyaluronic acid
HAB	harmful algal bloom
hADSC	human adipose-derived stem cell
HAP	hydroxyapatite
HBME	human brain microvascular endothelial
HBV	hepatitis B virus
HCMV	human cytomegalovirus
HCN	hydrogen cyanide
HCT	human colorectal tumor
HCT116	human colorectal carcinoma cell line
HCV	hepatitis C virus
HDAC	histone deacetylase
HDL	high density lipoprotein
He	helium
HeLa	human cervix carcinoma cells
HeLa	Henrietta Lacks
Hep3B	human liver carcinoma
HepG2	hepatoma cell line
HepG2	Hepatoma Growth2
HF	hydrofluoric
HF	hollow fiber
HF-MBR	hollow-fiber membrane bioreactor
HF-MFBR	hollow-fiber microfiltration bioreactor
HF-sMBR	hollow-fiber submerged MBR
HFAR	hybrid flow-through anaerobic reactor
HFBR	hollow-fiber bioreactor
HGF	hepatocyte growth factor
hGH1	human growth hormone 1
His-tag	hexahistidine-tag
HIV	human immunodeficiency virus
HIV-1	human immunodeficiency virus
HJ	hydrogen supply
HLE	human leukocyte elastase
hli	high light inducible gene
HM	high methoxy
HMT	histone methyltransferases
HNE	human neutrophil elastase
HP-CI	high-pressure continuous incubation system
HP-MI	high-pressure manifold incubation system
HPAEC-PAD	high-performance anion-exchange chromatography with pulsed amperometric detection
HPBBR	high-pressure batch bioreactor
HPBR	high-pressure bioreactor
HPHT	high-pressure/high-temperature
HPHTBR	high-pressure/high-temperature bioreactor
HPLC	high-performance liquid chromatography
HPLC-DAD	high-performance liquid chromatography with diode-array detection
HPTGS	high-pressure thermal gradient system
HPU	high-pressure unit
HPV	hepatopancreatic parvovirus
HPX	hypoxanthine
HR-SEM	high-resolution scanning electron microscopy
HRM	high resolution melting
HRMS	High Resolution Mass Spectrometry
HRP	horseradish peroxidase
HRT	hydraulic retention time
HRTEM	high-resolution transmission electron microscope
HS	heparin sulfate
HS	high-solid
HS	heparan sulfate
HSC70	heat-shock cognate protein 70
HSDH4	17β-hydroxysteroid dehydrogenase type 4
HSV	herpes simplex virus
HT-29	human colon adenocarcinoma
HTHPBR	high-temperature, high-pressure bioreactor
HTLV	T-cell leukaemia virus
HTS	high throughput screening
HUVEC	human umbilical vein endothelial cells

I

I-CTX	Indian-CTX
i.p.	intraperitoneal
I/O	inlet/outlet
IAA	imidazole acetic acid
IAAC	IAA-coupled chitosan
IAC	internal amplification control
IAEA	International Atomic Energy Agency
IAST	ideal adsorbed solution
IBPCS	integrated biomass production conversion system
ICC	intrahepatic cholangiocarcinoma
Ichip	isolation chip
ICP	ion concentration polarization
ICP-MS	inductively coupled plasma mass spectrometry
ICR	imprinting control region
IDO	indoleamine 2,3-dioxygenase

IEMBR	ion exchange membrane bioreactor
IgE	immunoglobulin E
IGF-IR	insulin-like growth factor-I receptor
IgG	immunoglobulin G
IgM	immunoglobulin M
IGR	insect growth regulator
IGS	intergenic spacer
IHHNV	infectious hypodermal and hematopoietic necrosis virus
IHNV	infectious hematopoietic necrosis virus
IISA	integrated in situ analyzer
IISA-Mn	integrated in situ analyzer for Mn
IKK	inhibitor of nuclear factor κ-B kinase subunit beta
IL	ionic liquid
IL	interleukin
IL-6	interleukin-6
IMG	Integrated Microbial Genomes
IMG/M	Integrated Microbial Genomes and Metagenomes System
IMNV	infectious myonecrosis virus
IMO	International Maritime Organization
IMPDH	inosine 5′-monophosphate dehydrogenase
INCI	International Nomenclature of Cosmetic Ingredients
INF	interferon
INF-γ	interferon-γ
InHA	immune inhibitor A precursor
iNOS	inducible nitric oxide synthase
IPCP	integrated pest control programs
IPG	immobilized pH gradient
IPNV	infectious pancreatic necrosis virus
IPP	isopentyl pyrophosphate
IR	Infrared Spectroscopy
IR	infrared
iRFP	near-infrared fluorescent protein
ISH	in situ hybridization
iso-DA A–H	isodomoic acids A, B, C, D, E, F, G, and H
IT	information technology
iTRAQ	isobaric tags relative absolute quantification
ITS	internal transcribed spacer
IUB	International Union of Biochemistry and Molecular Biology
IUPAC	International Union of Pure and Applied Chemistry
IV	intravenous injection
IV1	injection vessel
IV2	injection vessel

J

JNK	c-Jun N-terminal kinase

K

K562	human chronic myeloid leukemia cells
KB	human epidermoid carcinoma
KC	keratinocyte-derived chemokine
KF	Kahalalide F
KGF	keratinocyte growth factor
KM	kinetic modeling
KR	ketoreductase
KS	ketosynthase
KS	ketoacyl synthase
KSa-KSb-ACP	ketosynthase alpha subunit-ketosyntahase beta subunit-acyl carrier protein
KSR1	kinase suppressor of Ras1

L

L/D	light/dark
LasR	transcriptional activator
LB	Luria-Bertani broth
LB	Luria Bertani
LC	liquid chromatography
LC_{50}	lethal concentration
LC-ESI-MS	liquid chromatography electrospray ionization mass spectrometry
LC-MS	liquid chromatography-mass spectrometry
LCA	life cycle
LCB	lactophenol cotton blue
LCFA	long-chain fatty acid
LD_{50}	lethal dose 50
LDL	low-density lipid
LDL	low-density lipoprotein
LDLR	low-density lipoprotein receptor
LDPE	low-density polyethylene
LFB	limited filamentous bulking
LHCII	light-harvesting chlorophyll protein complex apoproteins associated with photosystem II
LM	low methoxy
LMA	low methoxy amidated
LN	lymphocytes
LOC	lab-on-a-chip
LOD	limit of detection
LoVo	human colon adenocarcinoma
LOVV	lymphoid organ vacuolization virus

LPS	lipopolysaccharide
LPV	lymphoidalparvo-like virus
LRT	larval rearing tank
LS	low-solid
LS	light source
LSNV	Laem–Singh virus
LSU	long sub-unit
LT	lipophilic toxin
LTA	lipoteichoic acid
LTR	long terminal repeat
LuxR	transcriptional activator
Lys	lysine

M

M	mannuronate
M	mitosis
M	mannuronic acid
M-PBR	membrane photobioreactor
M/G	molar ratio between mannuronic and glucuronic acids in alginates
M3–M9	trimer to nonamer of M
MA	maslinic acid
MAA	mycosporine-like amino acid
mAb	monoclonal antibodies
MABV	marine birnavirus
MAE	microwave-assisted extractions
MALDI	mass spectrometry using assisted laser desorption ionization
ManA	β-D-mannuronic acid
MAP	mussel adhesive protein
MAP	mitogen-activated protein
MAPK	mitogen-activated protein kinase
MAR	microautorardiography
Mb	met-myoglobin
MBA	mouse bioassay
MBBR	moving bed bioreactor
MBC	minimum bactericidal concentration
Mbp	million base pairs
MBR	membrane bioreactor
MBT	magnetotactic bacteria
MBV	monodon baculovirus
MCF	Michigan Cancer Foundation-7
MCF7	breast carcinoma cell
MCF7	human breast cancer cell line
MCH	melanin-concentrating hormone
MCP	monocyte chemoattractant protein
MCP	marine collagen peptide
MCP	monocyte chemotactic protein
MCP-1	monocyte chemoattractant protein-1
MDA	multiple displacement amplification
MDA-MB-231	human breast carcinoma
MDD	major depressive disorder
MDR	multi-drug resistant
MEGAN	meta genome analyzer
MegDB	Microbial Ecological Genomics DataBase
MeHg	methylmercury
MeHgCy	MeHg-cysteine
MEL-28	human melanoma cell
MEMS	microelectromechanical system
MEMS/NEMS	micro- and nanoelectromechanical systems
MetaBioME	Metagenomic BioMining Engine
MF	microfiltration
MG	heteropolymers of mannuronate and guluronate
MG	alternating blocks in alginates
MG-RAST	meta genomics rapid annotation using subsystem technology
MG-RAST	meta genomics rapid annotation using subsystems technology
MGB	minor groove binder
MHDS	multihead deposition system
MIC	minimum inhibitory concentration
MicroScope	microbial genome annotation & analysis platform
MIP	molecularly imprinted polymer
MIP	macrophage inflammatory protein
MISS	microbially induced sedimentary structures
MLVSS	mixed liquor volatile suspended solids
MM	homopolymeric blocks in alginates
MMAE	monomethyl auristatin E
MMP-9	metalloproteinase-9
MMP2	matrix metalloproteinase 2
MNP	marine natural product
MO	morpholino antisense oligo
MO	morpholino phosphorodiamidate oligonucleotide
MOLT	molybdate uptake transporter
MoMLV	Moloney murine leukemia virus
Mon	manganese
MoV	Mourilyan virus
Mp-TX	*Millepora platyphylla*
MPA	mycophenolic acid
MPBR	membrane photobioreactor
MPL	maximum permitted level
MRBC	Modified roller bottle cultivation
MRE	metal response element
MRI	magnetic resonance imaging

mRNA	messenger RNA
MRSA	methicillin-resistant *Staphylococcus aureus*
MS	mass spectrometry
MS	mass spectroscopy
MS^n	de novo tandem MS
MS/MS	tandem mass spectrometry
MSA	multiple sequence alignment
MSC	mesenchymal stromal cell
MSC	mesenchymal stem cell
MSSA	methicillin-susceptible *Staphylococcus aureus*
MT	shrimp maturation tank
MT	metallothionein
MT	metric ton
mTOR	mammalian target of rapamycin
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MTT	microculture tetrazolium assay
MTX	maitotoxin
MTZ	mass transfer zone
MUF-diNAG	4-methylumbelliferyl β-D-*N*,*N*′-diacetylchitobioside
MUFA	monounsaturated fatty acid
MurNAc	*N*-acetylmuramic acid
MVL	mevalonate
MW	molecular weight
MW	microwave
MWT	microwave treatment

N

NADH	nicotiamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
nanoSIMS	nanometer-scale secondary-ion mass spectrometry
NaPDoS	natural product domain seeker
NASBA	nucleic acid sequence-based amplification
NB-L	nitrobenzene with linoleic acid
NBC	nitrifying bacterial consortia
NCBI	National Center for Biotechnology Information
NCE	new chemical entity
NCI	human tumor cell line
NCI	National Cancer Institute
NCI-H460	human lung cancer cell line
NCN-Pt	platinum-dimethylamino pincer complex
NDGA	nordihydroguaiaretic acid
NER	nucleotide excision repair
NF	nuclear factor
NF-κB	nuclear factor-κB
NFT	neurofibrillary tangles
NGS	next generation sequencing
NHase	Nitrile hydratase
NHE	normal hydrogen reference electrode
NHP	*N*-(2-hydroxyphenyl)-2-phenazinamine
Ni	nickel
Ni-NTA	nickel nitrilotriacetic acid
NICD	Notch intracellular domain
NIO	National Institute of Oceanography
nirS	nitrite reductase
nirS	nitrification gene
NK	natural killer
NLase	Nitrilase
NMC-g-PEI	*N*-maleated chitosan-graft-PEI
NMDA	*N*-metyl-D-aspartic acid
NMNP	new marine natural product
NMP	*N*-methylpyrrolidone
NMR	nuclear magnetic resonance
NO	nitric oxide
NOAA	National Oceanic and Atmospheric Administration
NOB	nitrite-oxidizing bioreactor
NOESY	nuclear Overhauser and exchange spectroscopy
NP	nanoparticle
NP	nonylphenol mixture
NPU	net protein utilization
NRPS	nonribosomal peptide synthase
NRPS	nonribosomal peptide synthetase
NS	neutral sugar
NS2	nonstructural protein-2
NSC-g-PEI	*N*-succinyl chitosan-graft-PEI
NSCLC	non-small cell lung cancer
NSOM	near field scanning optical microscopy
NSP	non-starch polysaccharide
NSP	neurotoxic shellfish poisoning
NTA	nitrilotriacetic acid
NTC	no template controls
NTG	*N*-methyl-*N*′-nitro-*N*-nitroso guanidine
NTS	nontranscribed spacer
NVSCC	N-type voltage-sensitive calcium channel

O

OA	okadaic acid
OA	ocean acidification

OECD	Organization for Economic Cooperation and Development
OHT	overhead tank
OLED	organic light emitting diode
OmpA	outer membrane protein A
OP	organophosphorus
OPCW	Organization for the Prohibition of Chemical Weapons
OPD	organic photodetector
OPF	orange fluorescent protein
OPG	osteoprotegerin
OPH	organophosphorus hydrolase
ORAC	oxygen radical absorbance capacity
ORF	open reading frame
OTAB	octyl-trimethyl ammonium bromide
OTC	over-the-counter

P

P-388	mouse lymphocytic leukemia
P-CTX	Pacific-CTX
PAA	peak antibiotic activity
PAAR	peak activity attainment rate
pAb	polyclonal antibodies
PAFP	photoactivatable fluorescent protein
PAGE	polyacrylamide gel electrophoresis
PAH	polycyclic aromatic hydrocarbon
PAMA	peak antimicrobial activity
PAMAM	poly(amido amine)
PAN	plane polyacrylonitrile
PAO	phosphorus accumulating organism
PAPS	3′-phosphoadenosine 5′-phosphosulfate
PAR	photosynthetically active radiation
PB-PBR	packed bed photobioreactor
PBBR	packed bed bioreactor
PBEL-ALBR	packed bed external loop airlift bioreactor
PBMC	peripheral blood mononuclear cells
PbNP	lead (plumbum) nanoparticle
PBR	photobioreactor
PBS	phosphate buffered saline
PbTx	brevetoxin
PC	phycocyanin
PCA	principal component analysis
PCD4	programmed cell death 4
PCL	polycaprolactone
PCNA	proliferative cell nuclear antigen
PCP	PEG-graft-chitosan-graft-PEI
PCP-Pd	paradium-diphenylphosphino pincer complex
PCPA	PEG-graft-chitosan-graft-polyarginine
PCR	polymerase chain reaction
PDMS	polydimetylsiloxane
PE	phycoerythrin
PEEK	poly-ether-ether-ketone
PEG	poly(ethylene glycol)
PEI	polyethylene imine
PEI-g-C	PEI-graft-chitosan
PEI-g-SAC	PEI-conjugated stearic acid-graft chitosan
PEP-C	phosphoenolpyruvate carboxylase
PER	protein efficiency ratio
PES	protein expression signature
PET	photoinduced electron transfer
Pfu DNA	thermostable DNA polymerase named after the thermophilic bacterium *Pyrococcus furiosus*
PG	homopolymer of guluronate
PG	propyl gallate
PG	pressure gauge
PGE2	prostaglandin E_2
PGM	personal genome machine
PHA	poly-β-hydoxyalkanoate
PHA	polyhydroxyalkanoate
PHPA	*p*-hydroxyphenyl acetic acid
pI	isoelectric point
PIA	polysaccharide intercellular adhesin
PIA	pyrrole-imidazole alkaloid
PK	polyketide synthase
PKC	protein kinase C
PKD	polycystic kidney domain-like
PKS	polyketide synthase
PLA	polylactide
PLGA/PCL	poly(D,L-lactide-co-glycolide)/polycaprolactone
PlTX	palytoxin
PM	homopolymer of mannuronate
PM	petrosaspongiolide M
PMMA	poly(methyl methacrylate)
PMSF	phenylmethylsulfonyl fluoride
PMT	photomultiplier
PMT	photomultiplier tube
PNAG	poly-*N*-acetyl glucosamine polysaccharide
PnTX	pinnatoxin
PNV	pneumatic valve
POC	particulate organic carbon
POM	poly-oxy-methylene
PON1	paraoxonase
PP	protein phosphatase
PP	piston pump

PP9	perfluoromethyldecalin
PPC	proparacaine
PPE	poly(*para*-phenylene ethynlyene)
ppm	parts per million
PPO	polyphenol oxidases
PPP	Pentose phosphate pathway
ppp-3	palmitoyl pentapeptide-3
PR	proteorhodopsin
Prialt1	ziconotide
ProLuCID	name of a tandem mass spectrabased protein identification program
PRRSV	porcine reproductive and respiratory syndrome virus
PS	polysaccharide
PSC	pepsin-solubilized collagen
PSF	polysulfone
PSI	photosystem I
PSII	photosystem II
PSP	paralytic shellfish poisoning
PST	paralytic shellfish poisoning toxin
PT	prothrombin time
PT	pressure transducer
PTFE	polytetrafluoroethylene
PtOEP	Pt(II)-octaethylporphin
PTP1B	protein tyrosine phosphatase 1B
PtTX	pteriatoxin
PTX	pectenotoxin
PTX	palytoxin
PTX2SA	PTX2 seco acid
PU	polyurethane
PU-g-SP	pullulan-graft-spermine
PUFA	polyunsaturated fatty acid
PV	polyvinyl
PVA	polyvinyl alcohol
PVC	polyvinylchloride
PVDF	polyvinylidene fluoride

Q

QCM-D	quartz crystal microbalance dissipation
qPCR	quantitative PCR
QQ	Quorum Quenching
QS	quorum sensing
QSI	quorum sensing inhibitor

R

r-silicatein	recombinant silicatein
RACE-PCR	rapid amplification of cDNA ends-PCR
RANK	receptor activator of NF-κB
RANKL	ligand of the receptor activator of NF-κB
RANTES	regulated upon activation, normal T-cell expressed and secreted
RAP	RNA III activating protein
RAPD	random amplified polymorphic DNA
RAS	recirculating aquaculture system
RAST	rapid annotation using subsystem technology
RAW	ATCC cell line
RB	Remazol Black B
RBA	Receptor Binding Assay
RBBR	Remazol Brilliant Blue R
RBC	human red blood cell
RBC	rotary biological contactor
RBE	rat brain endothelial
RDBR	rotating disk bioreactor
rDNA	ribosomal DNA
RDP	Ribosomal Database Project
RFLP	restriction fragment length polymorphism
RFP	red fluorescent protein
RGD	arginylglycylaspartic acid
RGY	Remazol Golden Yellow
RHR	resting heart rate
RIA	radioimmunoassay
RIP	RNA III-inhibiting peptide
RiPP	ribosomally synthesized and post-translationally modified peptide
RISC	RNA-induced silencing complex
RNA	ribonucleic acid
RNAi	RNA interference
RO	reverse osmosis
ROS	reactive oxygen species
RP	magnetically driven vapor recirculation pump
RP-HPLC	reversed phase high performance
RPS	relative percent survival
RPS	rhabdovirus of penaeid shrimp
RR	Remazol Red RR
rRNA	ribosomal RNA
RSM	response surface methodology
RT	reverse transcriptase
RT-PCR	reverse transcription-polymerase chain reaction
RuBisCo	ribulose-1,5-bisphosphate carboxylase-oxygenase
RuBP	ribulose-1,5-bisphosphate

S

S	synthesis
SAG	single amplified genome
SAHA	suberoylanilide hydroxamic acid
SAM	S-adenosyl-L-methionine
SAR	structure-activity relationship
SAV	surface area volume
SBM	soybean meal
SC	stratum corneum
SCA	Sabouraud chloromphenicol agar
SCG	single-cell genomics
SCIE	science citation index expanded
SCO	single-cell oil
SCUBA	self-contained under water breathing apparatus
SDS	sodium dodecyl sulfate
SDS-PAGE	polyacrylamide gel electrophoresis
Se	selenium
SEAP	secreted form of alkaline phosphatase
Sec-tRNA	Selenocysteine-transfer RNA
SELDI	surface enhanced laser desorption/ionization
SEM	scanning electron microscope
SEM	scanning electron microscopy
SeN	selenoneine
SEQUEST	tandem mass spectrometry data analysis program used for protein identification
SERS	surface enhanced Raman scattering
SF	surfactin
SF-268	human CNS cancer cell line
SFE	supercritical fluid extraction
SFF	solid freeform fabrication
SGC 7901	human gastric cancer cell line
SGNH	a motif representing amino acid residues
SGP	polysaccharide
SGR	specific growth rate
SHRV	snakehead rhabdovirus
SIGEX	substrate-induced gene expression
SIP	stable isotope probing
Sir	strontium
siRNA	small (short) interfering RNA
SIV	subintestinal vessel plexus
SL	level of significance
SLM	St. Lawrence Mesocosm
SLRP	small leucine-rich repeat proteoglycan
SLS	sodium laureth sulfate
SMase 1	sphingomyolinase 1
sMBR	submerged-membrane bioreactor
SMF	submerged fermentation
SMTH	serine hydroxymethyltransferase
SMV	spawner-isolated mortality virus
SNP	single nucleotide polymorphisms
SOC	store-operated Ca^{2+}
SOD	superoxide dismutase
SONAR	sound navigation and ranging
SP	splenocyte
SP	sulfated polysaccharide
SPR	surface plasmon resonance
SPS	sulfated polysaccharide
SPX	spirolide
SR	scavenger receptors
SR	sulfate reduction
SRA	Sequence Read Archive
SRB	sulfate-reducing bacteria
SRT	solids retention time
SSAP	*Sebastes schlegeli*
SSCI	social sciences citation index
SSCP	single stranded conformation polymorphism
SSF	solid-state fermentation
SSR	short sequence repeats
ssRNA	single-stranded RNA
SSSF	semi-solid state fermentation
SSU	short subunit
ST-PBR	stirred-tank photobioreactor
STS	secondarily treated sewage
STX	saxitoxin

T

T-DNA	transfer DNA
t-PA	tissue plasminogen activator
TAG	triacylglyceride
TAG	triacylglycerol
TALE	transcription activator-like effector
TALEN	transcription activator-like effector nuclease
TAN	total ammoniacal nitrogen
Taq DNA	thermostable DNA polymerase named after the thermophilic bacterium *Thermus aquaticus*
TARC	thymus and activation regulated chemokine
TBA	thiobarbituric acid
TBHQ	*tert*-butylhydroquinone
TBT	tri-*n*-butyltin
TCA	tricarboxylic acid
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin

TCP	tricalcium phosphate
TDD	transdermal drug delivery
TDDFT	time-dependent density functional theory
TeBactEn	Text Mining for Bacterial Enzymes
TEM	transmission electron microscope
TEM	transmission electron microscopy
TEOS	tetraethoxysilane
TEOS	tetraethyl orthosilicate
TFA	total fatty acid
TG	triglyceride
TGB	thermal gradient block
TGF-β	transforming growth factor beta
TGI	total growth inhibition
TH	tilapia hepcidin
Th2	T helper 2 cells
Ti(BALDH)	titanium (IV) bis(ammonium lactato) dihydroxide
TLC	thin layer chromatography
TLR	toll-like receptor
TMA	transcription mediated amplification
TMOS	tetramethoxysilane
TMP	transmembrane pressure
TMTD	trimethyltridecanoic acid
TNF	tumor necrosis factor
TNF-α	tumor necrosis factor-α
TOCSY	total correlation spectroscopy
TOF	time of flight
TPA	12-O-tetradecanoylphorbol-13-acetate
TPR	tetratricopeptide repeat domain encoding protein
TR-PBR	tubular recycle photobioreactor
TRAP	total radical antioxidant parameter
TRAP	target of RAP
TRSA	tetracycline-resistant *Staphylococcus aureus*
tsa-FISH	tyramide signal amplification FISH
TSV	Taura syndrome virus
TT	thrombin time
TTE	triethylenetetraamine
TTX	11-[^{3}H]-tetrodotoxin

U

u-PA	urokinase plasminogen activator
UA	urocanic acid
UA	uronic acid
UAC	UA-coupled chitosan
UASB	upflow anaerobic sludge blanket
UCP1	uncoupling protein 1
UDP-sugar	nucleoside sugar diphosphate
UF	ultrafiltration
ULS-RDBR	ultralow speed rotating disk bioreactor
USP	ultra-short pulses
UTR	untranslated region
UV	ultraviolet
UVB	ultraviolet B radiation

V

VDA	vascular disrupting agent
VEGF	vascular endothelial growth factor
VFA	volatile fatty acids
VGCC	voltage-gated calcium channel
VGIC	voltage-gated ion channels
VGSC	voltage-gated Na^+ channel
VHSV	viral hemorrhagic septicemia virus
Vira-A1	Vidarabine
VLC	very long-chain
VLDL	very low density lipoprotein
VLP	virus-like particle
VP	viral protein
VRE	vancomycin-resistant *Enterococcus faecium*
VREF	vancomycin-resistant *Enterococcus faecium*
VS	volatile solid
VSV	vesicular stomatitis virus
VTG	estrogen inducible vitellogenin promoter

W

WAP	whey acidic protein
Wap65	warm temperature acclimation-related protein 65
WAT	white adipose tissue
WBC	white blood cell
WGA	whole genome amplification
WHO	World Health Organization
WoRMS	World Register of Marine Species
WSD	white spot disease
WSSV	white spot syndrome virus
ww	wet weight

X

XANES	X-ray adsorption near edge spectroscopy
XOD	xanthine oxidase

Y

YHV	yellow head virus
YTX	yessotoxin

Z

ZFN	zinc finger protein
ZFN	zinc-finger nuclease
ZFP	zinc finger peptide

1. Introduction to Marine Biotechnology

Se-Kwon Kim, Jayachandran Venkatesan

Marine biotechnology is an innovative field of research in science and technology concerning the support of living organisms with marine products and tools. To understand the *omics* of the living species: it is a novel way to produce genetically modified food, drugs, and energy to overcome global demand. The exploitation of biotechnology for drug discovery, including enzymes, antibiotics, and biopolymers, chemical compounds from marine sources are deliberated in this book. The concepts of marine microbiology and molecular biology are explored extensively in the present book. Biomedical applications of marine biomaterials such as tissue engineering, drug delivery, gene delivery, and biosensor areas are thoroughly discussed. Bioenergy from marine sources is a groundbreaking achievement in the field of marine biotechnology and is also covered in this book. Finally, industrial uses of marine-derived products are explored for mankind.

1.1 Marine Biotechnology – Definition

More than 80% of living organisms on earth are found in the aquatic ecosystem. The largest ecosystem on the planet is the ocean; it can be divided into photic, pelagic, benthic, epipelagic, and aphotic zones. More than 40 000 different kinds of species are present in the marine environment, and they are classified as microorganisms, seagrasses, algae, corals, and animals [1.1]. The marine world is considered as a huge reservoir of various biological active compounds. Marine organisms have the capacity to produce unique compounds due to exposure to exceptionally different oceanic environments, such as temperature, chlorophyll content, salinity, and water quality [1.2, 3]. The oldest known fossils are marine stromatolites, which have been evolving for 3.5 billion years; land fossils are about 450 million years old [1.4, 5]. Although the marine world represents nearly $\frac{3}{4}$ of the earth's surface, it is one of most underutilized biological resources.

Biotechnology is the most powerful tool to discover the many secrets of marine organisms and their compounds. There are numerous definitions and explanations that have been given to marine biotechnology since the day its term was coined [1.6, 7]. According to *Food and Agricultural Organization* (FAO, #8), biotechnology can be defined as [1.8]:

> *any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.*

J. Grant Burgess suggested that marine biotechnology is [1.4]:

> *biotechnology carried out using biological resources which have come from the marine environment rather than from the terrestrial environment.*

Alternatively, marine biotechnology is also defined as *the industrial use of living organisms or biological techniques developed through basic research*. In another words, The Organization for Economic Cooperation and Development (OECD) defines biotechnology as

> *the application of science and technology to living organisms, as well as parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services.*

Thakur et al. defined the Marine biotechnology as [1.9, 10]

> *the application of scientific and engineering principles to the processing of materials by marine biological agents provide good and services.*

Another possibility to define the marine biotechnology is that it might be derived from marine bio (techno) logy.

1.2 Marine Biotechnology – Tools

In recent years, advances in instrumentation and a combination of proteomic and bioinformatics are accelerating our ability to harness biology for commercial improvement [1.4]. The marine biotechnological process has significant capacity to improve human life. Several biotechnological tools have been developed for cost-effective products that can be used for medical, industrial, and environmental applications. A variety of biotechnological methods have been adopted from marine sources such as transgenic methods, genomics, fermentation, gene therapy, bioprocess techniques, bioreactor methods, etc. Marine biotechnology is more often considered in terms of molecular or genomic biological application to generate desirable products. It encompasses the production and application of living organisms and is expected to have numerous impacts on our economy. Marine biotechnology promises breakthroughs in areas such as aquaculture, microbiology, metagenomics, nutraceuticals, pharmaceutical, cosmeceuticals, biomaterials, biomineraliza-

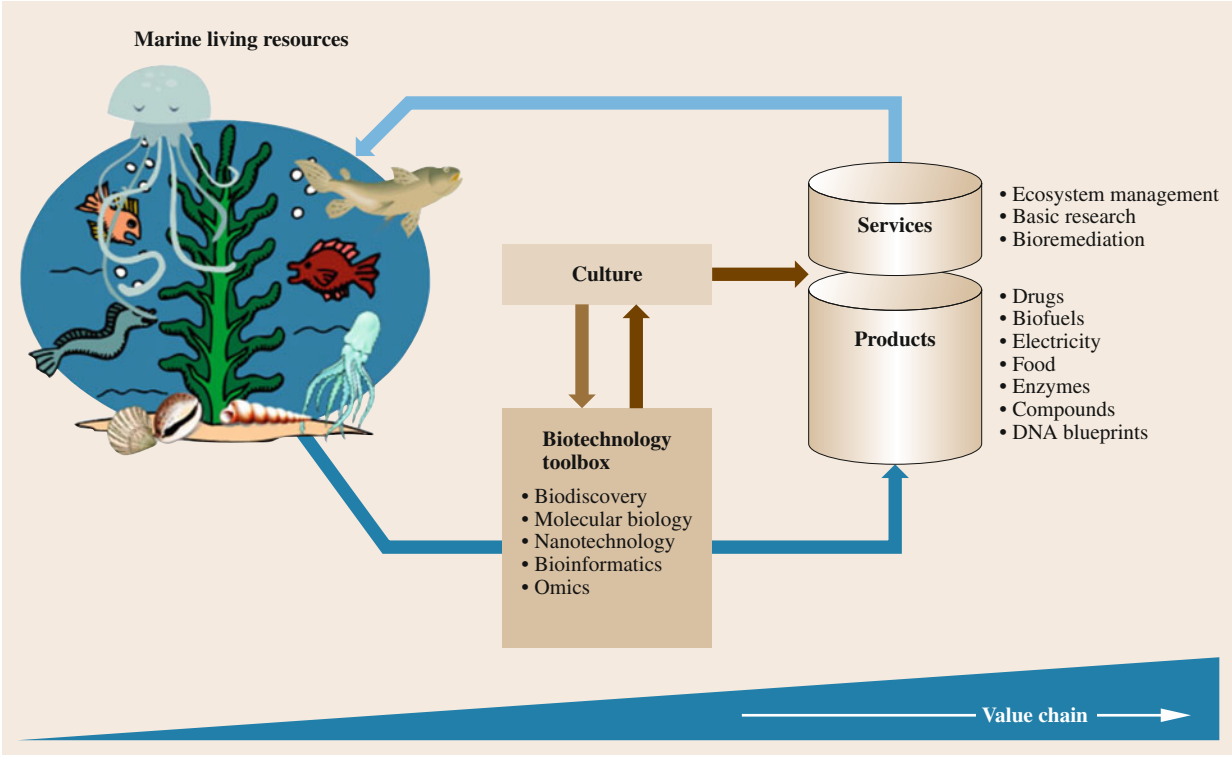

Fig. 1.1 Examples of products and services developed by technological applications using marine bioresources. After [1.11]

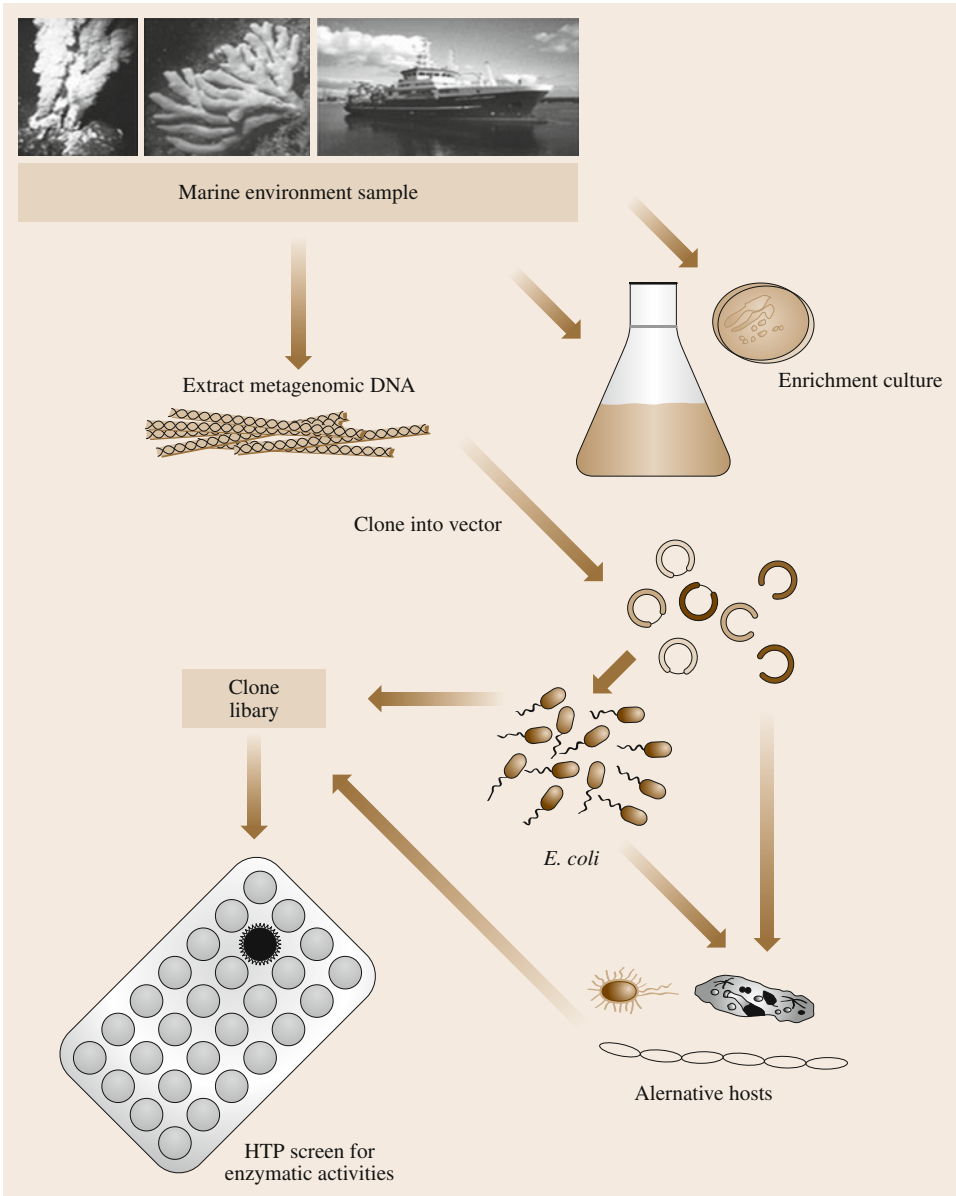

Fig. 1.2 Schematic depiction of functional metagenomic approaches for identification of novel biocatalysts

tion, biofouling, and bioenergy. Products derived by biotechnological methods are commonly more cost efficient with regards to production and also in pure form (Fig. 1.1).

An important consideration in transgenic research is the choice of promoter for regulating the expression of a foreign gene. The discovery of novel processes and techniques in marine biotechnology will create fresh opportunities for the development of innovative materials. The science of biotechnology has given us new tools and tremendous power to create genes and genotypes of plants, animals, and fish. *Lakra* and *Ayyappan* explored the use of synthetic hormones in fish breeding, the production of monosex, uniparental, and polyploid individuals, molecular biology and transgenesis, biotechnology in aquaculture nutrition and health management, gene banking, and marine natural products [1.12].

The biosynthesis and regulation route of many secondary metabolites in marine organisms should be addressed. It is possible, with the recent development of a novel transcriptome profiling methodology that allows for rapid and high-throughput screening of changes in messenger ribonucleic acid (mRNA) sequence pools. The application of genomics-based techniques and the integration of both biochemical and molecular data sets in marine organisms complement ongoing drug discovery efforts [1.13].

Metagenomic-based strategies are powerful tools to isolate and identify enzymes with novel biocatalytic activities from the uncultivable component of microbial communities [1.14]. The recent advances in biotechnological tools such as bioreactors, fermentations, and bioprocessing are useful in the production of functional ingredients, including enzymes that can be used in the food industry [1.15]. Molecular biology is playing a major role in marine biotechnology for an understanding of the genome level. Genomic analysis of marine organisms should be identified to utilize novel genes, proteins, enzymes, and small molecules. The knowledge of metabolic pathways and their genomics is the novel way to understand the mechanism behind the production of the compounds. Metabolic engineering is defined as the optimization of genetic and regulatory pathways to increase the production of compounds by cells (Fig. 1.2).

1.3 Marine Sources and Research Areas

Science and technology continues to move forward in making different technological tools to develop new products from the marine source. Important marine sources in the research are microorganisms, algae, and sponges. Various biotechnological products have been commercialized, ranging from novel drugs, chemicals, and enzymes to bioenergy [1.16–22]. Marine biotechnology plays an important role in the development of various biomaterials, biosensors, seafood safety, aquaculture, bioremediation, and biofouling (Table 1.1). Several drugs are obtained from natural sources, and researchers are still searching for potential organisms from marine sources.

1.4 Applications of Marine Biotechnology

1.4.1 Marine Aquaculture

Marine aquaculture is one of the best examples of marine biotechnology. Fish is one of the most important marine sources for protein supplement in human food. Overfishing and changes in the global environment are contributing to the slow disappearance of this important food resource. By applying marine biotechnological tools, we may be able to provide or improve aquaculture procedures through recombinant technology to develop genetically modified organisms [1.23–26], which could be useful to overcome the global food demand.

1.4.2 Marine Natural Products for Medicine

Marine bioresources are huge reservoirs for various potential biological molecules, which have tremendous potential as human medicines. Natural products are both a fundamental source of a new chemical diversity and an integral component of today's pharmaceutical collection [1.27–33]. Numerous marine compounds are isolated from marine animals, algae, fungi, and bacteria with antibacterial, anticoagulant, antifungal, antimalarial, antiprotozoal, antituberculosis, and antiviral activities. There are now 4 approved products, 13

Table 1.1 Important marine sources and research areas

Research area	Marine source	Aims
Food	Algae, invertebrates, fish	Development of innovative methods, to increase aquaculture production and zero waste recirculation systems
Energy	Algae	Biofuel production, biorefineries
Health	Algae, sponges, microorganisms	To find novel bioactives
Environment	Marine microorganisms	Biosensing technologies for marine environment monitors and non-toxic antifouling technology
Industrial products	Algae	Production of marine biopolymers for food, cosmetics, health

Table 1.2 Examples of market level marine-derived products

Products	Source	Application
Ara-A	Marine sponge	Antiviral
Ara-C	Marine sponge	Anticancer
Okadaic acid	Dinoflagellate	Molecular probe
Manoalide	Marine sponge	Molecular probe
Vent TMA polymerase	Deep-sea hydrothermal vent bacterium	PCR enzyme
Aequorin	Bioluminescent jelly fish	Bioluminescent calcium indicator
Green fluorescent protein	Bioluminescent jelly fish	Reporter gene
Phycoerythrin	Red algae	Conjugated antibodies used in ELISAs and flow cytometry
Cephalosporins	*Cephalosporium* sp., marine fungi	Antibiotic

in clinical trials, and large number of pre-clinical investigations, coming from a wide range of marine sources from many different parts around the world. Prialt ziconotide, a painkiller originally isolated from a Pacific (Philippines) cone snail, Yondelis trabectidin, an anticancer molecule from the Caribbean tunicate *Ecteinascidia turbinata*, and 3-(2,4-dimethoxybenzylidene)-anabaseine (DMXBA) from the ribbon worm *Paranemertes peregrina*, from the Pacific Rim, are a few examples [1.10] (Table 1.2). 59 marine compounds have been reported to affect the cardiovascular, immune, and nervous systems, as well as to possess anti- inflammatory effects. 65 marine metabolites have been shown to bind to a variety of receptors and miscellaneous molecular targets, and thus upon further completion of the mechanism of action studies, will contribute to several pharmacological classes [1.34]. The route to market involves isolation and chemical characterization, followed by synthesis or semi-synthesis of the molecule or an active analog.

Natural product lead compounds from sponges have often been found to be promising pharmaceutical agents. Most of these drugs are used in the treatment of the human immunodeficiency virus (HIV) and the herpes simplex virus (HSV). The most important antiviral lead of marine origin reported thus far is a nucleoside Ara-A (vidarabine), isolated from the sponge *Tethya crypta*. Marine compounds that act on the six hallmarks of cancer presented self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of apoptosis, limitless replication, sustained angiogenesis and tissue invasion, and metastasis [1.35–39].

Marine microbes have a huge biochemical diversity and are likely to become a rich source of novel drugs. Marine microbial compounds are an important source for drug development [1.22]. Marine bacteria are one of the important sources for many bioactive compounds, antibiotics, and pharmaceuticals. They are usually found in marine sediments and are also found to be associated with marine organisms [1.40]. Marine fungi are also reported to be a potential source for bioactive compounds. Polyketide synthases are a class of enzymes that are involved in the biosynthesis of secondary metabolites (erythromycin, rapamycin, tetracycline, lovastatin, and resveratrol).

Actinomycetes are one of the most efficient groups of secondary metabolite producers; they exhibit a wide range of biological activities, including antibacterial, antifungal, anticancer, and insecticidal, and enzyme inhibition. Several species have been isolated and screened from the soil in the past decades. Among its various genera, *Streptomyces, Saccharopolyspora, Amycolatopsis, Micromonospora*, and *Actinoplanes* are the major producers of commercially important biomolecules [1.41]. Actinomycetes are virtually unlimited sources of new compounds with many therapeutic applications and hold a prominent position due to their diversity and proven ability to produce novel bioactive compounds; 70% of which are produced by actinomycetes, 20% from fungi, 7% from *Bacillus* sp. and 1−2% by other bacteria [1.42]. Antimicrobial peptides are promising candidates, because their initial interaction with microbes is through binding to lipids [1.43].

Dinoflagellate toxins and bioactives are of increasing interest because of their commercial impact [1.44]. Functional screens to isolate novel cellulases, lipases and esterases, proteases, laccases, oxidoreductases, and biosurfactants have been described [1.45]. Enzyme inhibitors have received increasing attention as useful tools for the study of enzyme structures and their mechanisms. Marine organisms have been documented as a productive source for the enzyme inhibitors. Several commercialized products are shown in Table 1.2. Arebinosyl cytosine (Ara-C) is currently sold by the Pharmacia and Upjohn company under the brand name Cytosar-R [1.10].

1.4.3 Marine Nutraceuticals

Marine nutraceuticals can be derived from a vast array of sources, including marine plants, microorganisms, and sponges. Marine nutraceutical products currently promoted to various countries include fish oil, chitin, chitosan, marine enzymes, chondroitin from shark cartilage, sea cucumbers, and mussels. As mentioned earlier, the marine world represents a largely unexploited reservoir of bioactive substances that can be used for food processing, storage, and protection. Enzymes extracted from fish and marine organisms can provide numerous advantages over traditional enzymes. Fish protein such as collagens and their gelatin derivatives operate at relatively low temperatures and can be used in heat sensitive processes such as gelling and clarifying. Polysaccharides derived from alga, including alginate, carrageenan, and agar types are widely used as thickeners and stabilizers in a variety of food ingredients. In addition, Omega-PUFA (polyunsaturated fatty acid) is an important ingredient in the nutraceutical industry [1.46]. It has been proven that Omega-PUFA, especially eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA) play a significant role in number of aspects of human health [1.47]. The application potential of chitin and chitosan are multidimensional, for example, in food and nutrition, biotechnology, material science, drugs and pharmaceuticals, agriculture and environmental protection, and gene therapy [1.48–52]. Fucoidan is a complex-sulfated polysaccharide, which can be derived from brown algae. Fucoidan has been used in several biological activities and is important for its high bioactive properties, for example, antibacterial, anticoagulant, antiviral, antitumor, etc., and many more yet to be explored [1.53].

1.4.4 Marine Biomaterials

In the recent years, much attention has been paid to marine-derived biomaterials for various biological, biomedical, and environmental applications. A recent report estimates that the global markets for marine biotechnological products might exceed US$ 4.1 billion by 2015, to which the following product segments, marine biomaterials (including food hydrocolloids) could contribute over 40%; marine bioactive substances for healthcare would be the most important and fastest-growing sector. Non-toxic, biocompatible, natural chitin and chitosan from crustaceans have potential use in cosmetics, food, and pharmaceuticals [1.54]. Seaweeds are the abundant source for polysaccharides, which are commercial products (alginate, agar, agarose, and carrageenan) [1.55–61].

1.4.5 Marine Bioenergy

Bioenergy from marine algae is a groundbreaking achievement in the field of marine biotechnology [1.62–67]. Biofuels derived from marine algae are a potential source of sustainable energy that can contribute to future global demands. The realization of this potential will require manipulation of the fundamental biology of algal physiology to increase the efficiency with which solar energy is ultimately converted into usable biomass [1.68]. Anaerobic digestion of microalgae is a necessary step to make microalgae biodiesel and biogas sustainable [1.69, 70]. The potential biomass sources for bioenergy are photosynthetic microalgae and cyanobacteria. There are versatile marine organisms that can be used in the production of biogas, biodiesel, bioethanol, and biohydrogen [1.71–76].

1.4.6 Marine Bioremediation

Bioremediation is also an important area of marine environmental biotechnology. Marine microorganisms have the capacity to degrade the variety of organic pollutants. *Pseudomonas chlororaphis* produces pyoverdin, which catalyzes the degradation of organotin compounds in seawater. Biopolymers and biosurfactants are also applied to environmental waste management and treatment [1.77–81].

1.5 Research Scope

Marine biotechnology plays a vital role in the exploration and study of various marine resources. Marine biotechnology comprises a broad range of subjects: marine bioactive substances, genetics, marine culture, fermentation engineering, and enzyme engineering. The marine biotechnology market is still in the promising stages; during the years 2008 and 2009, the global marine biotechnology market witnessed a slowdown owing to the global economic meltdown. The market gained drive in 2010 with the recovery of the economic situation and is expected to post substantial growth in ensuing years.

Table 1.3 Countries and their marine biotechnology research priorities

Countries		Research priorities
Africa	Mozambique, Nigeria, South Africa, Tunisia and Kenya	Biofuels and bioactives
Central and South America	Brazil, Chile, Argentina, Mexico, Costa Rica	Biodiscovery, bioenergy, bioremediation and biofouling
North America	USA, Canada	Biodiscovery, aquaculture and biofuels
Asia	China, India, South Korea, Japan, Taiwan	Biofuels, biodiscovery for human pharmaceuticals, food, feed, cosmetics
Middle East	Israel	Sponge biotechnology, marine bioactives and biofuels
South East Asia, Indian Islands	Thailand, Vietnam, Indonesia, Malaysia, Singapore, Sri Lanka, the Philippines	Biodiversity for novel bioactives and aquaculture,
Australia Pacific	Australia and New Zealand	Aquaculture and marine bioactives

The research drive on marine biotechnology is high in countries like USA, Brazil, Canada, China, Japan, the Republic of Korea, and Australia, as well as in other countries where activities are grow from a smaller base (Thailand, India, Chile, Argentina, Mexico, and South Africa), and where there are signs that marine biotechnology is increasing in importance as a research priority. It is notable that the major international effort, the Census of Marine Life (CoML), involved 2700 researchers, about 31% from Europe, 44% from USA and Canada, and 25% from the rest of the world, notably Australia, New Zealand, Japan, China, South Africa, India, Indonesia, and Brazil (Table 1.3) [1.82].

The United States is the world leader and represents the single largest region for marine biotechnology worldwide. The marine bioactive substances market is forecasted to register the fastest growth rate of more than 4.0% during the period 2009–2015. Healthcare/biotechnology constitutes the largest, as well as fastest growing, end use for marine biotechnology. Very few countries have initiated national R&D programs to exploit the benefits of biotechnology in the marine sector. However, advances in aquaculture, drug discovery and fisheries are expected to encourage applications of marine biotechnology. The research report titled as *Marine Biotechnology: A Global Strategy Business Report* provides a comprehensive review of the marine biotechnology market, recession on the markets, current market trends, key growth deliverers, introductions of recent products, recent activity in the industry (Aker Bio Marine ASA, CP Kelco US Inc., Cyanotech Corp., Elan Corporation plc, FMC Corp., FMC Biopolymers AS, GlycoMar Ltd., Integrin Advanced Biosystems, International Specialty Products Inc., Lonza Group Ltd., MariCal, Marinova, Martek Biosciences Corp., Mera Pharmaceuticals Inc., New England Biolabs Inc., PharmaMar S.A, PML Applications Ltd., Primex Ltd., Prolume Ltd., Sea Run Holdings Inc., and Tequesta Marine Biosciences), and the profile of major global as well regional market participants. The harnessing of marine resources through biotechnology and development of products and services should be a serious target for any country with significant aquatic biodiversity.

A major task of marine biotechnology is to develop an efficient process for the discovery of novel molecules from the marine environment. The high level of marine biodiversity of marine organisms makes them a prime target for bioprospecting; these are enzymes, bioactive molecules, and biopolymers with varied industrial applications. Biochemical studies of marine organisms are an important task for the discovery of new drug molecules and biological tools and management of biodiversity.

1.6 Organization of the Handbook

This handbook combines the knowledge of sea flora and fauna, biotechnological methods, product development and industrial applications. It is divided into 10 parts. The introduction of the book comprises the definition, history and research scope of marine biotechnology. The first part introduces marine flora and fauna in detail, such as fungi, phototrophs, viruses, microalgae, seaweed, coral, and sponges. In this part, a detailed explanation is given on the production, cultivation, and processing of flora and fauna. The second part of the book introduces the tools and method of marine biotechnology; it covers, bioprocess engineering,

bioinformatics techniques, bioreactors, transgenic technology, quorum sensing, and molecular methods for the detection of invasive species. The third part of the book provides details about marine metagenomics, proteomics, marine metagenomics and supporting technology, microfluidic systems, and genomic mining. The fourth part of the book deals with algal biotechnology, starting with the structure and biological activities of marine algal polysaccharides, two centuries of research on iodine in seaweeds, marine macrophytes, and heavy metal removal by marine algae. The fifth part of the book covers marine microbiology, marine microbial biotechnology, and marine actinomycetes.

The sixth part of the book provides details about marine-derived metabolites, starting with marine natural products, biocatalysts, antimicrobial peptides, marine-derived fungal metabolites, dinoflagellates, carotenoids, Cnidarians, fatty acids, biotoxins, microbial enzymes, and polysaccharides. The seventh part of the book focuses on applications of marine biotechnology, starting with pharmaceuticals, functional food, nutraceuticals and cosmeceuticals. The eighth part of the book covers bioenergy and biofuels; here, the lead authors discuss marine bioenergy, marine algal biotechnology for bioenergy, and biofuels. Biomedical applications are extensively discussed in the ninth part of this book; the topics are marine biomaterials, gene delivery, biosensors, and biomineralization. Finally, the last part of this book focuses on industrial applications of marine biotechnology.

References

1.1 J.W. Nybakken: *Marine Biology: An Ecological Approach* (Harper Collins, New York 1993)

1.2 X. Irigoien, J. Huisman, R.P. Harris: Global biodiversity patterns of marine phytoplankton and zooplankton, Nature **429**, 863–867 (2004)

1.3 J.H. Steele: A comparison of terrestrial and marine ecological systems, Nature **313**, 355–358 (1985)

1.4 J.G. Burgess: New and emerging analytical techniques for marine biotechnology, Curr. Opin. Biotechnol. **23**, 29 (2012)

1.5 D.A. Caron, P.D. Countway, A.C. Jones, D.Y. Kim, A. Schnetzer: Marine protistan diversity, Annu. Rev. Mar. Sci. **4**, 467–493 (2012)

1.6 D. Regan: Marine biotechnology and the use of arid zones, Search **11**, 377–381 (1980)

1.7 R.R. Colwell: Biotechnology in the marine sciences, Science **222**, 19–24 (1983)

1.8 FAO: FAO statement on biotechnology. Biotechnology in food and agriculture, available online at http://www.fao.org/Biotech/stat.asp (2000)

1.9 R.A. Zilinskas, R. Colwell, D. Lipton, R. Hill: *The Global Challenge of Marine Biotechnology: A Status Report on the United States, Japan, Australia, and Norway* (Maryland Sea Grant College, College Park, MD 1995)

1.10 N.L. Thakur, A.N. Thakur: Marine Biotechnology: an overview, Indian J. Biotechnol. **5**, 263 (2006)

1.11 Flanders Marine Institute (VLIZ): http://www.coastalwiki.org/wiki/Category:Marine_Biotechnology

1.12 W. Lakra, S. Ayyappan: Recent advances in biotechnology applications to aquaculture, Asian Australas. J. Anim. Sci. **16**, 455–462 (2003)

1.13 C.A. Hoover, M. Slattery, A.G. Marsh: A functional approach to transcriptome profiling: Linking gene expression patterns to metabolites that matter, Mar. Biotechnol. **9**, 411–419 (2007)

1.14 J. Kennedy, J.R. Marchesi, A.D. Dobson: Marine metagenomics: Strategies for the discovery of novel enzymes with biotechnological applications from marine environments, Microb. Cell Fact. **7**, 27 (2008)

1.15 A.C. Freitas, D. Rodrigues, T.A. Rocha-Santos, A.M. Gomes, A.C. Duarte: Marine biotechnology advances towards applications in new functional foods, Biotechnol. Adv. **30**, 1506–1515 (2012)

1.16 M.A. Borowitzka: Commercial production of microalgae: Ponds, tanks, and fermenters, Prog. Ind. Microbiol. **35**, 313–321 (1999)

1.17 P. Spolaore, C. Joannis-Cassan, E. Duran, A. Isambert: Commercial applications of microalgae, J. Biosci. Bioeng. **101**, 87–96 (2006)

1.18 R.J. Radmer: Algal diversity and commercial algal products, Bioscience **46**, 263–270 (1996)

1.19 D. Leary, M. Vierros, G. Hamon, S. Arico, C. Monagle: Marine genetic resources: A review of scientific and commercial interest, Mar. Policy **33**, 183–194 (2009)

1.20 J.J. Milledge: Commercial application of microalgae other than as biofuels: A brief review, Rev. Environ. Sci. Bio/Technology **10**, 31–41 (2011)

1.21 T. Rustad, I. Storrø, R. Slizyte: Possibilities for the utilisation of marine by-products, Int. J. Food Sci. Technol. **46**, 2001–2014 (2011)

1.22 J.F. Imhoff, A. Labes, J. Wiese: Bio-mining the microbial treasures of the ocean: New natural products, Biotechnol. Adv. **29**, 468–482 (2011)

1.23 B.H. Buck: *Marine Biotechnology in Germany: Aquaculture in the Open Ocean* (King Mongkuts University of Technology North, Bangkok 2011)

1.24 Y. Tal, H.J. Schreier, K.R. Sowers, J.D. Stubblefield, A.R. Place, Y. Zohar: Environmentally sustainable land-based marine aquaculture, Aquaculture **286**, 28–35 (2009)

1.25 R.J. Ritchie, K. Guy, J.C. Philp: Policy to support marine biotechnology-based solutions to global challenges, Trends Biotechnol. **31**, 128–131 (2013)

1.26 M.C. Alvarez, J. Bejar, S. Chen, Y. Hong: Fish ES cells and applications to biotechnology, Mar. Biotechnol. **9**, 117–127 (2007)

1.27 W. Fenical, P.R. Jensen: Developing a new resource for drug discovery: Marine actinomycete bacteria, Nat. Chem. Biol. **2**, 666–673 (2006)

1.28 L. Bohlin, U. Göransson, C. Alsmark, C. Wedén, A. Backlund: Natural products in modern life science, Phytochem. Rev. **9**, 279–301 (2010)

1.29 J.W. Blunt, B.R. Copp, R.A. Keyzers, M.H. Munro, M.R. Prinsep: Marine natural products, Nat. Prod. Rep. **30**, 237–323 (2013)

1.30 R.A. Hill: Marine natural products, Annu. Rep. B (Organ. Chem.) **108**, 131–146 (2012)

1.31 W.H. Gerwick, B.S. Moore: Lessons from the past and charting the future of marine natural products drug discovery and chemical biology, Chem. Biol. **19**, 85–98 (2012)

1.32 E. Fattorusso, W.H. Gerwick, O. Taglialatela-Scafati: *Handbook of Marine Natural Products* (Springer, New York 2012)

1.33 S. Agatonovic-Kustrin, D. Morton, C. Kettle: Structural characteristics of bioactive marine natural products. In: *Marine Biomaterials: Characterization, Isolation, and Applications*, ed. by S.-K. Kim (Taylor Francis, Boca Raton 2013)

1.34 A. Mayer, A.D. Rodríguez, R.G. Berlinck, N. Fusetani: Marine pharmacology in 2007–8: Marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous system, and other miscellaneous mechanisms of action, Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. **153**, 191–222 (2011)

1.35 M. Schumacher, M. Kelkel, M. Dicato, M. Diederich: Gold from the sea: Marine compounds as inhibitors of the hallmarks of cancer, Biotechnol. Adv. **29**, 531–547 (2011)

1.36 I. Orhan, B. Şener, M. Kaiser, R. Brun, D. Tasdemir: Inhibitory activity of marine sponge-derived natural products against parasitic protozoa, Mar. Drugs **8**, 47–58 (2010)

1.37 D.S. Dalisay, B.I. Morinaka, C.K. Skepper, T.F. Molinski: A tetrachloro polyketide Hexahydro-1H-isoindolone, Muironolide A, from the marine sponge *Phorbas* sp. Natural products at the nanomole scale, J. Am. Chem. Soc. **131**, 7552 (2009)

1.38 P. Proksch, A. Putz, S. Ortlepp, J. Kjer, M. Bayer: Bioactive natural products from marine sponges and fungal endophytes, Phytochem. Rev. **9**, 475–489 (2010)

1.39 I. Wijesekara, N.Y. Yoon, S.-K. Kim: Phlorotannins from *Ecklonia cava* (Phaeophyceae): Biological activities and potential health benefits, Biofactors **36**, 408–414 (2010)

1.40 N.L. Thakur, U. Hentschel, A. Krasko, C.T. Pabel, A.C. Anil, W.E.G. Müller: Antibacterial activity of the sponge *Suberites domuncula* and its primmorphs: potential basis for epibacterial chemical defense, Aquat. Microb. Ecol. **31**, 77–83 (2003)

1.41 R. Solanki, M. Khanna, R. Lal: Bioactive compounds from marine actinomycetes, Indian J. Microbiol. **48**, 410–431 (2008)

1.42 R. Subramani, W. Aalbersberg: Marine actinomycetes: An ongoing source of novel bioactive metabolites, Microbiol. Res. **167**, 571–580 (2012)

1.43 S.V. Sperstad, T. Haug, H.-M. Blencke, O.B. Styrvold, C. Li, K. Stensvåg: Antimicrobial peptides from marine invertebrates: Challenges and perspectives in marine antimicrobial peptide discovery, Biotechnol. Adv. **29**, 519–530 (2011)

1.44 F.G. Camacho, J.G. Rodríguez, A.S. Mirón, M. Garcia, E. Belarbi, Y. Chisti, E.M. Grima: Biotechnological significance of toxic marine dinoflagellates, Biotechnol. Adv. **25**, 176–194 (2007)

1.45 J. Kennedy, N. O'Leary, G. Kiran, J. Morrissey, F. O'Gara, J. Selvin, A. Dobson: Functional metagenomic strategies for the discovery of novel enzymes and biosurfactants with biotechnological applications from marine ecosystems, J. Appl. Microbiol. **111**, 787–799 (2011)

1.46 R.S. Rasmussen, M.T. Morrissey: Marine biotechnology for production of food ingredients, Adv. Food Nutr. Res. **52**, 237–292 (2007)

1.47 M. Venegas-Calerón, O. Sayanova, J.A. Napier: An alternative to fish oils: Metabolic engineering of oilseed crops to produce omega-3 long chain polyunsaturated fatty acids, Prog. Lipid Res. **49**, 108–119 (2010)

1.48 R. Tharanathan, K. Prashanth: Chitin/chitosan: modifications and their unlimited application potential-dan overview, Trends Food Sci. Technol. **18**, 117–131 (2007)

1.49 J.-K. Francis Suh, H.W. Matthew: Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: A review, Biomaterials **21**, 2589–2598 (2000)

1.50 G. Crini, P.-M. Badot: Application of chitosan, a natural aminopolysaccharide, for dye removal from aqueous solutions by adsorption processes using batch studies: A review of recent literature, Prog. Polym. Sci. **33**, 399–447 (2008)

1.51 M.L. Kang, C.S. Cho, H.S. Yoo: Application of chitosan microspheres for nasal delivery of vaccines, Biotechnol. Adv. **27**, 857–865 (2009)

1.52 S. Bahmani, G. East, I. Holme: The application of chitosan in pigment printing, Color Technol. **116**, 94–99 (2000)

1.53 V. Morya, J. Kim, E.-K. Kim: Algal fucoidan: Structural and size-dependent bioactivities and their perspectives, Appl. Microbiol. Biotechnol. **93**, 71–82 (2012)

1.54 J. Venkatesan, S.-K. Kim: Chitosan composites for bone tissue engineering—An overview, Mar. Drugs **8**, 2252–2266 (2010)

1.55 T.H. Silva, A.R. Duarte, J. Moreira-Silva, J.F. Mano, R.L. Reis: Biomaterials from marine-origin Biopoly-

mers. In: *Biomimetic Approaches for Biomaterials Development*, ed. by J.F. Mano (Wiley, Weinheim 2012) pp. 1–23

1.56 J.H. Waite, C.C. Broomell: Changing environments and structure–property relationships in marine biomaterials, J. Exp. Biol. **215**, 873–883 (2012)

1.57 S.-K. Kim, F. Karadeniz, M.Z. Karagozlu: Treatment of obesity and diabetes with marine-derived biomaterials. In: *Marine Biomaterials: Characterization, Isolation, and Applications*, ed. by S.-K. Kim (Taylor Francis, Boca Raton 2013) p. 437

1.58 S.-K. Kim: *Marine Biomaterials: Characterization, Isolation, and Applications* (Taylor Francis, Florida 2013)

1.59 C. Zhang, X. Li, S.-K. Kim: Application of marine biomaterials for nutraceuticals and functional foods, Food Sci. Biotechnol. **21**, 625–631 (2012)

1.60 N. Nwe, T. Furuike, H. Tamura: Isolation and characterization of chitin and chitosan as potential biomaterials. In: *Marine Biomaterials: Characterization, Isolation, and Applications*, ed. by S.-K. Kim (Taylor Francis, Florida 2013) p. 45

1.61 S.-K. Kim, I. Bhatnagar, R. Pallela: Microbial biomaterials and their applications. In: *Marine Biomaterials: Characterization, Isolation, and Applications*, ed. by S.-K. Kim (Taylor Francis, Florida 2013) p. 457

1.62 C. Bogen, V. Klassen, J. Wichmann, M.L. Russa, A. Doebbe, M. Grundmann, P. Uronen, O. Kruse, J.H. Mussgnug: Identification of Monoraphidium contortum as a promising species for liquid biofuel production, Bioresour. Technol. **133**, 622–626 (2013)

1.63 A.F. Ferreira, L.A. Ribeiro, A.P. Batista, P.A.S.S. Marques, B.P. Nobre, A.M.F. Palavra, P.P. da Silva, L. Gouveia, C. Silva: A biorefinery from Nannochloropsis sp. microalga – Energy and CO2 emission and economic analyses, Bioresour. Technol. **138**, 235–244 (2013)

1.64 J.C. Frigon, F. Matteau-Lebrun, R. Hamani Abdou, P.J. McGinn, S.J.B. O'Leary, S.R. Guiot: Screening microalgae strains for their productivity in methane following anaerobic digestion, Appl. Energy **108**, 100–107 (2013)

1.65 A.D. Hughes, K.D. Black, I. Campbell, K. Davidson, M.S. Kelly, M.S. Stanley: Does seaweed offer a solution for bioenergy with biological carbon capture and storage?, Greenh. Gases: Sci. Technol. **2**, 402–407 (2012)

1.66 J. Lu, Y. Zhang: Spatial distribution of an invasive plant *Spartina alterniflora* and its potential as biofuels in China, Ecol. Eng. **52**, 175–181 (2013)

1.67 J.H. Park, H.C. Cheon, J.J. Yoon, H.D. Park, S.H. Kim: Optimization of batch dilute-acid hydrolysis for biohydrogen production from red algal biomass, Inter. J. Hydrog. Energy **38**, 6130–6136 (2013)

1.68 P.G. Stephenson, C.M. Moore, M.J. Terry, M.V. Zubkov, T.S. Bibby: Improving photosynthesis for algal biofuels: Toward a green revolution, Trends Biotechnol. **29**, 615–623 (2011)

1.69 B. Sialve, N. Bernet, O. Bernard: Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable, Biotechnol. Adv. **27**, 409–416 (2009)

1.70 A.D. Hughes, M.S. Kelly, K.D. Black, M.S. Stanley: Biogas from Macroalgae: Is it time to revisit the idea?, Biotechnol. Biofuels **5**, 1–7 (2012)

1.71 C.S. Jones, S.P. Mayfield: Algae biofuels: versatility for the future of bioenergy, Curr. Opin. Biotechnol. **23**, 346–351 (2012)

1.72 T. Burton, H. Lyons, Y. Lerat, M. Stanley, M.B. Rasmussen: *A Review of the Potential of Marine Algae as a Source of Biofuel in Ireland* (Sustainable Energy Ireland-SEI, Dublin 2009)

1.73 T. Matsunaga, M. Matsumoto, Y. Maeda, H. Sugiyama, R. Sato, T. Tanaka: Characterization of marine microalga, *Scenedesmus* sp. strain JPCC GA0024 toward biofuel production, Biotechnol. Lett. **31**, 1367–1372 (2009)

1.74 T.T.Y. Doan, B. Sivaloganathan, J.P. Obbard: Screening of marine microalgae for biodiesel feedstock, Biomass Bioenergy **35**, 2534–2544 (2011)

1.75 M. Matsumoto, H. Sugiyama, Y. Maeda, R. Sato, T. Tanaka, T. Matsunaga: Marine diatom, *Navicula* sp. strain JPCC DA0580 and marine green alga, *Chlorella* sp. strain NKG400014 as potential sources for biodiesel production, Appl. Biochem. Biotechnol. **161**, 483–490 (2010)

1.76 P.M. Schenk, S.R. Thomas-Hall, E. Stephens, U.C. Marx, J.H. Mussgnug, C. Posten, O. Kruse, B. Hankamer: Second generation biofuels: High-efficiency microalgae for biodiesel production, Bioenergy Res. **1**, 20–43 (2008)

1.77 W.R. Jones: Practical applications of marine bioremediation, Curr. Opin. Biotechnol. **9**, 300–304 (1998)

1.78 M. Milanese, E. Chelossi, R. Manconi, A. Sara, M. Sidri, R. Pronzato: The marine sponge *Chondrilla nucula* Schmidt, 1862 as an elective candidate for bioremediation in integrated aquaculture, Biomol. Eng. **20**, 363–368 (2003)

1.79 K. Watanabe: Microorganisms relevant to bioremediation, Curr. Opin. Biotechnol. **12**, 237–241 (2001)

1.80 T. Matsunaga, H. Takeyama, T. Nakao, A. Yamazawa: Screening of marine microalgae for bioremediation of cadmium-polluted seawater, Prog. Ind. Microbiol. **35**, 33–38 (1999)

1.81 Y. Cohen: Bioremediation of oil by marine microbial mats, Int. Microbiol. **5**, 189–193 (2002)

1.82 Marine Biotechnology: ERA-NET and Marine Biotechnology international summary, available online at http://www.marinebiotech.eu/wiki/Marine_Biotechnology_international_summary

Marine F Part A

Part A Marine Flora and Fauna

2. Marine Fungal Diversity and Bioprospecting

Kalaiselvam Murugaiyan

Marine fungi are a large group of eukaryotic organisms. Marine fungi, and particularly wood-inhabiting fungi, have been extensively studied since 1944. These have been termed lignicolous fungi and constitute more than 50% of the total 450 species of obligate marine fungi described so far. Marine fungi occur not only in water and sediment, but also as parasites on plants and animals, as well as symbionts in marine lichens, plants, and algae. A rich pool of fungal species is yet to be discovered and investigated over the coming years. About 150 species have been found exclusively on decaying mangrove wood, aerial roots, and seedlings, and are categorized as *Manglicolous fungi*; most of the species belong to the class of *Ascomycetes*. Fungi in mangroves play a significant role in litter decomposition and nutrient cycling, thereby contributing to the fertility of the environment. Fungal biomass along with detritus contributes significantly to the food chain of detritus-feeding organisms found in mangroves. *Aspergillus* and *Penicillium* are the dominant fungi involved in litter decomposition of mangroves. Fungal endophytes are microfungi; they colonize the internal tissues of vascular plants without producing any apparent disease symptoms and are considered as an important component of biodiversity. The distributions of endophytic mycoflora differ with the host and modify the host plants at genetical, physiological, and ecological levels. These modifications induce profound changes in how plants respond to their environments. The environmental and biological factors such as the availability of substrates or hosts, salinity, hydrostatic pressure, temperature, and the availability of oxygen control the distribution of marine fungi. The adaptation of marine fungi in an extreme environment suggests that they are promising sources for screening natural products.

2.1 Preamble

Fungi are eukaryotic and non-vascular organisms. The cells of fungi contain nuclei with chromosomes. A fungus is neither a plant nor an animal. The cell walls of fungi are similar to the structure of the cell walls of plants. The only different is in the chemical composition. The fungi's cell wall is composed mainly of chitin, while plant cell walls are mostly made of cellulose. Their cytoplasmic ultrastructure is also similar to that

of plant cells and differs only in the organelles and their structures. Furthermore, fungi are heterotrophic, feeding on other organisms. Unlike animals (heterotrophic) which ingest and then digest food, fungi will first digest food then ingest it.

Most fungi are multicellular; some are unicellular and are composed of filaments called hyphae. Hyphae may contain internal cross walls called septa. Septa may have pores allowing cytoplasmic contents to flow freely from one cell to another, through which they obtain their nutrients from the host. Hyphae may be branched; a dense mass of hyphae is called a mycelium. To maximize nutrient uptake, filamentous fungi form mats of fine hyphae. Fungi reproduces using spores through wind-dissemination. Both types of spores (sexual and asexual) may be reproduced, depending on the species and conditions. They primarily function as recyclers of organic material.

2.2 Diversity of Fungi

The kingdom Fungi is the second largest group after insects and is widely distributed in nature. A conservative estimate of the total number of fungal species thought to exist is 1.5 million [2.1], with only 71 000 having been described so far [2.2]; the vast majority of all extant fungi is yet to be named. Assuming a relatively constant rate at which new species are described, it will take more than 1100 years to catalog and describe all remaining fungi. However, many of these fungi are likely to become extinct before they are ever discovered, given the current rates of habitat and host loss. For example, up to 2% of tropical forests are destroyed globally each year. These habitats are exceedingly rich in fungal species. It should be considered that this estimate is based only on plant parasitic fungi and does not take into account other ecological groups of fungi such as saprotrophs.

2.2.1 Current Status of Marine Fungi

The first scientific report on marine mycology was published in the early 1900s; therein marine fungi are distinct from their terrestrial and freshwater counterparts [2.3, 4]. 24 species of marine and terrestrial fungi have been found on wood-blocks submerged in water from brackish water lakes in Japan [2.5]. Very little scientific information is available on the occurrence and distribution of fungi inhabiting marine environments, including marine mangroves [2.6]. A saprophytic and facultative parasitic fungus was reported in the coastal waters and adjacent pelagic areas of the Hawaiian Islands [2.7]. More than 800 microorganisms have been isolated from marine sediments in Italy [2.8].

Fungal diversity on prop roots, seedlings, and wood of *Rhizophora apiculata*, and wood, roots, and pneumatophores of *Avicennia* spp. are found in the deltaic mangroves of Godavari and Krishna rivers, on the east coast of India [2.9]. Frequency of occurrence and biodiversity of the fungi have been reported from mangroves of the Godavari and Krishna deltas, on the east coast of India [2.10]. The assemblage and diversity of filamentous fungi on leaf and woody litter accumulated on the floor of two mangrove forests (Nethravathi and Udyavara) on the southwest coast of India have been studied [2.11].

The diversity and ecology of fungi colonizing litter of mangroves in the Bay of Bengal region has been studied in the mangroves of the Godavary and Krishna deltas of Andhra Pradesh, Pichavaram of Tamilnadu, Andaman, and Nicobar islands [2.12]. The occurrence of fungi and a checklist of fungi have been prepared based on the pilot study in the Pichavaram mangroves of southeast India [2.13]. After the tsunami on December 26, 2004, the occurrence of filamentous fungi on woody debris by means of short-term (1 month) and long-term (12 months) damp incubation were investigated along five coastal locations on the southeast coast of India [2.14]. Marine fungi associated with decaying wood samples in the brackish water mangrove ecosystem and shoreline ecosystem was reported in south India [2.15]. Filamentous fungal diversity from the sediments of the continental slope has been investigated along the Bay of Bengal [2.16]. Floristic diversity and phorophyte specificity of lichens have been studied along the southeast coast of India, revealing that the latex bearing *Excoecaria agallocha* bears a maximum of lichen diversity followed by *Rhizophora* sp. [2.17, 18]. The above global and Indian scenario of fungal diversity provides unique opportunities for mycologists to explore fungal diversity and exploit the ecological, medicinal, and industrial potentials of fungi.

2.2.2 Major Groups of Fungi

True fungi belong to the Eukaryota kingdom, which has four phyla, 103 orders, 484 families, and 4979 gen-

era. The Deuteromycotina is not accepted as a formal taxonomic category. About 205 new genera have been described from India. Of these, approximately 27 000 species are reported to colonize diversified habitats. The major groups (phyla) of true fungi are *Ascomycota, Basidiomycota, Chytridiomycota*, and *Zygomycota*. Recently, studies have provided support for the recognition of additional phyla, such as *Glomeromycota*, a group of fungi once placed in *Zygomycota*, which form an association with the roots of most plants. A group of parasitic organisms called *Microsporidia* that live inside the cells of animals are also now considered to belong in the fungal kingdom.

A comprehensive classification of the kingdom Fungi was published as the result of collaboration among many fungal taxonomists [2.19]. This classification is used in the *Dictionary of the Fungi* [2.1] and other fungal references and databases. However, the classification system will undergo additional changes as scientists use new methods to study fungi. For example, *Cryptomycota*, a potentially new phylum of organisms within the fungi kingdom has been described. Recent findings suggest that a total of 625 fungi exist on a global scale (278 *Ascomycetes*, 277 anamorphic taxa, 30 *Basidiomycetes*, and 14 *Oomycetes*) and about 150 species of mangrove fungi have been reported from the mangroves of the Indian subcontinent.

Zygomycotina

Zygomycotina undergo asexual reproduction and are ubiquitous in soil and dung. Most of them are saprophytes and very few are parasitic on plants and animals. Trichomycetous fungi colonize the guts of arthropods. More than 1000 fungal species belonging to *Zygomycotina* have been reported from India, which are of high industrial value. For example, *Saksenea vasiformis*, a unique, indigenous fungus, has received special attention in medical mycology.

Ascomycotina

It is estimated that the *Ascomycetes* form approximately 40–45% of total fungi. Ascomycetous fungi include a number of fungi which have been show variations in their morphological diagnostic features (anamorphes and teleomorphs), an ascus-like organizational nature in ascospores, conidiophores and other characters. *Ascomycotina* forms the largest subdivision with 2700 genera and 28 500 species. Yeasts are of significant importance in industrial fermentations, such as brewing and baking. They are vital in decomposition processes to degrade cellulose and other plant polymers. On other hand, they can parasitize plants and can cause serious damage to the crop which leads to economic loss.

Basidiomycotina

Fleshy fungi, which include toadstools, bracket fungi, fairy clubs, puff balls, stinkhorns, earthstars, bird's nest fungi, and jelly fungi, fall into the category of *Basidiomycotina*. Toadstools exist as a symbiont, as well as parasites, where most of them are saprophytic in nature. They can be poisonous, however, the majority is harmless and some of them have higher nutritive values. More than 2000 species of edible mushrooms are reported from different parts of the world.

Mushrooms and Other Macrofungi

Among fungi, especially *Basidiomycetes* has been exploited more owing to its pharmacological properties such as antibiotic, antiviral, phytotoxic and cytostatic activities. Out of the 41 000 species of mushrooms, 850 species are recorded from India. Isolation of antitumor and immunomodulatory compounds of unusual polysaccharides of these macrofungi are being targeted nowadays.

Rust and Smut Fungi

The rust and smut are largest group of plant parasitic fungi in Basidiomycotina, which could cause drastic damage to economically important crop plants like wheat, corn, cereals, legumes, beans, and grasses. More than 160 genera of rusts have been recorded, out of which 46 are monotypic comprising 7000 species. The maximum number of species is seen in temperate and near temperate regions. Rust fungi occur on dicots rather than monocot plants. They parasitize plants that range from ferns to orchids and mints to composites. Several smuts can also cause considerable economic loss to cultivated plants, mainly on angiospermic monocots. Nearly 1450 smut species are distributed in 77 genera and about 3500 synonyms. Teliospore forming smuts (*Ustilaginomycetes*) are parasites on herbaceous, non-woody plants, while those lacking teliospores (*Microstromatales, Exobasidiales*) mostly parasitize woody (*Microstromatales, Exobasidiales*) plants.

Deuteromycotina

Deuteromycetes constitute an artificial group, which represents the asexual phases of *Ascomycotina* and *Basidiomycotina*. The multiplication occurs through the production of mitotic spores or conidia from special-

ized hyphae called conidiophores. Conidial ontogeny forms the basis for identification and segregation of *Fungi imperfecti*. It comprises 1700 genera of *Hyphomycetes*, and 700 genera of *Coelomycetes* that cover some 20 000 known species. They colonize, survive, and multiply in air, litter, soil, and other substrates and contribute extensively towards bio-degradation and recycling of organic matter, enzyme production, and industrial production including antibiotics, immunoregulators, and bio-control agents, in addition to causing profound mycoses, allergies, and plant diseases. About 8000 *Fungi imperfecti* are reported from India. Very few thermophilic fungi are reported from India with growth optima of around 45 °C.

2.3 Habitats of Fungi in the Marine Ecosystem

Different marine habitats support very different fungal communities. Fungi can be found in niches ranging from ocean depths and coastal waters to mangrove swamps and estuaries with low salinity levels. It is more than five decades since the marine mycology has evolved and it evidently states that marine fungi show a great variance not only in their taxonomy but also in their morphological, physiological and ecological adaptation capabilities from both terrestrial and freshwater fungi. Although *marine fungi* is a common term used to refer all fungi that are present in the sea, they are distinctly referred as marine, oceanic, manglicolous, arenicolous, or estuarine, based on their habitat.

2.3.1 Marine Fungi

Marine fungi form an ecological and not a taxonomic group. According to their origin marine fungi are categorized into two types i. e., obligate marine fungi and facultative marine fungi. Obligate marine fungi grow and sporulate exclusively in seawater, and their spores are capable of germinating in seawater. However, facultative marine fungi are those from fresh water or a terrestrial source that have undergone physiological adaptations that allow them to grow and also sporulate in the marine environment [2.4].

Marine fungi comprise an estimated 1500 species, excluding those that form lichens [2.20]. This number is much smaller when compared to the number of named and undescribed terrestrial fungi, which has been estimated to be 250 000 or more [2.4]. So far less than 500 filamentous higher marine fungi have been described and only 79 are associated with algae as parasites or symbionts, and 18 with animal hosts [2.21].

2.3.2 Fungi in Mangroves

Among the tropical marine ecosystems, mangroves and coral reefs are the major habitats in gross productivity [2.22]. Mangrove forests are spread over 181 000 km^2 in 112 countries in the tropics and subtropics [2.23, 24]. Through detritus production and decomposition mangroves support a variety of planktonic, benthic, and fish communities [2.25–27]. Up to 41% of mangroves exist in South East Asia [2.24]. The Indian subcontinent ranks fourth (6700 km^2) after Indonesia, Bangladesh, and Malaysia in mangrove vegetation cover [2.28]. Mangroves are intertidal forested wetland confined to tropical and subtropical regions, and mangrove vegetation is considered to be a dynamic ecotone (or transition zone) between terrestrial and marine habitats [2.29].

Mangrove ecosystems command intensive attention among coastal ecosystems, not only due to their peculiar habitat characteristics but also due to their rich biodiversity. Mangrove forests are biodiversity *hotspots* for marine fungi because the bases of mangrove trunks and aerating roots are permanently or intermittently submerged. Because the upper parts of roots and trunks are rarely or never reached by salt water, terrestrial fungi and lichens occupy the upper part of the trees and marine species occupy the lower part. At the interface, there is an overlap between marine and terrestrial fungi [2.30]. Mangrove fungi constitute the second largest ecological group of marine fungi. The latest estimate of marine fungi is 1500 species, in which 339 are found in mangrove ecosystems [2.31].

The fungi in mangroves play a significant role in litter decomposition and nutrient cycling, thereby contributing to the fertility of the environment. Fungal biomass along with detritus contributes significantly to the food chain of the detritus-feeding organisms found in mangroves. *Aspergillus* and *Penicillium* are dominant fungi involved in litter decomposition of mangroves. Since 1944, marine fungi have been extensively studied, particularly wood-inhabiting fungi [2.3]. About 150 species are found exclusively on decaying mangrove wood, aerial roots, and seedlings, and are categorized as *Manglicolous fungi*; most of the species belong to the class of *Ascomycetes*.

Of the 150 species, more than 30 species of marine fungi are found in the mangroves in the New World. Only a few seem to be host specific [2.4]. For example, *Avicennia germinans* specifically acts as a host for the fungal species *Rhabdosphora avicenniae* and *Mycosphaerella pneumatophorae*, whereas *Rhizophora mangle* forms another host for *Didymosphaeria rhizophora* and *Leptosphaeria australensis*. They are quite abundant in mangroves due to the easy availability of wood as their bait. Large numbers of fungi are found on *Avicennia marina, A. officinalis, Rhizophora mucronata, R. apiculata*, and *Sonneratia alba*. The fungal biodiversity in mangroves may reflect the age of the plants. A well-developed mangrove habitat provides a larger number of fungal species than new mangrove sites.

2.3.3 Fungi as Endophytes

Fungal endophytes are microfungi, mostly belonging to the family *Ascomycetes*. They colonize the internal tissues of vascular plants without producing any apparent disease symptoms. Fungal endophytes are now considered an important component of biodiversity. The distribution of endophytic mycoflora differs with the host. The fungal endophytes can modify the host plants at genetic, physiological, and ecological levels. It has been suggested that the association of fungi as endophytes with mangrove roots would confer protection from adverse environmental conditions and would allow the latter to successfully compete with saprophytic fungi that decompose senescent roots.

Mangroves are a special kind of host plant and also an abundant resource for endophytic fungi diversity. More than 200 species of endophytic fungi have been isolated and identified from mangroves, this being the second largest community of marine fungi. Most endophytic fungi have wide range of hosts, and a few only have a single host. Although several temperate plants have been studied for their endophyte assemblages, very few tropical plants have been screened for endophytes [2.32, 33]. Recently, endophytic fungi were isolated from mature green leaves of *A. officinalis* along the southeast coast of India and it was observed that the occurrences of dominant endophytic fungi are *Rhizopus* sp. (51.7%), *Penicillium lividum* (45%), and *A. ochraceus* (45%). Species richness and diversity are high in the leaves of *R. mucronata*, and the most dominant endophytes are *P. lividum* (45%) and sterile mycelia (40%). *F. moniliforme* (45%), *Alternaria* sp. (46.7%) are the dominant endophytes in leaves of *R. annamalayana* [2.34]. However, the composition and dominant species on each mangrove plant are different and their colonization varies with different parts (leaves, twigs, and stems), the age of the host plants, and with the seasons [2.35].

Scientists have been astonished at the adaptation of mangrove fungi in extreme environments and have suggested that fungi, in particular endophytes, are promising sources for screening new products. Sampling and characterization of endophytic fungal diversity is an emerging challenge and promises to lead to the discovery of new species, novel compounds, and a better understanding of their role in ecosystems.

2.4 Habitat Characteristics and Their Effect on Fungal Diversity

Despite of the insufficiency of data, generalizations have been made on habitat characteristics [2.2]. Five littoral mycogeographic zones such as arctic, temperate, subtropical, tropical, and Antarctic zones, have been proposed based on the sea-surface temperature. Temperature holds the first position among environmental and biological factors controlling the distribution of marine fungi, and then come the availability of substrates or hosts, salinity, hydrostatic pressure, and the availability of oxygen.

2.4.1 Effects of the Substratum

Marine fungi are commonly colonized in different substrata, which includes leaves and drift mangrove woods, *Avicennia alba, Bruguiera cylindrica*, and *Rhizphora apiculata* [2.35–37]. An abundance of the *Hyphomycetes* group of fungi on marine and mangrove substrates has been reported [2.20]. This might be due to the adaptation of their spores to the marine ecosystem by way of production of appendages, which provide buoyancy in water, entrapment, and adherence to the substrates, as reported in mangrove wood driftwood and animal substrates [2.38–40]. In addition to the above, *Hyphomycetes* also possess an enzyme producing ability and hence, they could potentially colonize lignocellulosic woody substrates. Maximum diversity of fungi on the woody litter of *Rhizophora* (64 spp.) and *Avicennia* (55 spp.) are reported from the Godavari and Krishna deltas of the east coast of India [2.41, 42]. The

nutritional features and persistent nature of the wood of *Avicennia* and *Rhizophora* in mangrove habitats might be responsible for yielding the rich mycoflora [2.43]. It is evident from the frequency distribution of fungi from Malaysian mangroves that driftwood supports a greater diversity of fungi than exposed test panels, e.g., 26 species on driftwood, 9 species on test blocks, while 104 species have been recorded on mangrove driftwood [2.44], yet only 77 on exposed test blocks of mangrove wood [2.45].

Differences in fungal counts may be attributed to a rhizosphere effect, which varies with the mangrove species. The effect of root exudation, which includes both promoters (sugars, amino acids, etc.) and inhibitors (phenolic compounds), and the ratio between the two types of compounds influence the fungal growth and multiplication [2.46]. It is also inferred that distributions of fungal species within the mangrove habitat vary with temperature, salinity, humidity, and organic contents [2.47]. Moreover, the frequency of occurrence and relative abundance of marine fungi from various mangrove forests of the world shows variations. This could be attributed to the difference in the species' diversity of the mangrove ecosystem, age, and preference of the host substrate, ecological factors, high temperature, abrasion, desiccation, variation in salinity, and exposure to UV light [2.48]. Mangrove leaf litter is an important substratum colonized by a very different fungal community to that of lignocellulosic materials [2.49, 50]. Higher marine fungi are not common on such leaf material [2.51].

2.4.2 Effects of Salinity

Salinity is an important factor that profoundly influences the abundance and distribution of fungi in the marine environment [2.52]. There seems to be continuous alterations in the intertidal amplitude and salinity, which can considerably affect fungal biodiversity. Earlier physiological studies of marine fungi led to the conclusion that they require sodium chloride at concentrations found in seawater for their growth. In fact, zoosporic fungi such as *Althornia, Haliphthoros* and *Thraustochytrium* species need sodium for growth at the macronutrient level [2.53]. Generally, changes in salinity in brackish water habitats such as estuaries, backwaters, and mangroves are due to the influx of freshwater from land run-off, caused by monsoons or tidal variations. The effects of salinity on fungal growth have been investigated by various authors, who observed only vegetative growth and depicted that most freshwater fungi cannot reproduce at salinities above 30% seawater and suggest that this is the major reason that they do not grow in the sea [2.19, 21, 54]. Further, it is interesting to note that the species diversity is much greater at the mangrove site when compared with the samples from the open ocean site, and this accounts for the larger number of terrestrial fungi recorded there [2.55].

In higher salinity, some species like *Curvularia lunata, Drechslera* sp., *A. terreus, Cladosporium herbarum*, and *Aurobasidium pullulans* are isolated [2.55]. This may be due to the fact that all these species are salt-tolerant fungi, although these species are mostly found in saltpan and seawater zones [2.56]. These species are mostly isolated from marine zones and rarely in fresh water and mangrove zones. These fungal cells employ two main mechanisms for adaptation to salt stress: accumulation of a polyol, glycerol, and maintenance of ion homeostasis [2.57]. When exposed to NaCl, the cells experience both osmotic stress and ion toxicity. To respond to a low external osmotic potential, the accumulating glycerol seemingly compensates for the difference between the extra and intracellular water potential [2.58]. To reduce sodium toxicity, fungal cells have to maintain low cytosolic Na^+ concentrations, and this is achieved by several mechanisms: by restricting Na^+ influx, rapidly extruding Na^+, and/or efficiently compartmentalizing sodium into vacuoles [2.59, 60]. Genetic evidence indicates that both mechanisms are essential for yeast salt tolerance [2.61].

2.4.3 Effects of Temperature

Temperature is the foremost important physical factor influencing the physicochemical characteristics and also the geographical distribution and abundance of mycoflora [2.52]. This may be due to direct solar heating and penetration of the warm surface water from the sea. Generally, the surface water temperature is influenced by the intensity of solar radiation, evaporation, freshwater influx and cooling, and mix up with ebb and flow from adjoining neritic waters. In general, marine fungi need high temperatures (usually between 25–30 °C) to reproduce [2.62]. At higher temperatures, *A. niger, A. terreus* and *Cladosporium herabrum* have been recorded in the marine zone of the Pichavaram mangrove forest [2.55]. The abundance of this group of fungi in the mangrove environment might be due to their spores, which show adaptation by way of pro-

duction of appendages that provide buoyancy in water, entrapment, and adherence to substrates, as reported in mangrove wood [2.63] and driftwood [2.64]. On the contrary, temperatures below 10 °C support the growth of wood fungi, *Digitatispora marina*, but when the temperature reaches 10 °C and above, the fungi stop fruiting on the wood [2.65]. Likewise, a few marine fungi have been recovered from Antarctic waters such as *Thraustochytrium antarticum, Leucosporidium anatartica*, and *Spathulospora antartica*, which leads us to the conclusion that the low temperature of the seawater and the availability of suitable substrata in the Antarctic region are responsible for the lower numbers of marine fungi [2.66].

There is clear evidence that temperature is responsible for sporulation, deposition, and germination of endophytic fungi. It has been demonstrated that endophytes enhance the thermotolerance of temperate plants, augmenting their potential to colonize extreme environments [2.67]. Further, temperature appears to be a major variable affecting the fluctuation of endophyte frequency in plant tissue [2.68]. Recent studies have provided evidence that the population density of leaves is influenced by summer temperatures; however, the number species of endophytic fungi increases with increasing air temperature [2.69]. It is interesting to note that seven isolates of *Corollospora maritime* have been investigated on the 18S gene [2.70], and it was found that five isolates from temperate localities grouped together; those from subtropical collections formed a separate group, while the strain from Aldabra separated from both groups.

2.5 Collection, Isolation, and Identification of Fungi

The water, sediments, substrates, and any solid material from the habitat can be collected in sterile polythene bags for fungal examination. The samples are transferred to the laboratory for further analysis. Isolation of fungi is performed by using a pour plate method with different fungal agar medium plates. The media is weighed out and prepared according to the manufacturer's specifications, with respect to the given instructions and directions. The isolated fungal species are identified up to species level by referring standard mycological manuals and books [2.4, 71–73]. After identification, the fungal species are maintained on a Sabouraud chloromphenicol agar (SCA) medium at 27 °C and they are subcultured at regular intervals using a sterile cork borer. Mycelial disks are cut at random and used for further studies.

2.5.1 Techniques for Sample Collection

Direct examination method. Fungi are directly examined under a dissection microscope for the presence of ascocarps, basidiocarps, or pycnidia. Such fruit bodies are transferred with a needle to a microscopic slide, torn apart in a drop of water to expose the spores, and carefully squeezed under a cover glass. To allow the development of fruit bodies, the litter samples can be incubated in a sterile moist chamber/petridish/polybag at room temperature. The incubated samples are examined under a microscope for fungal fruit bodies.

Another method is called the *wood baiting technique*, where terrestrial wooden logs measuring 6 × 3 × 2 cm are immersed in mangrove waters for certain period and then collected to examine the fungal colonization. To determine the fungal biota in any habitat, grab samples are satisfactory. At the chosen sites, samples are obtained scooping the water, mud, sand, or soil to be examined into the sample container. Plastic vials, soil cans, or other containers may be used to collect the samples. The container should be clean and the sample should be handled aseptically. It is not possible to obtain samples from the bottom of a body of water without water; to obtain samples from the bottom of a body of water deeper than elbow depth any of the available sampling devices can be used. The container is closed with its proper cover, excess sludge, dirt, etc., is wiped or rinsed off after which it is returned to the laboratory. The container should not be completely airtight.

2.5.2 Media Preparation for Isolation of Fungi

Various culture media are used for the isolation of fungi from the samples. The most commonly used media are Sabouraud dextrose agar or potato dextrose agar, along with chloramphenicol to avoid bacterial contamination. The chemical should be added in proper proportion along with agar (for solidification), autoclaved at 121 °C for 15 min, and plated in a sterile Petri dish that is free from contamination. Media used in plating the samples may be prepared the day before the samples are collected. The most convenient method of storage is in 10 ml lots in culture tubes in culture tube

racks in a hot water bath (45–55 °C). Instead of this, the medium may be placed in agar storage bottles and kept in the refrigerator or on the laboratory shelf. If the latter technique is used, the medium should be remelted just before use and pipetted onto the plate with 10 ml wide-mouth pipettes.

2.5.3 Isolation of Fungi

For the best results in the reading of plates, each plate should contain 40 to 60 colonies of fungi. To accomplish this, the sample must be diluted before it is plated. In general, liquids may be diluted only 1 : 10, a fairly rich sewage may be diluted 1 : 100, sludge containing about 4–6% dry matter and relatively poor clayey soils 1 : 1000, and richer, fairly dry materials (dry matter 30–60%), as much as 1 : 10 000. When the sample reaches the laboratory, a series of dilution flasks is prepared. The samples are then serially diluted and inoculated in the medium, and plating is done by various methods as per convenience. Different culture media are available to culture the yeast and other groups of fungi.

Incubation and Subculture of Isolated Fungi

Commonly, fungi can be grown at room temperature. 25 °C is a standard temperature for the growth of fungi and the temperature can be varied in the case of thermophilic fungi when it requires more than 50 °C to grow. For subcultures of fungi, a sterile cork-borer is used. The media block is taken out and inoculated into an another freshly prepared medium, and incubated at a suitable temperature.

2.5.4 Identification of Fungal Isolates

Several techniques are available for the identification of fungi. Because the nature of spore production apparatus and the spores associated with it is fragile, there are two techniques that are usually used. These are described below.

Direct Mounting Technique or LCB Mount

A small portion of mycelium is taken from the medium with a help of teasing needle. The needle must be incinerated and allowed to cool in laboratory conditions. Care must be taken when the fungus is taken out from the medium for mounting because the spores will be diffused into the environment and this may affect the individual dealing with the fungus; the laminar airflow must be in off mode. A drop of lactophenol cotton blue (LCB) solution is placed on the center part of the microscopic slide. The fungal mycelia should be mounted over the LCB. The mycelia should be gently handled as can break into pieces, which results in difficulties during the identification. Later a cover slip is placed over the mount without air bubbles. The slide is placed under a high power objective and the structure of the fungi is observed and identified.

Agar Block Technique

Fungal media are prepared and cut into 1 × 1 cm blocks with a sterile scalpel blade and placed over a slide. A small piece of mycelia is transferred to the agar block over the slide; a cover slip should be placed. The whole setup is placed inside the petri dish and small cotton soaked in water is also placed inside the dish for maintaining the moisture. The Petri dish is incubated at 25 °C for a certain period of time. Once the spores and mycelia have developed, the cover slip is removed aseptically from the agar block placed over the slide mounted with LCB, and observed under a high power objective. Identification is carried out with the help of a standard atlas and monograph using key characters (Table 2.1).

Macroscopic Morphology of Fungi

1. Rate of growth: slow (7–14 days)
   Rapid (2–7 days)
2. Topography: flat, regularly-folded, tangled
3. Texture: creamy, powdery cottony, mucoid, waxy
4. Color: front color of the colony, back color of the colony.

Microscopic Examination of Slide Cultures

This will be done for the following characteristics:

A. Hyphae-pigmentation of hyphal elements, shapes
B. Asexual spores – simple or specialized asexual spores
C. Conidiophores, conidia, macroconidia, and microconidia
D. Sporangiospores
E. Blastospores or chlamydospores
F. Spore size, shape, attachment to the mycelium, unicellular or multicellular, number of compartments
G. Rhizoids, columella.

2.5.5 Molecular Taxonomical Identification

Assembling taxa based primarily on morphological similarities does not necessarily reflect phylogenetic re-

Table 2.1 Key to the classes of fungi

S. No.	Major classes	Key characters
1.	*Zygomycetes*	Hyphal filaments, usually one-celled, rarely septate usually multinucleate; aquatic species propagating by zoospores, terrestrial species by zoospores, conidia, or conidia-like sporangia; sex cells when present forming oospores or zygospores
2.	*Phycomycetes*	Hyphal filaments, when present, multicellular, cell with one, two, or several nuclei, without zoospores, with or without sporangia, usually with conidia; sexual reproduction absent or culminating in the formation of asci or basidia
2a.	*Ascomycetes*	Sexual spores born in asci
2b.	*Basidiomyctes*	Sexual spores born in basidia
2c.	*Fungi imperfecti* (*Deuteromycetes*)	Without a sexual stage in the life cycle, or with sexual stage rare or obscure; spores born on conidiophores, which may produced at random, in clusters, or within pycnidia

lationships but is rather a convenient scheme which hinders exploration of marine fungi that might produce microbial metabolites for therapeutic use. This necessitates the careful identification and selection of species unique to a particular host before the high-throughput screening of metabolites for desired industrial applications. Therefore, taxonomy of fungi is a formidable challenge for most applications.

Fungal taxonomy is a dynamic, progressive discipline that consequently requires changes in nomenclature. The baseline of traditional fungal taxonomy and nomenclature is morphological criteria or their phenotypes. Numerous alternative approaches have been developed, including nutritional and physiological studies, serologic tests, secondary metabolites, and fatty acids. Although some of these are very useful for identifying poorly differentiated fungi such as yeast and black yeast, in most cases they are only complementary tools of morphological data. The fungus as a whole comprises a teleomorph (sexual state) and one or more anamorphs (asexual states). Traditional fungal classification is possible when the fungus is at its teleomorphic stage (asexual spore morphology also helps); however, the snag occurs in cases of fungi where only the anamorphic stage is available. The dual modality of fungal propagation, i. e., sexual and asexual, has led to a dual nomenclature. The anamorph and the teleomorph generally develop at different times and on different substrates. Species identification by morphological traits is often problematic because mycelial pigmentation, and the shape and size of conidia, which are unstable and highly dependent on the composition of media and environmental conditions. Further, subspecies level of identification is usually based upon pathogenic or physiological race reactions on a set of differential cultivars [2.74]. These processes of identification of fungi are time consuming, labor intensive, and subject to varying environmental or cultural growth conditions during the experiments [2.75], and also lead to inappropriate and unreliable application of species [2.76].

Thus methods are needed to distinguish between closely related species that occur in different habitats. Hence, molecular techniques for fungal identification and to investigate genetic variability within species have been increasingly used during the last decade. Molecular techniques based on polymerase chain reaction (PCR) have been used as a tool in genetic mapping, molecular taxonomy, evolutionary studies, and diagnosis of several fungal diseases [2.77, 78].

Differentiation of the *Fusarium* species/subspecies based on comparison of deoxyribonucleic acid (DNA) sequences of the ribosomal DNA (rDNA) and internal transcribed spacer (ITS) regions have been reported [2.79, 80]. The sequenced rDNA region of *F. oxysporum* (Accession no. JX 840353) isolated from sediments of mangroves along the southeast Indian coast covered the 18S ribosomal ribonucleic acid (rRNA) gene, partial sequence; internal transcribed spacer1; the 5.8S ribosomal RNA gene and ITS2; complete sequences; and the 28S ribosomal RNA gene, partial sequence. In the constructed phylogenetic tree, the strains *F. oxysporum* (GU205817), *F. oxysporum* (EF495237), *F. oxysporum* (EF495230), and *Fusarium* sp., (JF429684) have 98% similarity with the isolated strain *F. oxysporum* (JX 840353) [2.55] and 97% similarity with *Fusarium* sp., (GU973787). Genotypic identification of the 18S ribosomal RNA gene of endophytic *Fusarium* sp. isolated from leaves of *Rhizophora annamalayana* has been analyzed (accession number JN681281) and the fungus was found to be the closest homolog to *Fusarium moniliforme* [2.34]. Ribosomal RNA genes (rDNA) possess characteristics that are suit-

able for the detection of fungi at the species level. These rDNA are highly stable and exhibit a mosaic of conserved and diverse regions within the genome [2.18]. ITS regions have been used successfully to generate specific primers capable of differentiating closely related fungal species [2.81].

2.6 Bioprospecting of Marine Fungi

The need for new and safe bioactive compounds to provide comfort in all aspect of human life is ever increasing. Due the emergence of new diseases, the development of drug resistant pathogenic microorganisms, the appearance of life threatening viruses, the management of post operative complications in patients with organ transplantations are some of the challenges to scientists. Synthetic antibiotics and drugs are extending antibiotic resistant microbes, however it is essential to investigate a new way to treat diseases [2.82]. This situation has forced scientists to explore different natural sources for safe and potent agents to meet the challenges of the twenty-first century. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources based on their use in traditional medicines or phytomedicines. Due to safety and environmental problems, many synthetic drugs and chemicals have been removed or are currently being targeted for removal from the market, which creates the need to find alternative ways to control farm pests and dermatophytes [2.83–86].

Marine fungi have provided new incentives for research on marine natural products over the past years; this research also continue to be the subject of vigorous chemical investigation [2.87–89]. The diversity of secondary metabolites reported in recent decades is fascinating. This highlights the importance of marine fungi as a source of natural products [2.90–92]. The marine environment is unique in terms of its specific composition in both organic and inorganic substances, as well as temperature ranges and pressure conditions. Ecological niches, e.g., deep-sea hydrothermal vents, mangrove forests, algae, sponge, and fish provide habitats for the evaluation of specific microorganisms.

2.7 Conclusions

Fungi are not only beautiful but play a significant role in the daily life of human beings in addition to their utilization in industry, agriculture, medicine, the food industry, textiles, bioremediation, biodegradation, biogeochemical cycle, biofertilizers, and in many other ways. Fungal biotechnology has become an integral part of human welfare. Fungal biotechnology or *mycotechnology* has advanced considerably in the last five decades. Terrestrial fungi are used in the production of various extracellular enzymes, organic acids, antibiotics, and anticholesterolemic statins. They have been used as expression by hosts, as well as a source of new genes. With modern molecular genetic tools, fungi have been used as *cell factories* for heterologous protein production and human proteins. The focus of future research is oriented towards fungi in special ecological niches, as a basic understanding of the ecology to help reveal the novelty of an organism and its properties. The emphasis is on the following aspects for top priority:

i) Fungi associated with endophytic fungi in marine algae, seagrasses, and mangroves, and the implications.
ii) Fungi associated with marine invertebrates, especially corals and sponges, and their potential for production of bioactive molecules.
iii) Fungi from extreme environments such as the deep sea with elevated hydrostatic pressure and low temperatures, hypersaline waters of the Dead Sea, and anoxic or hypoxic (oxygen deficient) sediments from the marine environment.
iv) Bioremediation of pollutants using salt-tolerant fungi and their salt-tolerant enzymes on a pilot scale and industrial scale.
v) Genomic and proteomic studies with novel organisms such as *Corallochytriumlima cisporum* as a model of animal fungal allies. It is hoped that this will help basic research on evolutionary biology.

References

2.1 P.M. Kirk, P.F. Cannon, D.W. Minter, J.A. Stalpers: *Dictionary of the Fungi*, 10th edn. (CAB International, Wallingford 2008)

2.2 D.L. Hawksworth: The fungal dimension of biodiversity: Magnitude, significance, and conservation, Mycol. Res. **95**, 641–655 (1991)

2.3 E.S. Barghoorn, D.H. Linder: Marine fungi: Their taxonomy and biology, Farlowia **1**, 395–467 (1944)

2.4 J. Kohlmeyer, E. Kohlmeyes: *Marine Mycology: The Higher Fungi* (Academic, New York 1979)

2.5 K. Tubaki, T. Ito: Other fungi occur in brackish water, Rep. Tottori Mycol. Inst. **10**, 523 (1973)

2.6 R.S. Upadhyay, D.B. Singh, B. Rai: Ecology of microfungi in a tropical coastal sand belt, Indian J. Mar. Sci. **7**, 187–190 (1978)

2.7 C.W. Steele: Fungus populations in marine waters and coastal sands of Hawaiian, Line, and Phoenix Islands, Pac. Sci. **21**, 317–331 (1967)

2.8 F. Sponga, L. Cavaletti, A. Lazzarini, A. Borghi, I. Ciciliato, D. Losi, F. Marinelli: Biodiversity and potentials of marine-derived microorganisms, J. Biotechnol. **70**, 65–69 (1999)

2.9 V.V. Sarma, B.P.R. Vittal: Biodiversity of mangrove fungi on different substrata of *Rhizophora apiculata* and *Avicennia spp.* from Godavari and Krishna deltas, east coast of India, Fungal Divers. **5**, 23–41 (2000)

2.10 V.V. Sarma, K.D. Hyde, B.P.R. Vittal: Frequency of occurrence of mangrove fungi from the east coast of India, Hydrobiology **455**, 41–53 (2001)

2.11 K. Ananda, K.R. Sridhar: Diversity of filamentous fungi on decomposing leaf and woody litter of mangrove forests in the Southwest coast of India, Current Sci. **87**(10), 1431–1437 (2004)

2.12 P.R. Vittal, V. Sarma: Diversity and ecology of fungi on mangroves of Bay of Bengal region – An overview, Indian J. Mar. Sci. **35**, 308–317 (2006)

2.13 R. Kandikere, Sridhar: Fungal diversity of pichavaram mangroves, Southeast coast of India, Nat. Sci. **7**(5), 67–75 (2009)

2.14 M. Ravikumar, K.R. Sridhar, T. Sivakumar, K.S. Karamchand, N. Sivakumar, R. Vellaiyan: Diversity of filamentous fungi on coastal woody debris after tsunami on the southeast coast of India, Czech Mycol. **61**(1), 107–115 (2009)

2.15 S. Nambiar, R. Gayatri, K. Raveendran, L. Jaleel, C. Abdul: Marine mycoflora of south India with special emphasis on lignicolous marine fungi, Front. Biol. China **4**(4), 436–441 (2009)

2.16 S. Das, P.S. Lyla, S.A. Khan: Filamentous fungal population and species diversity from the continental slope of Bay of Bengal, India Acta Oecol. **35**, 269–279 (2009)

2.17 A.R. Logesh, D.K. Upreti, M. Kalaiselvam, S. Nayaka, K. Kathiresan: Lichen flora of Pichavaram and Muthupet mangroves, Mycosphere **3**(1), 884–888 (2012)

2.18 A.R. Logesh, M. Kalaiselvam, D.K. Upreti: A new species of non-lichenized ascomata from India, Mycosphere **4**(1), 152–154 (2013)

2.19 D.S. Hibbett: Ribosomal RNA and fungal systematics, Trans. Mycol. Soc. **33**, 533–556 (1992)

2.20 K.D. Hyde, E.B.G. Jones, E. Leano, S.B. Pointing, A.D. Poonyth, L.L.P. Vrijmoed: Role of fungi in marine ecosystems, Biodivers. Conserv. **7**, 1147–1161 (1998)

2.21 J. Kohlmeyer, B. Volkmann-Kohlmeyer: Fungi from coral reefs: A commentary, Mycol. Res. **107**(4), 386–387 (2003)

2.22 S.Z. Qasim, M.V.M. Wafar: Marine resources in the tropics, Resource Manag. Optim. **7**, 141–169 (1990)

2.23 J.S. Bunt: Introduction. In: *Tropical Mangrove Ecosystem*, ed. by A.I. Robertson, D.M. Alongi (American Geophysical Union, Washington 1992) pp. 1–6

2.24 M.D. Spalding: *The global distribution and status of mangrove ecosystems', Mangrove Edition, International Newsletter of Coastal Management (Intercoast Network) Special Edition #1 (Coastal Resources Center, University of Rhode* (Island, Narragansett 1997) pp. 20–21

2.25 A.I. Robertson, D.M. Alongi: *Coastal and Estuarine Studies: Tropical Mangrove Ecosystems* (American Geophysical Union, Washington 1992), pp. 41, 43–62

2.26 D.M. Alongi, F. Tirendi, P. Dixon, L.A. Trott, G.J. Brunskill: Mineralization of organic matter in intertidal sediments of a tropical semi-enclosed deltam, Estuar. Coast. Shelf Sci. **48**, 451–467 (1999)

2.27 K. Kathiresan, B.L. Bingham: Biology of mangroves and mangrove ecosystems, Adv. Mar. Biol. **40**, 81–251 (2001)

2.28 F. Blasco, M. Aizpuru: Classification and evolution of the mangroves of India, Trop. Ecol. **38**, 357–374 (1997)

2.29 B. Gopal, M. Chauhan: Biodiversity and its conservation in the Sundarban mangrove, Ecosyst. Aquat. Sci. **68**, 338–354 (2006)

2.30 V.V. Sarma, K.D. Hyde: A review on distribution of marine fungi on *Rhizophora* sp. at frequently occurring fungi in mangroves. Fungal Kampong Danau Mangrove, Brunei, Asian Mar, Divers. **8**, 1–34 (2001)

2.31 K.R. Sridhar: Mangrove fungi in India, Current Sci. **86**, 1586–1587 (2004)

2.32 K.F. Rodrigues, O. Petrini: Biodiversity of endophytic fungi in tropical regions. In: *Biodiversity of Tropical Microfungi*, ed. by K.D. Hyde (Hong Kong Univ. Press, Hong Kong 1997) pp. 57–69

2.33 J. Fröhlich, K.D. Hyde: Biodiversity of palm fungi in the tropics: Are global fungal diversity estimates realistic?, Biodivers. Conserv. **8**, 977–1004 (1999)

2.34 A. Elavarasi: Studies on mangrove endophytic fungi from Vellar estuary along southeast coast of India (Annamalai University, Tamil Nadu 2013), pp. 187

2.35 L.S. Yang, Y.J. Huang, Y.J. Zheng, Z.H. Song, S. Yang, S.W. Jin, S.Y. Mao: The population fluctuation and

bioactivity of endophytic fungi from mangrove plants in different seasons, J. Xiamen Univ. Nat. Sci. **45**, 95–99 (2006)
2.36 J. Kohlmeyer: Ecological notes on fungi in mangrove forests, Trans. Br. Mycol. Soc. **53**, 237–250 (1969)
2.37 T.K. Tan, W.F. Leong: Mangrove fungi in Singapore and some possible factors influencing their occurrence, Trans. Mycol. Soc. Japan **31**, 35–44 (1990)
2.38 R. Sundari, S. Vickineswary, M. Yusoff, E.B.G. Jones: Observations on tropical arenicolous marine fungi on driftwood from Malaysia and Singapore, Bot. Marina **39**, 361–375 (1996)
2.39 D.R. Ravikumar, B.P.R. Vittal: Fungal diversity on decomposing biomass of mangrove plant *Rhizophora* in Pichavaram estuary, East coast of India, Indian J. Mar. Sci. **25**, 142–144 (1996)
2.40 K. Ananda, K.R. Sridhar: Diversity of filamentous fungi on decomposing leaf and woody litter of mangrove forests in the Southwest coast of India, Current Sci. **87**(10), 1431–1437 (2004)
2.41 K. Prasannarai, K. Ananda, K.R. Sridhar: Intertidal fungi in Mangalore Harbour, Southern India, Bot. Marina **42**(3), 117–122 (1999)
2.42 V. Sarma, B.P.R. Vittal: Biodiversity of manglicolous fungi on selected plants in the Godavari and Krishna deltas, east coast of India, Fungal Divers. **6**, 115–130 (2001)
2.43 V.V. Sarma, K.D. Hyde: A review on frequently occurring fungi in mangrove, Fungal Divers. **8**, 1–34 (2001)
2.44 J.O. Miller, N.J. Whitney: Fungi from the Bay of Fundy. I: Lignicolous marine fungi, Can. J. Bot. **59**, 1128–1133 (1981)
2.45 S.A. Alias, A.J. Kuthubutheen, E.B.G. Lones: Frequency occurrence of fungi on wood in Malaysian mangroves, Hydrobiologia **295**, 97–106 (1995)
2.46 S. Ravikumar, K. Kathiresan: Influence of tannins, sugars and amino acids on fungi of marine halophytes, Mahasagar **26**, 21–24 (1993)
2.47 S. Ravikumar, K. Kathiresan, S. Thadedus Maria Ignatimmal, M. Babuselvam, S. Shanthy: Nitrogen fixing azotobacters from mangrove habitat and their utility as marine biofertilizers, J. Exp. Mar. Biol. Ecol. **312**(1), 5–17 (2004)
2.48 J. Kohlmeyer, B. Volkmann-Kohlmeyer: Biogeographic observations on Pacific marine fungi, Mycologia **85**, 337–346 (1993)
2.49 S.Y. Newell, J.W. Fell: Parallel testing of media for measuring frequencies of marine oomycetes (*Halophytophthora* spp.) in mangrove ecosystems, Mycol. Res. **96**, 851–856 (1994)
2.50 S.Y. Newell, J.W. Fell: Competition among mangrove oomycetes, and between oomycetes and other microbes, Aquat. Microb. Ecol. **12**, 21–28 (1997)
2.51 A. Nakagiri, S.Y. Newell, T. Ito, T.K. Tan, C.L. Pek: Biodiversity and ecology of the oomycetous fungus, *Halophytophthora*. In: *Biodiversity and the Dynamics of Ecosystems*, DIWPA Series, Vol. 1, ed. by L.M. Turner, C.H. Diong, S.S.L. Lim, P.K.I. Ng (DIWPA, Ohtu 1996) pp. 273–280
2.52 T. Booth, N. Kenkel: Ecological studies of lignicolous marine fungi: A distribution model based on ordination and classification. In: *The Biology of Marine Fungi*, ed. by S.T. Moss (Cambridge Univ. Press, Cambridge 1986) pp. 297–310
2.53 D.J. Alderman, E.B.G. Jones: Physical requirement of two marine Phycomycetes, *Althornia erouchii* and *Ostracoblade implexa*, Trans. Br. Mycol. Soc. **57**, 213–225 (1971)
2.54 J.L. Harrison, E.B.G. Jones: The effect of salinity on sexual and asexual sporulation of member of the Saprolegniaceae, Trans. Br. Mycol. Soc. **65**, 339–394 (1975)
2.55 K. Sharmila: Studies on mycofloral diversity in mangroves of Pichavaram and Vellar estuary along southeast coast of India (Annamalai Univ., Tamil Nadu 2013), pp. 195
2.56 D.H. Jennings: Fungal growth in the sea. In: *The Biology of Marine Fungi*, ed. by S.T. Moss (Cambridge University Press, Cambridge 1986) pp. 1–10
2.57 I.M. Wethered, E.C. Metcalf, D.H. Jennings: Carbohydrate metabolism in fungus *Dendryphiella salina*. VIII. The contribution of polyols and ions to the mycelial solute potential in relation to the external osmoticum, New Phytol. **101**, 631–649 (1985)
2.58 A.D. Brown: *Microbial Water Stress Physiology, Principles and Perspectives* (Wiley, New York 1990)
2.59 T. Yagi, K. Tada: Isolation and characterization of salt-sensitive mutants of a salt-tolerant yeast *Zygosaccharomyces rouxii*, FEMS Microbiol. Lett. **49**, 317–321 (1988)
2.60 K. Ushio, H. Ohtsuka, Y. Nakata: Intracellular Na^+ and K^+ contents of *Zygosaccharomyces rouxii* mutants defective in salt tolerance, J. Ferment. Bioeng. **73**, 11–15 (1992)
2.61 B. Garciadeblas, F. Rubio, F.J. Quintero: Differential expression of two genes encoding isoforms of the ATPase involved in sodium efflux in *Saccharomyces cerevisiae*, Mol. Genet. Genomics **236**, 363–368 (1993)
2.62 D.H. Griffin: *Fungal Physiology* (Wiley, Canada 1981) p. 383
2.63 A.A. Aleem: Distribution and ecology of marine fungi in Sierra Leone (Tropical West Africa), Bot. Marina **23**, 679–688 (1980)
2.64 K. Prasannarai, K. Ananda, K.R. Sridhar: Intertidal fungi in Manglore Harbour, Southern India, Bot. Marina **42**(3), 117–122 (1999)
2.65 P.J. Byrne, E.B.G. Jones: Lignicolous marine fungi, Veröff. Inst. Meeresforsch. Bremerhav. Suppl. **5**, 301–320 (1974)
2.66 G.J.F. Pugh, E.B.G. Jones: Antarctic marine fungi, a preliminary account. In: *The Biology of Marine Fungi*, ed. by S.T. Moss (Cambridge Univ. Press, Cambridge 1986) pp. 323–330
2.67 R.S. Redman, K.B. Sheehan, R.G. Stout, R.J. Rodriguez, J.M. Henson: Thermotolerance conferred to plant host and fungal endophyte during mutualistic symbiosis, Science **298**, 1581 (2002)

2.68 H.J. Ju, N.S. Hill, T. Abbott, K.T. Ingram: Temperature influences on endophyte growth in tall fescue, Crop Science **46**, 404–412 (2006)

2.69 Y. Hashizume, F. Kenji, S. Norio: Effects of summer temperature on fungal endophyte assemblages in Japanese beech (*Fagus crenata*) leaves in pure beech stands, Bot. Marina **88**(3), 266–274 (2010)

2.70 P.L. Roberts, J.L. Hitchell, S.T. Moss, E.B.G. Jones: Morphological and molecular taxonomy of marine ascomycetes Corollospora, 6th Int. Mar. Mycol. Symp. (1995), p. 54

2.71 J.C. Gilman: *A manual of soil fungi* (Oxford and IBH Publishing Company, Calcutta 1959)

2.72 M.B. Ellis, J.P. Ellis: *Micro fungi on Land Plants: An Identification Handbook* (Croom Helm, London 1985)

2.73 C.V. Subramanian, S. Raghukumar: Ecology of higher fungi in soils of marine and brackish environments in the around Madras, Veröff. Inst. Meeresforsch. Bremerhav. Suppl. **5**, 377–402 (1974)

2.74 W.C. Snyder, H.N. Hansen: The species concept in *Fusarium*, Am. J. Bot. **27**, 64–67 (1940)

2.75 S.L. Woo, A. Zoina, G. Sorbo, M.D. Lorito, N.B.F. Scala, C. Noviello: Characterization of *Fusarium oxysporum f.* sp. *phaseoli* by pathogenic races, VCGs, RFLPs and RAPD, Phytopathology **86**, 966–973 (1996)

2.76 J.W. Taylor, D.J. Jacobson, S. Kroken, T. Kasuga, D.M. Geiser, D.S. Hibbett, M.C. Fisher: Phylogenetic species recognition and species concepts in fungi, Fungal Genet. Biol. **31**, 21–32 (2000)

2.77 J.G.K. Williams, A.R. Kubelik, K.J. Livak, J.A. Rafalski, S.V. Tingey: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers, Nucleic Acids Res. **18**, 6531–6535 (1990)

2.78 B.A. McDonald: The population genetic of fungi: Tools and techniques, Phytopathology **87**, 448–453 (1997)

2.79 T.D. Bruns, T.J. White, J.W. Talyor: Fungal molecular systematics, Annu. Rev. Ecol. Syst. **22**, 525–564 (1991)

2.80 C. Yao, R.A. Frederiksen, C.W. Magill: Length heterogeneity in ITS2 and the methylation status of CCGG and GCGC sites in the rRNA genes of the genus Peronosclerospora, Current Genet. **22**, 415–420 (1992)

2.81 G.T. Bryan, M.J. Daniels, A.E. Osbourn: Comparison of fungi within the Gaeumannomyces-Phialophora complex by analysis of ribosomal DNA sequence, Appl. Environ. Microbiol. **61**, 681–689 (1995)

2.82 G. Strobel, B. Daisy: Bioprospecting for microbial endophytes and their natural products, Microbiol. Mol. Biol. Rev. **67**, 491–502 (2003)

2.83 A.L. Demain: Pharmaceutically active secondary metabolites of microorganisms, Appl. Microbiol. Biotechnol. **52**, 455–463 (1999)

2.84 N. Arivuselvan, M. Kalaiselvam, P. Anantharaman: vitro antidermatophytic activity of seaweed from Mandapam Coast, Br. J. Pharmacol. Taxicol. **1**(2), 62–66 (2011)

2.85 N.B. Dhayanidhi, T.T. Ajithkumar, M. Kalaiselvam, T. Balasubramanian, N. Sivakumar: Antidermatophytic activity of marine sponge, *Sigmadocia carnosa* (Dendy) on clinically isolated fungi, Asian Pac. J. Trop. Biomed. **2**(1), 1–5 (2012)

2.86 A. Dhinakaran, M. Kalaiselvam, R. Rajasekaran: Isolation of *Penicillium* sp. and its antagonistic activity against dermatophytes from volcano soil of Baratang Island, Andaman, Int. J. Pharm. Sci. Res. **3**(2), 564–568 (2012)

2.87 K. Liberra, U. Lindequist: Marine fungi – a prolific resource of biologically active natural products, Pharmazie **50**(9), 583–588 (1995)

2.88 D.J. Faulkner: Marine natural product, Nat. Prod. Rep. **19**, 1–48 (2002)

2.89 T. Ramesh, M. Kalaiselvam: An experimental study on citric acid production by *Aspergillus niger* using *Gelidiella acerosa* as a substrate, Indian J. Microbiol. **51**(3), 289–293 (2011)

2.90 K. Sharmila, K.A. Thillaimaharani, A.R. Logesh, A. Sathishkumar, M. Kalaiselvam: Production of cyclosporin-A by saprophytic filamentous fungus *Fusarium oxysporum*, Int. J. Phar. Pharm. Sci. **4**(4), 149–153 (2012)

2.91 A. Elavarasi, G. Sathiya Rathna, M. Kalaiselvam: Studies on Taxol Production of Mangrove Endophytic Fungi *Fusarium oxysporium*, Asian Pac. J. Trop. Biomed. **1**(2), 1–5 (2012)

2.92 A.R. Logesh, K.A. Thillaimaharani, K. Sharmila, M. Kalaiselvam, S.M. Raffi: Production of chitosan from endolichenic fungi isolated from mangrove environment and its antagonistic activity, Asian Pac. J. Trop. Biomed. **2**(2), 140–143 (2012)

3. Diversity of Marine Phototrophs

Hideaki Miyashita

Phototrophs transform the energy of solar irradiation into chemical-bond energy in organic substances. Those organic substances provide biological energy not only for the activities of life on Earth, but also the formation and maintenance of regional and/or global ecosystems. They also provide biotechnological resources for materials, energy, and so on. Since their energy transformation is the starting point of the energy flow in the marine ecosystem, the understanding of *phototrophs* is important not only for the consideration of the homeostasis of the marine environment, but also for the development of new marine biotechnologies. Traditionally, diatoms and flagellates had been understood to be the major primary producers in the ocean. However, the developments of technologies for phytoplankton research within the past three decades revealed a diversity of phototrophs serving as *primary producers* and contributing to the transformation of light-energy into the chemical energy. The contribution by those diverse phototrophs to the energy flow in the ocean seemed much larger than that by diatoms and flagellates, which indicated the necessity of reconsidering energy flow or primary production. In this paper, the author discusses the diversity of planktonic phototrophs that serve as the *primary producers* (the starting point of the energy flow in the marine environments) based on research results in chronological order.

3.1 Traditional Understanding of Primary Producers (≈1970s)

Until the mid 1970s, primary production in marine environments was understood to be performed mainly by diatoms and flagellates [3.1]. It was obvious that they contribute to primary production, since they are the major organisms that can be collected by a phytoplankton net (small mesh-size net). Moreover, phytoplankton are distributed in a wide range of water quality, including eutrophic and oligotrophic environments. Dinoflagellates are also widely distributed as a major member of phytoplankton in the oligotrophic ocean. Symbiotic dinoflagellates in corals at tropical reefs play a significant role as the major primary producer in those oligotrophic environments. Thus, diatoms and flagellates were understood to be the major primary producers in marine environments (Fig. 3.1).

The reason why those two microalgae were considered to be the major primary producers was easily speculated from the approach to phytoplankton sampling and the methods for phytoplankton detection at the time. Except for phytoplankton bloom such as red

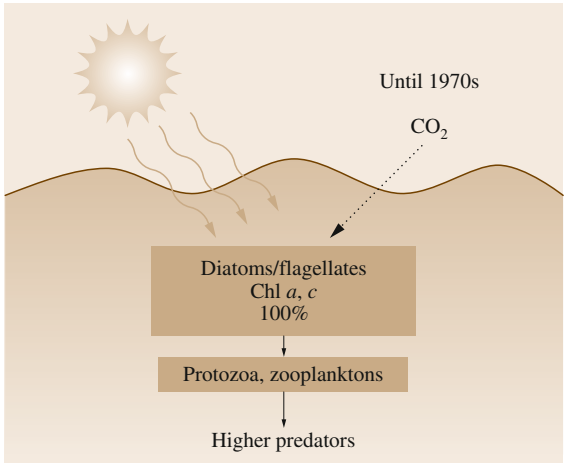

Fig. 3.1 Classical understanding of primary production

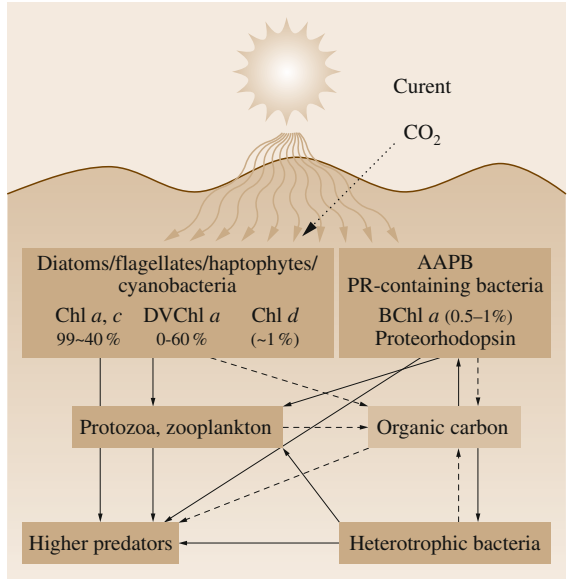

Fig. 3.2 Current understanding of the flow of energy and carbon. AAPB: aerobic anoxygenic photosynthetic bacteria, PR-containing bacteria: proteorhodopsin-containing bacteria

tide or cyanobacterial blooms, plankton nets had been used for phytoplankton sampling. A plankton net is field-equipment used to trap plankton. It is a conical polyethylene net with a defined mesh size and has a collecting bottle attached to the tip end. The mesh size of the net determines the size range of the plankton trapped. In phytoplankton research, nets with mesh sizes of 100 μm (XX13), 70 μm (XX17), and 60 μm (XX25) were used. The result was that only phytoplankton with a cell or colony size of more than 60 μm were used in phytoplankton research until the 1970s (Fig. 3.2).

However recent phytoplankton research using deoxyribonucleic acid (DNA) detection and flow cytometry has shown that the predominant phytoplankton in marine environments is much smaller than those collected by plankton nets. For example, haptophytes including coccolithophores, which are phytoplankton that deposit calcareous plates called coccoliths, are one of the major constituents of primary producers in marine environments. However, most of these pass through a phytoplankton net that is usually used for phytoplankton research, since the cell size of most haptophytes is less than 50 μm. Plankton nets with a smaller mesh size of around 10 μm have been employed for phytoplankton research in recent years. However, the dominant phytoplankton in marine environments has much smaller cell size than the cells that can be caught by those smaller mesh-sized nets.

3.2 Recognition of Picocyanobacteria Dominance (1970s–2000s)

In the late 1970s, the development of observation techniques by fluorescence microscopy and accumulation of knowledge from electron microscopic observations revealed that unicellular cyanobacteria *Synechococcus* spp. are predominantly distributed in the marine environments, especially in tropical and subtropical regions [3.2, 3]. They are very tiny cells with 0.5−2.0 μm in length and 0.5−1.0 μm in diameter, called picocyanobacteria. They play a significant role as a primary producer in those environments. In the late 1980s, the developments of the high performance liquid chromatography (HPLC) technique for pigment analysis and of the cell detection technique using flow cytometry brought new insight in marine picocyanobacteria. Apart from picocyanobacteria, which was recognized as *Synechococcus* spp., it contained divinyl-chlorophyll (DVChl) *a* which had an absorption spectrum slightly different from that of the usual Chl *a* [3.4–6]. Furthermore, those cells did not contain phycobilipigments, which were typical light-harvesting antenna in com-

mon *Synechococcus*. The cyanobacterium named as the genus *Prochlorococcus* is coccoid to ellipsoidal unicellular cells with 0.4–0.6 in diameter and 0.5–0.8 in length. They perform oxygenic photosynthesis using DVChl a/b which can efficiently absorb the blue light dominating in the ocean. They are the dominant picophytoplankton in tropical and subtropical regions between the latitudes of 30° north and 30° south. In several marine regions, the amount of DVChl *a* is three times higher than that of usual Chl *a*, indicating that *Prochlorococcus* play a significant role as a primary producer in those environments.

3.3 Discovery of Ubiquitous Photoheterotrophs (2000–Current Times)

In the early 2000s, two significant findings were reported with respect to the fact that microorganisms other than traditional phytoplankton could possibly contribute significantly to the transformation of light energy into chemical energy in marine environments. One was the wide distribution of aerobic anoxygenic photosynthetic bacteria (AAPB) which contained bacteriochlorophyll (BChl) *a*, that had mainly been recognized as a photosynthetic pigment in anaerobic anoxygenic photosynthetic bacteria (AnAPB) distributed in anaerobic environments [3.7]. The other was the wide distribution of proteorhodopsin-containing bacteria [3.8, 9]. Both bacteria are photoheterotrophs without carbon fixation and had never been recognized as *primary producers*.

APB were first reported in 1979 [3.10]. Phylogenetically, AAPB was derived from non-sulfur purple bacteria [3.11], the anaerobic anoxygenic photosynthetic bacteria (AnAPB) in the α-Proteobacteria. AnAPB had been traditionally understood to distribute in an anaerobic environment and perform photosynthesis without oxygen evolution. They employ bacteriochlorophylls (BChls) for their photosynthesis instead the Chls detected in algae and plants. The amount of primary production by AnAPB reached up to 30% of the total primary production in some specific lakes [3.12]. However, their contribution was negligible on global scale, since such environments were restricted. On the other hand, AAPB grow (photo-)heterotrophycally in an aerobic environment. AAPB are the same with AnAPB on the point that they contain BChl *a*. However, AAPB are aerobic and require molecular oxygen for their growth and BChl *a* synthesis [3.11]. AAPB distributes widely in the euphotic zone of marine environments in amounts with from ranging from 1–24% of the total bacterial count [3.13]. It had been already reported that the phototrophic growth of some APPB was faster than those growing heterotrophically in the dark [3.14]. Moreover, the in situ growth rate of APPB in seawater was faster than that of other heterotrophic bacteria. The detailed physiological role and activity of photosystems in all AAPB have not clarified yet, but AAPB must contribute to the transformation of solar energy in marine environments and act as *primary producer*.

Proteorhodopsin is the rhodopsin-like protein detected in marine Proteobacteria. It is a retial-opsin complex in a member of rhodopsin. Opsin is a bundle of seven transmembrane α-helices. Retinal, vitamin A aldehyde binds to the lysine residue at the central pocket of the opsin. Rhodopsin is well known as a photoreceptor in vertebrates as a light sensor. One rhodopsin-like protein was known to contribute to adenosine triphosphate (ATP) synthesis. Bacteriorhodopsin of *Halobacteria* in *Archea* can transform light energy to transmembrane proton electrochemical gradients ($\Delta\mu H^+$) for ATP synthesis [3.15]. Conformation of retinal molecule changes by absorbing light in synchronization with structure changes of ligand in the central part of opsin. The conformation change lead a serial proton to deliver from cytoplasmic to periplasmic space, and results in a formation of $\Delta\mu H^+$ for ATP synthesis. However, such ATP synthesis using bacteriorhodopsin had been thought to be a special mechanism that is restricted in special Archaean bacterium-distributed extremely salty environments.

However, a large amount and wide variety of rhodopsin-like genes were detected in seawater by metagenomic analysis [3.8, 9]. Moreover the gene was expressed in *E. coli* and acted to form $\Delta\mu H^+$ for ATP synthesis under light [3.16]. The genes of proteorhodopsin are contained in a diversity of bacterial taxa [3.17]. The total amount of proteorhodopsin-containing bacteria is equivalent to 13–80% of the total amount of bacteria and *Archea* in marine environments [3.18, 19]. These results indicate that proteorhodopsin was widely and significantly distributed and play a significant role in light energy transformation in the marine environments. Actually, light-dependent ATP synthesis using proteorhodopsin was

reported in a Flavobacterium strain [3.20]. However, this light-dependent growth was not observed in all proteorhodopsin-containing bacteria. A role of proteorhodopsin other than ATP synthesis was indicated, such as the advantage for the recovery from a starvation situation [3.21]. Detailed research on the roles of proteorhodopsin will clarify its contribution to the light energy conversion process in marine environments.

3.4 Oxygenic Photosynthesis Using Far-Red Light (1990s–2011)

Chl *a* is indispensable for oxygenic photosynthesis, since it plays an essential role in light harvesting, energy transfer, and charge separation in the primary reaction in photosynthesis. This is based on the results from photosynthesis research using land plants and green algae as model organisms. Quantification of the amount of oxygenic phototrophs and the activity of oxygenic photosynthesis are, therefore, estimated based on the amount of Chl *a*. The in situ light absorption range by Chl *a*, from 400 to 700 nm (or from 380 to 710 nm), is defined as photosynthetically active radiation (PAR). PAR is used for the evaluation of light strength for the activity of oxygenic photosynthesis. However, a cyanobacterium *Acaryochloris marina*, which performs oxygenic photosynthesis using Chl *d*, was reported in 1996 [3.22]. Chl *d* is a red-shifted chlorophyll which can absorb far-red light for an almost 30 nm longer wavelength than Chl *a* [3.23]. The cyanobacterium employs Chl *d* not only for the light-harvesting antenna but also for the chlorophyll in the reaction centers [3.24, 25]. As a result, the cyanobacterium can perform oxygenic photosynthesis using far-red light from 700 to 740 nm, which cannot be effectively used by common phototrophs. *Acaryochloris* spp. are widely distributed in coastal areas as epiphytes on seaweed [3.26]. The amount of Chl *d* on the seaweed ranges between about 1−13% versus total Chl *a* content in seaweed. Moreover, chlorophyll was universally detected to be about 1% (versus the Chl *a* amount) in the bottom sediment of coastal areas and lakes in the Antarctic and Arctic [3.26]. The energy transfer efficiency of the photosynthesis using Chl *d* is equivalent to that using Chl *a* [3.27]. Photosynthesis using far-red light, which has not been estimated at all to date, must contribute to primary production in marine environments.

Moreover, in 2010, another red-shifted chlorophyll, Chl *f* was reported from a cyanobacterium isolated from the microbial mat on stromatolite at the coast of Australia [3.28]. Chl *f* has its absorption maximum at around 720 nm and it can absorb far-red light up to around 760 nm in vivo. Because we have to wait for further research to know the contribution of Chl *f* to oxygenic photosynthesis in marine environments, the activity of Chl *f* was not taken into account in the estimation of primary production in marine environments.

These findings on far-red utilization using the red-shifted chlorophylls, Chl *d* or Chl *f*, for oxygenic photosynthesis require reconsideration of the PAR concept and primary production in marine environments.

3.5 Discovery of Picoeukaryotic Phytoplankton (1990s–2011)

In recent years, very diverse eukaryotic picophytoplankton species have been revealed to be widely distributed in significant amounts in marine environments. However, only some dozens of species in chlorophyta, heterokontophyta, and haptophyta were known as eukaryotic phytoplankton until the early 2000s.

Around 2010, several reports on marine picophytoplankton analysis using the techniques of metagenome and flow cytometry were published. These showed that a significant amount of picohaptophytes, including many undescribed species, were widely distributed in marine environments. By pigment composition analysis of marine waters, a carotenoid 19′-hexanoyloxyfucoxanthin (19HF) was predominantly detected in the picoplankton fraction less than 3 μm at a high latitude area from 40° to 60° of both north and south [3.29]. Since picocyanobactria, which is well known as a predominant pico-phytoplankton in tropical and subtropical marine environments, do not contain 19HF, and 19HF is a specific pigment to the algae in the Haptophyta, the amount of picophytoplankton belonging to

Haptophyta accounted for 50% of the total amount of the picophytoplankton fraction [3.29]. A similar estimate was also reported; picohaptophytes formed 5% of the global picophytoplankton biomass and contribute significantly to primary production in the marine environment [3.30].

Furthermore, it was also reported that a significant amount of microalgae in unknown phylum (unisolated phylum to date) also exists in marine environments. In 2007, phytoplankton which did not belong to any known phyla of algae were discovered by metagenomic analysis of a plankton fraction of less than 3 μm in size [3.31]. Its DNA sequence was close to the cryptophycean linage. It was tentatively named *picobiliphytes*. The *picobiliphytes* distribute widely in coastal areas of Europe and the North Atlantic, and the cell number was reaches up to 1.6% of the total eukaryotic phytoplankton. In 2011, the new candidate phylum was also detected by metagenomic analysis targeting plastid DNA. *Rappemonads*, the name tentatively given to the algae in the candidate phylum, were close to haptophytes, but they did not belong to any known phyla phylogenetically [3.32]. *Rappemonads* also widely distribute in coastal areas of Europe, and the Atlantic and Pacific oceans. Since algae in both candidate phyla have not been isolated and cultivated, the characteristics in morphology, physiology, life cycle, photosynthesis, and productivity are not yet known. It might possible to find a further new phylum of photoplankton which has not yet been discovered. The accumulation of knowledge on the diversity of phytoplankton including unknowns might contribute to a better understanding of primary production in marine environments.

3.6 Strange Phototrophic(?) Microorganisms (1990–2011)

The cyanobacterium-like particle UCYN-A was detected by metagenomic analysis of nitrogen-fixing bacteria in marine waters. It was first detected as a cyanobacterium which had a nitrogenase gene for nitrogen fixation in pelagic waters. The wide distribution of UCYN-A indicated that the particle contributes to the nitrogen cycle in pelagic waters. Usual cyanobacteria grow by an oxygenic photosynthesis process photosystems, a respiration system, and genes for a carbon fixation system. However, UCYN-A lacks photosystem II (one of the two photosystems that split water into protons, electrons, and molecular oxygen), RuBisCo (ribulose-1,5-bisphosphate carboxylase-oxygenase), the primary enzyme for fixing CO_2 and the tricarboxylic acid (TCA) cycle for respiration [3.33]. The lacking of these essential genes for free living suggests that UCYN-A might be a symbiont in some organisms. Since it possesses a complete gene set for photosystem I and contains chlorophyll, it must use light energy for some activity of the particle. While it is unclear how the light energy absorbed by photosystem I is used in the particle, it must be contribute to the transformation of light energy into chemical energy.

3.7 Diversity of Light Energy Transformation Systems and Reconsideration of *Photosynthesis*

Organisms that can grow using light as its energy source are called *phototrophs*. Representatives of phototrophs are photosynthetic organisms that can perform *photosynthesis*. *Photosynthesis* is classically the reaction that can reduce carbon dioxide into organic compounds such as sugars using sunlight as a energy source. *Primary producers* are organisms that synthesize organic compounds using inorganic carbon as their carbon source. Their representatives are photosynthetic organisms (such as land plants, algae, cyanobacteria, and some photosynthetic bacteria) and chemoautotrophs. Since the productivity of chemoautotrophs in a general environment is considered to be much smaller than that of photoautotrophs. *Primary producers* has been used synonymously with *photosynthetic organisms*. Similarly, it has been considered that phototrophs and photosynthetic organisms are almost the same so far. However, the recent discoveries of new marine microbes which utilize light energy suggest that the conventional understanding of the terms *phototrophs*, *photosynthetic organisms* and *primary producers* may not necessarily be suitable for

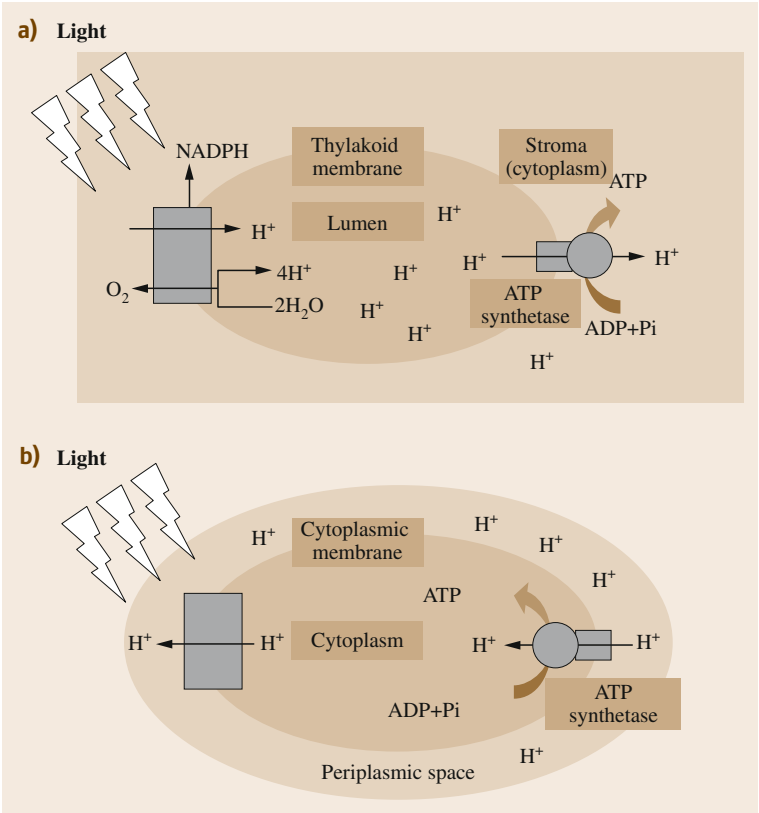

Fig. 3.3a,b Formation of transmembrane electrochemical potential of proton ($\Delta\mu H^+$) using sunlight. **(a)** Cyanobacteria or plastids in algae and land plants; **(b)** photosynthetic bacteria, halorhodopsin and proteorhodopsin-containing bacteria

the understanding of the energy and carbon flow in marine environments.

In this paper, the author defined *phototrophs* as *organisms which can synthesize ATP using light as its energy source*. In another words, phototrophs are

> *the organisms which can synthesize ATP by ATP synthase using the transmembrane electrochemical potential of proton ($\Delta\mu H^+$) by pumping up protons from the membranous one side to another side based on light energy.*

That is, in a chloroplast or cyanobacteria, protons are pumped into thylakoid lumen (the inside of thylakoid) from stroma (cytoplasm in the case of cyanobacteria) (Fig. 3.3a). In cases of non-oxygenic photosynthetic bacteria or proteorhodopsin-containing bacteria, protons are pumped out from the cytoplasmic space to the periplasmic space (Fig. 3.3b). Protons pumped into the thylakoid lumen or pumped out from cytoplasm flow back into the stroma or the cytoplasm, respectively, through ATP synthase, which results in the synthesis of ATP. Here, phototrophs are all organisms that can perform such light-dependent ATP synthesis.

Therefore, photosynthetic organisms are only a part of phototrophs. Primary producers are not only the organisms that can produce organic compounds from inorganic carbon, but also the organisms that can produce ATP using sunlight energy. As a result, phototrophs are almost equal to primary producers in this chapter. Table 3.1 shows primary producers under this concept. Further investigations on how these organisms transform light energy will develop the understanding of energy flows in the marine environment.

Table 3.1 Diversity of phototrophs

Phototrophs	Phylogenetic position	Pigment	Niche	Trophic mode	Carbon source
Anoxygenic phototrophs					
Purple bacteria (including aerobic bacteria)	Bacteria Proteobacteria	Bacteriochlorophyll	Anaerobic ~ Aerobic	Photoheterotroph Photoautotroph	Organic carbon CO_2
Green non-sulfur bacteria (filamentous anoxygenic photosynthetic bacteria)	Bacteria Chloroflexi	Bacteriochlorophyll	Anaerobic ~ Aerobic	Photoheterotroph Photoautotroph	Organic carbon CO_2
Green sulfur bacteria	Bacteria Chlorobi	Bacteriochlorophyll	Obligate anaerobic	Photoautotroph	CO_2
Heliobacteria	Bacteria Firmicutes	Bacteriochlorophyll	Obligate anaerobic	Photoheterotroph	Organic carbon
Chloroacidobacteria	Bacteria Acidobacteria	Bacteriochlorophyll	Aerobic	Photoheterotroph	Organic carbon
Halobacteria	Archaea Euryarchaeota	Bacteriorhodopsin	Aerobic ~ Anaerabic	Heterotroph Photoheterotroph	Organic carbon
PR-containing bacteria	Bacteria (polyphyly)	Proteorhodopsin	Aerobic	Heterotroph Photoheterotroph	Organic carbon
Oxygenic phototrophs					
Cyanobacteria	Bacteria Cyanobacteria	Chlorophyll	Aerobic	Photoautotroph	CO_2
Algae	Eukarya (polyphyly)	Chlorophyll	Aerobic	Photoautotroph Mixotroph	CO_2 Organic carbon
Land plants	Eukarya Streptophyta	Chlorophyll	Aerobic	Photoautotroph	CO_2

3.8 Conclusion

In relation to the food web in marine environments, studies on energy flow are important and relevant to global environment change, preservation and protection of living marine resources, and the diversity of marine microbes. Energy flow in the marine environments has been thought based on the food chain, starting with oxygenic photosynthesis with carbon fixation. However, recent studies have revealed that the energy flow through the microbial loop is very important, in addition to the traditional understanding of the food web, since it is possible that a large amount of bacteria can transform light energy into chemical energy.

To shift to a new paradigm and create a new concept of the energy flow in marine environments, it is required to reveal the diversity of marine phototrophs and their roles in the energy and material flow in marine environments.

References

3.1 C.M. Lalli, T.R. Parsons: *Biological Oceanography: An introduction* (Elsevier, Oxford 1993)

3.2 J.B. Waterbury, S.W. Watson, R.R.L. Guillard, L.E. Brand: Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium, Nature **277**, 293–294 (1979)

3.3 P.W. Johnson, J.M. Sieburth: Chroococcoid cyanobacteria in the sea: A ubiquitous and diverse phototrophic biomass, Limnol. Oceanogr. **24**, 928–935 (1979)

3.4 W.W.C. Gieskes, G.W. Kraay: Unknown chlorophyll a derivatives in the North Sea and the tropical Atlantic Ocean revealed by HPLC analysis, Limnol. Oceanogr. **28**, 757–766 (1983)

3.5 S.W. Chisholm, R.J. Olson, E.R. Zettler, R. Goericke, J.B. Waterbury, N. Welschmeyer: A novel free-living prochloropyte abundant in the oceanic euphotic one, Nature **334**, 340–343 (1988)

3.6 F. Partensky, W.R. Hess, D. Vaulot: *Prochlorococcus*, a marine photosynthetic prokaryote of global

significance, Microbiol. Mol. Biol. Rev. **63**, 106–127 (1999)
3.7 Z.S. Kolber, C.L. Van Dover, R.A. Niederman, P.G. Falkowski: Bacterial photosynthesis in surface waters of the open ocean, Nature **407**, 177–179 (2000)
3.8 O. Béjà, L. Aravind, E.V. Koonin, M.T. Suzuki, A. Hadd, C. Nguyen, S.B. Jovanovich, C.M. Gates, R.A. Feldman, J.L. Spudich, E.N. Spudich, E.F. DeLong: Bacterial rhodopsin: evidence for a new type of phototrophy in the sea, Science **289**, 1902–1906 (2000)
3.9 O. Béjà, E.N. Spudich, J.L. Spudich, M. Leclerc, E.F. DeLong: Proteorhodopsin phototrophy in the ocean, Nature **411**, 786–789 (2001)
3.10 T. Shiba, U. Simidu, N. Taga: Distribution of aerobic bacteria which contain bacteriochlorophyll *a*, Appl. Environ. Microbiol. **38**, 43–45 (1979)
3.11 V.V. Yurkov, J.T. Beatty: Aerobic anoxygenic phototrophic bacteria, Microbiol. Mol. Biol. Rev. **62**, 695–724 (1998)
3.12 J. Overmann, F. Garcia-Pichel: The phototrophic way of life. In: *The Prokaryotes 2*, ed. by M. Dworkin (Springer, New York 2006) pp. 32–85
3.13 R. Lami, M.T. Cottrell, J. Ras, O. Ulloa, I. Obernosterer, H. Claustre, D.L. Kirchman, P. Lebaron: High abundances of aerobic anoxygenic photosynthetic bacteria in the South Pacific Ocean, Appl. Environ. Microbiol. **13**, 4198–4205 (2007)
3.14 Z.S. Kolber, F.G. Plumley, A.S. Lang, J.T. Beatty, R.E. Blankenship, C.L. VanDover, C. Vetriani, M. Koblizek, C. Rathgeber, P.G. Falkowski: Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean, Science **292**, 2492–2495 (2001)
3.15 E. Bamberg, J. Tittor, D. Oesterhelt: Light-driven proton or chloride pumping by halorhodopsin, Proc. Natl. Acad. Sci. USA **90**, 639–643 (1993)
3.16 T. Friedrich, S. Geibel, R. Kalmbach, I. Chizhov, K. Ataka, J. Heberle, M. Engelhard, E. Bamberg: Proteorhodopsin is a light-driven proton pump with variable vectoriality, J. Mol. Biol. **321**, 821–838 (2002)
3.17 J.R. de la Torre, L. Christianson, O. Béjà, M.T. Suzuki, D. Karl, J.F. Heidelberg, E.F. DeLong: Proteorhodopsin genes are widely distributed among divergent bacterial taxa, Proc. Natl. Acad. Sci. USA **100**, 12830–12835 (2003)
3.18 E.F. DeLong, O. Béjà: The light-driven proton pump proteorhodopsin enhances bacterial survival during tough times, PLoS Biol. **8**, e1000359 (2010)
3.19 M.A. Moran, W.L. Miller: Resourceful heterotrophs make the most of light in the coastal ocean, Nat. Rev. Microbiol. **5**, 792–800 (2007)
3.20 S. Yoshizawa, A. Kawanabe, H. Ito, H. Kandori, K. Kogure: Diversity and functional analysis of proteorhodopsin in marine Flavobacteria, Environ. Microbiol. **14**, 1240–1248 (2012)
3.21 L. Gomez-Consarnau, N. Akram, K. Lindell, A. Pedersen, R. Neutze, D.L. Milton, J.M. Gonzalez, J. Pinhass: Proteorhodopsin phototrophy promotes survival of marine bacteria during starvation, PLoS Biol. **8**, e1000358 (2010)
3.22 H. Miyashita, H. Ikemoto, N. Kurano, K. Adachi, M. Chihara, S. Miyachi: Chlorophyll *d* as major pigment, Nature **383**, 402 (1996)
3.23 H. Miyashita, K. Adachi, N. Kurano, H. Ikemoto, M. Chihara, S. Miyachi: Pigment composition of a novel oxygenic photosynthetic prokaryote containing chlorophyll d as the major chlorophyll, Plant Cell Physiol. **38**, 274–281 (1997)
3.24 Q. Hu, H. Miyashita, I. Iwasaki, N. Kurano, S. Miyachi, M. Iwaki, S. Itoh: A photosystem I reaction center driven by chlorophyll *d* in oxygenic photosynthesis, Proc. Natl. Acad. Sci. USA **95**, 13319–13323 (1998)
3.25 T. Tomo, T. Okubo, S. Akimoto, M. Yokono, H. Miyashita, T. Tsuchiya, T. Noguchi, M. Mimuro: Identification of the special pair of photosystem II in a chlorophyll *d*-dominated cyanobacterium, Proc. Natl. Acad. Sci. USA **104**, 7283–7288 (2007)
3.26 Y. Kashiyama, H. Miyashita, S. Ohkubo, N.O. Ogawa, Y. Chikaraishi, Y. Takano, H. Suga, T. Toyofuku, H. Nomaki, H. Kitazato, T. Nagata, N. Ohkouchi: Evidence of Global Chlorophyll *d*, Science **321**, 658 (2008)
3.27 S. Miyachi, K. Strassdat, H. Miyashita, H. Senger: Quantum requirement of photosynthesis in the primarily chlorophyll *d*containing prokaryote *Acaryochloris marina*, Z. Naturforsch. **52c**, 636–638 (1997)
3.28 M. Chen, M. Schliep, R.D. Willows, Z.L. Cai, B.A. Neilan, H. Scheer: A red-shifted chlorophyll, Science **329**, 1318 (2010)
3.29 H. Liu, I. Probert, J. Uitz, H. Claustre, S. Aris-Brosou, M. Frada, F. Not, C. de Vargas: Haptophyta rule the waves: Extreme oceanic biodiversity in non-calcifying prymnesiophytes explains the 19-Hex paradox, Proc. Natl. Acad. Sci. USA **106**, 12803–12808 (2009)
3.30 M.L. Cuvelier, A.E. Allen, A. Monier, J.P. McCrow, M. Messie, S.G. Tringe, T. Woyke, R.M. Welsh, T. Ishoey, J.H. Lee, B.J. Binder, C.L. DuPont, M. Latasa, C. Guigand, K.R. Buck, J. Hilton, M. Thiagarajan, E. Caler, B. Read, R.S. Lasken, F.P. Chavez, A.Z. Worden: Targeted metagenomics and ecology of globally important uncultured eukaryotic phytoplankton, Proc. Natl. Acad. Sci. USA **107**, 14679–14684 (2010)
3.31 F. Not, K. Valentin, K. Romari, C. Lovejoy, R. Massana, K. Toebe, D. Vaulot, L. Medlin: Picobiliphytes: A marine picoplanktonic algal group with unknown affinities to other eukaryotes, Science **315**, 253–255 (2007)
3.32 E. Kim, J.W. Harrison, S. Sudek, M.D. Jones, H.M. Wilcox, T.A. Richards, A.Z. Worden, J.M. Archibald: Newly identified and diverse plastid-bearing branch on the eukaryotic tree of life, Proc. Natl. Acad. Sci. USA **108**, 1496–1500 (2011)
3.33 H.J. Tripp, S.R. Bench, K.A. Turk, R.A. Foster, B.A. Desany, F. Niazi, J.P. Affourtit, J.P. Zehr: Metabolic streamlining in an open-ocean nitrogen-fixing cyanobacterium, Nature **464**, 90–94 (2010)

4. Marine Viruses

Jeeva Subbiah

Marine viruses are the most abundant *life-forms* in the ocean and exist wherever life is found. The estimated virus count in the ocean is 10^{30}, and every second about 10^{23} viral infections occur in the ocean. These infections are a major source of disease and mortality in organisms ranging from shrimp to whales. Each infection potentially introduces new genetic information into an organism or into progeny viruses. These new genetic changes allow evolution of the host and virus population. Viruses represent the planet's largest pool of genetic diversity. Our understanding of the impact of viruses on global systems, however, is still incomplete. This chapter will present a general review of marine viruses; their impact on marine organisms, such as cyanobacteria, mollusks, and more detailed about shrimp viruses, diagnostics and the control of shrimp viral diseases.

4.1 Viruses

Viruses are more common in air, soil, and water, including the ocean environment, and exist wherever life is found. They are obligate intracellular parasites and infect all living organisms such as vertebrates, invertebrates, plants, fungi, algae, archaea, and bacteria. The marine environment is the major habitat for many living organisms which have a large virus population, and the interaction between virus and their hosts allow the evolution of both viruses and host organisms. Furthermore, each and every progeny virus has the potential to evolve as a new virus, which may involve a huge impact on living organisms or become an emerging virus [4.1]. The evolved new viral strains change in virulence and transfer viruses between ecosystems. In some circumstances, gene transformation between viruses and their hosts may produce dramatic effects on the hosts. For example, the well-known phage cholera toxin (CTX) gene originated from CTXf or CTXφ bacteriophage through horizontal gene transfer. The bacterium *Vibrio cholera* is normally harmless but could become one of humanity's greatest scourges by incorporating CTX genes of filamentous phage (*Inovirus*) from the family *Inoviridae* [4.2, 3]. Marine viruses are not only the major cause of mortality in marine organisms but are also involved in global biogeochemical cycle changes. Moreover, they are a huge reservoir of the greatest genetic diversity on Earth. The estimated virus count in the ocean is

10^{30}, and every second about 10^{23} viral infections occur in the ocean. Therefore, the monitoring of the virus population in the marine environment and an understanding of the virus replication and host specificity are vital and emerging fields in the study of marine viruses. However, our understanding of the impact of viruses on global systems is still incomplete [4.4].

4.2 General Characteristic Features of Viruses

Viruses consist of genetic materials such as nucleic acids, which are either DNA or RNA, surrounded by a protein coat. They can replicate as obligate intracellular parasites by taking on the biosynthetic functions within a host cell. All forms of cellular life are susceptible to virus infection. Therefore, we can speculate that every type of marine organism is host to at least one type of virus [4.5]. The genome of the DNA virus may be single or double-stranded, and the structure may be linear with free 5′ and 3′ ends or the ends have a covalently closed circular form. RNA viruses can also be categorized based on their genome structure: single-stranded RNA (ssRNA), double-stranded (dsRNA), linear, and circular. Single-stranded genomes can be designated as positive (exactly the same as messenger RNA (mRNA) except thymine replaces uracil in the ssDNA genome) or negative (complementary to the mRNA) sense genomes [4.6]. The genome size of viruses varies from a smaller size of about 1.7 kb (Porcine circovirus (ssDNA) and hepatitis delta virus (ssRNA)) to a larger genome size of approximately 1.2 Mb in mimivirus double-stranded DNA (dsDNA) [4.7]. The virus gene expression differs depending on the viral genome nature. For instance, dsDNA viruses encode genes similarly to cellular organisms such as plants, animals, and bacteria. Nevertheless, ssDNA, ssRNA, and dsRNA viruses following a unique gene expression. Furthermore, most prokaryote, plant, and fungal virus genomes are dsDNA, ssRNA, and dsRNA, respectively [4.6]. The structure and morphology of viruses are also comparable with other virus groups. Some of viruses have a lipid layer or compound which is responsible for the development of an envelope along with proteins on the surface of virions. The envelope involves the attachment and entry of virions into the host cells.

4.3 Host Specificity

Every virus has a host specificity and a particular virus can infect a particular species or cells. However, some viruses can infect multiple organisms or a wide range of species. The wide host range suggests that viruses might be involved in different host shift events. Viruses must adapt to the new genetic and immunologic environment of their hosts in order to replicate [4.8]. Furthermore, the interactions between virus and host are crucial for viral replication. Moreover, the proteins or receptor molecules, which are not uniform for all species for virus attachments, may play an important role in host specificity [4.9, 10]. Thus, viruses must often co-evolve with their hosts, which often leads to a species and host specificity [4.8]. Marine environments contain huge life-forms and many different species living in close proximity, which may allow the possibility of viral host shift events.

4.4 Viral Families in Marine Ecosystems

About 15 DNA viral families persist in the marine environment; however, the majority of the families are of the dsDNA virus family rather than ssDNA viruses. *Microviridae* and *parvoviridae* belong to the ssDNA virus family among the DNA virus families in the marine environment. *Microviridae* is one of the families of bacteriophages with single-stranded circular DNA genome. The virions are nonenveloped, icosahedral with spikes and a size of about 25–27 nm in diameter and lack tails. The genome sizes range

from 4.5 to approximately 6 kb, which can encode about 11 genes, and most of them have overlapping reading frames [4.11]. Most of the viruses and morphology are mentioned in Table 4.1. The most common dsDNA viruses include, *Baculoviridae, Herpesviridae, Iridoviridae, Lipothrixviridae, Nimaviridae, Papovaviridae, Phycodnaviridae, Corticoviridae, Tectiviridae, Myoviridae, Podoviridae*, and *Siphoviridae*. *Baculoviridae, Nimaviridae*, and *Parvoviridae* virus families are responsible for infection in crustaceans. Molluscs or mollusks, a large phylum of invertebrates, are the most predominant host for virus families like *Herpesviridae, Birnaviridae* (dsRNA), *Iridoviridae*, and *Papovaviridae*. *Iridoviridae* is a round, icosahedral-shaped virus, which can also infect fish. *Herpesviridae*, an pleomorphic, icosahedral, and enveloped virus, has a wide range of hosts, including mollusks, fish, turtles, and mammals. *Lipothrixviridae* and *phycodnaviridae* infect archaea and algae, respectively. The lipothrixvirus is an envelope and rod shaped, while phycodnavirus is icosahedrally-shaped. *Phycodnaviridae* is a group of large double-stranded DNA viruses and it can infect important taxa of marine primary producers including toxic bloom formers and macroalgae. A marine brown algae infecting phycodnavirus is the *Ectocarpus siliculosus* virus (EsV-1), which can encode about 231 proteins from a 336 kb size of the genome. EsV-1 is a lysogenic virus and the accumulating evidence indicates that the phycodnaviruses and their genes are ancient [4.12]. RNA viruses are a dynamic, widespread, and persistent component of the marine virus community. Although a vast number of marine RNA viruses have not been isolated, the most recognizable marine RNA viruses are positive-sense ssRNA viruses.

4.5 Marine Phages

Viruses can be arranged in terms of the types of cellular organisms that they infect. Marine phages are viruses that live as obligate parasitic agents in marine bacteria. The concentration of viruses is approximately 10^7 viruses/mL of surface seawater and the majority of the viruses are phages. Marine phages control bacterial abundance and impact the biogeochemical cycle through lysing their host (bacteria). In addition, marine phages are extremely diverse and influence their hosts through selection for resistance, horizontal gene transfer, and manipulation of the bacterial metabolism [4.13]. Sometimes, bacteriophages are responsible for altering the bacterial genome, gene expression, and development as emerging bacterial diseases or virulent strain through viral gene transfer. For instance, a major shrimp pathogen *Vibrio harveyi*, converted a nonvirulent strain into a virulent strain by DNA viruses like *Myoviridae* and *Siphoviridae* by transmission of a toxin gene [4.14]. *Corticoviridae, Tectiviridae, Myoviridae, Podoviridae, Siphoviridae, Microviridae, Cystoviridae*, and *Leviviridae* are common marine phages in the marine environment.

4.5.1 Types of Phage Infection

Phage infections can be categorized into the following types: lytic, lysogenic, chronic, pseudolysogenic, restricted, and abortive. In lytic infection, the produced phages release by destroying or lysing the bacterial cells and only the phage can survive. Lysogeny is a phage infection in which the phage genome can replicate continually without phage virion formation; in this case both bacteria and phages survive. The chronic infection effect is similar to lysogeny infection, both bacteria and phage survive, but it is a productive cycle; the produced mature phages are released from infected bacteria by extrusion or budding [4.15]. Some reports suggest that lysogeny is widespread in marine bacteria, and furthermore, up to 50% of bacterioplankton are lysogenic [4.16, 17]. The pseudolysogenic stage is a latent or quiescent state; the infecting phage genome does not replicate and does not produce phage progeny. This phenomenon is usually induced due to the unfavorable growth condition for bacteria, and this cycle ends with either the lytic or the lysogenic cycle, when it turns into favorable condition. This stage indicates phage and bacteria interactions, which may be crucial for the starving viral genome in the host and may involve the virulence of bacteria [4.18]. The bacterium (host) causes death to the infecting phage via host restriction endonuclease activity, thus there is no chance for phage progeny replication and production; this type of infection is called a restricted infection, and only the host only survives not the phage. Abortive infection involves the death of both host and infecting phage without production of phage progeny [4.15].

Table 4.1 Viruses infecting marine organisms

Virus family	Nucleic acid	Morphology	Size (nm)	Host
Baculoviridae	dsDNA	Enveloped, rods	200–450 × 100–400	Crustacea
Herpesviridae	dsDNA	Enveloped icosahedral	150–200	Mollusks, fish, mammals
Iridoviridae	dsDNA	icosahedral	190–200	Mollusks, fish
Lipothrixviridae	dsDNA	Enveloped, rod-shaped	40 × 400	Archaea
Nimaviridae	dsDNA	Enveloped, rod-shaped with tail-like appendage	120 × 275	Crustacea
Papovaviridae	dsDNA	Round, icosahedral	40–50	Mollusks
Phycodnaviridae	dsDNA	Icosahedral	130–200	Algae
Corticoviridae	dsDNA	Icosahedral with spikes	60–75	Bacteria
Tectiviridae	dsDNA	Icosahedral with spikes	60–75	Bacteria
Myoviridae	dsDNA	icosahedral	80–200	Bacteria
Podoviridae	dsDNA	Icosahedral with noncontractile tail	60	Bacteria
Siphoviridae	dsDNA	Icosahedral with noncontractile tail	60	Bacteria
Microviridae	ssDNA	Icosahedral with spikes	25–27	Bacteria
Parvoviridae	ssDNA	Round, icosahedral	20	Crustacea
Birnaviridae	dsRNA	Round, icosahedral	60	Mollusks, fish
Reoviridae	dsRNA	Icosahedral, some with spikes	50–80	Crustacea (?), mollusks, fish
Cystoviridae	dsRNA	Icosahedral with lipid coat	60–75	Bacteria
Bunyaviridae	ssRNA	Round, enveloped	80–120	Crustacea (?)
Caliciviridae	ssRNA	Round, icosahedral	35–40	Fish, mammals
Coronaviridae	ssRNA	Rod-shaped with projections	200 × 42	Crustacea
Dicistroviridae	ssRNA	Round, icosahedral	30	Crustacea
Leviviridae	ssRNA	Round, icosahedral	26	Bacteria
Marnaviridae	ssRNA	Round, icosahedral	25	Algae
Nodaviridae	ssRNA	Round, icosahedral	30	Fish
Orthomyxoviridae	ssRNA	Round, with spikes	80–120	Fish
Paramyxoviridae	ssRNA	Enveloped, filamentous	60–300 × 1000	Mammals
Picornaviridae	ssRNA	Round, icosahedral	27 × 30	Algae, crustacea (?), thraustochytrids
Rhabdoviridae	ssRNA	Bullet-shaped with projections	45–100 × 100–430	Fish

4.5.2 Cyanophages

Cyanobacteria are photosynthetic prokaryotes; they are one of the largest and most important groups of bacteria on earth. Cyanobacteria play an important role as primary producers in the ocean world. Examples are the cyanobacterial genera *Synechococcus* and *Prochlorococcus*, which together account for about 25% of global photosynthesis [4.19]. However, some groups of viruses infect cyanobacteria and cause mortality, that is, those groups of viruses called cyanophages. At the beginning, the causes of cyanobacteria mortality was poorly understood; it was assumed that it was due to protozoan infection rather than viral infection. However, the recent research data suggests that up to 7% of the heterotrophic bacteria and 5% of the cyanobacteria were associated with phages, and approximately 70% of prokaryotes might be infected by viruses [4.20]. In the 1990s, the first viral infection of marine unicellular cyanobacteria was reported and subsequently the cyanophages were isolated and characterized [4.20, 21].

Horizontal Gene Transfer

The interaction of phages and cyanobacteria may possible for new pathways of carbon and nitrogen cycling in marine food webs through gene transfer between marine organisms [4.20]. Metagenomic analysis of viral fractions reveals that cyanophages are widely distributed in the marine environment and interaction between cyanophages and cyanobacteria have been observed in the ocean [4.22]. Especially, most of the

cyanophage genes are involved in cyanobacteria photosynthesis [4.23]. Photosynthesis genes such as high light inducible genes (*hli*), psbA, and psbD are found in cyanophages. These genes encode the photosystem II (PSII) core reaction-center proteins D1 and D2, respectively. PSII, which catalyzes the light-dependent oxidation of water to molecular oxygen in chloroplasts, is a large pigment–protein complex in the thylakoid membrane. The D1 and D2 proteins of PSII bind the pigments and cofactors necessary for primary photochemistry. PSII is very sensitive to photo inhibition, and the D1 protein of the PSII reaction center is the main target for light-induced damage among the PSII proteins. The damaged D1 proteins are degraded and subsequently replaced with newly synthesized polypeptides in a repair cycle. This efficient repair mechanism is crucial to maintain PSII in a functional state. Cyanophages shut down the most of the host's gene expression during the lytic cycle infection and the proton motive force must be maintained if they are to lyse the host. Therefore, it is necessary to prolong photosynthesis of the hosts during the infection cycle. Thus, it is intended that the phage generates the energy for viral production by encoding *psbA* and other genes involved in photosynthesis. The phylogenetic analysis of the cyanophage *psbA* gene provides evidence that the acquisition of these genes by horizontal gene transfer from their cyanobacterial hosts (*Synechococcus* and *Prochlorococcus*) and gene acquisition were not very recent [4.24].

Types of Cyanophages

Cyanophages have been classified into three-tailed phage families: *Myoviridae, Podoviridae*, and *Siphoviridae*; all of them are dsDNA phages. These viruses can be isolated from both marine and fresh water environments [4.25]. Myoviruses and siphoviruses have broad host ranges and have frequently been isolated from a natural marine ecosystem. However, podoviruses have a very narrow host range with a short noncontractile tail. Myoviruses are nonenveloped, have a head with icosahedral symmetry, and a tail with tubular and helical symmetry, which is separated by a neck. The head diameter is 50–110 nm, while the tail is 16–20 nm, and the capsid is made up of 152 capsomers. Furthermore, morphological evidence supports that marine and freshwater myoviruses are more closely related each other than other bacteriophages. Only six cyanophages (S-PM2, P-SSM2, P-SSM4, P-SSP7, P60, and Syn9) infect cyanobacteria in the marine environment and all of these cyanophage sequences are available in GenBank. Interestingly, all those six cyanophages have been isolated either from *Synechococcus* or *Prochlorococcus*. S-PM2, P-SSM2, and P-SSM4 are more similar in morphology to the *Myoviridae*, However P-SSP7 and P60 belong to the *Podoviridae* family. The genomes of podoviral cyanophages are small and compact compared to myoviral cyanophages. For instance, the genome size of P-SSP7 and P60 are found to be about 44 to 47 kb. Nevertheless, myoviral cyanophages have relatively large genomes; 196, 280 bp, 178, 249 bp, and 252, 401 bp are found in S-PM2, P-SSM4, and P-SSM2, respectively [4.26].

4.5.3 Phage Therapy

Natural bacterial viruses or bacteriophages have been applied to control bacterial diseases. For example, instead of antibiotics, phages are used as a common therapy for human gastrointestinal diseases such as salmonellosis in Russia [4.27]. In 1915, the phage was discovered by the English microbiologist F.W. Twort and subsequently by d'Herelle, who introduced the term bacteriophage [4.28]. Phage research was an important field of research in the 1920s and it was desired to treat bacterial diseases [4.28]. Despite phages having been used as an antibacterial agent in the United States and Europe during the 1920s and 1930s, it has been abandoned in the western countries for various reasons, including the discovery of antibiotics. Although the commercial production of therapeutic phages has ceased in most of the Western world, phages continue to be used therapeutically in Eastern Europe and in the former Soviet Union. Moreover, several institutions in these countries are actively involved in therapeutic phage research and production [4.27].

Antibiotics have been widely used as an antibacterial agent. However, antibiotic drugs allow the development of mutated drug-resistant bacteria. Therefore, an effective alternative therapy is necessary to control the rise of anti-drug-resistant bacteria. Bacteria have the tendency to mutate against antibiotics once in every 10^6 divisions, but the antibiotics are immutable chemicals, which are not effective against the new antibiotic resistant bacterium. Although bacteria are also becoming resistant to phages, the rate of developing resistance against phages is approximately once in every 10^7 divisions, which is approximately tenfold lower compared to antibiotics. Furthermore, phages are living organisms and evolve along with hosts by mutation. Thus mutated phages can overcome the bacterial mutations within either a few days or weeks.

Additionally, phages could be the ideal strain for co-therapy along with antibiotics to prevent the emergence of bacterial resistance to antibiotics. Especially lytic phages are the most suitable candidates for phage therapy, due to the fact that they reproduce rapidly within the bacteria and lyse these in their specific host range [4.29].

Since 2006, the United States Food and Drug Administration and United States Department of Agriculture have approved several bacteriophage products for use on all food products. In marine aquaculture, the use of bacteriophages for the control of bacterial diseases in prawns in Asia has been considered. Phage therapy has been applied to control the bacterial pathogen *Vibrio harveyi*, which causes luminous bacterial disease in shrimp larvae [4.30]. Due to their host specificity, this phage therapy may be applicable to control many bacterial diseases in the future.

4.6 Impact of Marine Viruses on Mollusks

Mollusks are invertebrates and one of the largest marine phyla. These bivalve mollusks are filter feeders, thus marine viruses can be easily accumulated in their tissues and may transfer or infect other species, including higher vertebrates and humans, through the food chain. Although the pathogenic viruses are harmful to other species, it gives serious effect on the bivalve mollusks [4.31]. In the 1960s a major epizootic gill necrosis was observed in the oyster *Crassostrea angulata*, and the first report of viral disease in mollusks was reported at the same time. Most mollusks are associated with many different types of viruses including *Herpesviridae, Papovaviridae, Togaviridae, Retroviridae, Reoviridae, Birnaviridae, Picornaviridae*, and the irido-like virus [4.32]. Marine molluscan viruses and their structure morphology and main hosts are given in Table 4.2. Although many viruses are associated with marine mollusks, herpes-like and birnavirus groups are the major threats to mollusks.

4.6.1 Herpesvirus

Herpes-like virus infections have been identified in various marine mollusks throughout the world. As a result of viral disease, European oyster fisheries were destroyed in the 1970s. Since the first observation of the herpesvirus in *Crassostrea virginica*, many reports suggest that mortality is associated with this virus in *Ostreaedulis* and *C. gigas* in France and New Zealand. Herpes-like viruses were also observed in haemocytes of *O. angasi* adults in Australia and in New Zealand in flat oysters, *Tiostreachilensi*. Ostreid herpesvirus 1 (OsHV1); a herpesvirus has been isolated and reported from several species of bivalve mollusks. Moreover, unlike most herpesviruses, this virus has a wide host range of marine bivalves [4.32]. Sequence analysis results have revealed that it has links with groups of herpesviruses isolated from mammals, birds, and fish [4.33].

4.6.2 Birnavirus

Marine birnaviruses (MABV) are icosahedral, non-enveloped viruses, belonging to *Birnaviridae*. The genome of this virus comprises 2 segments of double-stranded RNA designated A and B [4.34]. MABVs had been responsible for the considerable losses in *Pictada fucata*, a commercially important pearl oyster in Japan [4.35]. MABVs infect a wide range of shellfish and fish, and it has been suspected that the mode of transmission may be due to zooplankton [4.36].

Viral diseases in bivalves are a serious concern without any specific chemotherapies and vaccination. According to the bivalve culture, a routine effective diagnostic tool is needed to monitor virus infection, and it is also important to control viral diseases.

4.7 Marine Viruses and Shrimp Aquaculture

Shrimp comprise a main aquaculture commodity worldwide. Many viruses affect shrimps, causing severe mortality of economically important shrimp species. Approximately 60% of disease losses in shrimp aquaculture is associated with viral pathogens, 20% of bacteria, and 20% by fungi and other pathogens [4.37].

Table 4.2 Viruses associated in marine mollusks

Viruses	Host species	Nucleic acid	Morphology	Symmetry
Herpesviridae	*Crassostrea virginicci*	DNA	90 nm capsid; 200 × 250 nm enveloped virion	Icosahedral
Togaviridae	*Ostrea lurida*	RNA	50 nm, enveloped virion	Icosahedral?
Retroviridae	*Crassostrea virginica*	RNA	100–110 nm, enveloped virion	Anisometric
Reoviridae	*Sepia officinalus*	RNA	75 nm, nonenveloped capsid	Icosahedral
Birnaviridae	*Pictada fucata*	RNA	60 nm, nonenveloped virion	Icosahedral
Iridoviridae	*Octopus vulgaris*	DNA	110–120 nm enveloped	Icosahedral
Papovaviridae	*Crassostrea virginica*	DNA	53 nm, nonenveloped virion	Icosahedral

The shrimp industry has grown rapidly and become a major global enterprise that serves the increasing consumer demand for seafood, and it plays an important role in improving food security, poverty mitigation, employment, and other economic activities in many countries [4.38]. Moreover, shrimp aquaculture has contributed significantly to national economies in Southeast Asia and Central and South America.

Since the early 1990s, shrimp aquaculture has expanded rapidly, reaching a worldwide production of 1 million metric tonnes (MT) in 2002 [4.39]. The global production of cultivated shrimp was about 3.5 million MT in 2009 and it continues to represent a vital source of export income for many countries [4.40]. Despite this success, viral diseases have caused billions of dollars' worth of losses for shrimp farmers.

More than 20 viruses, including white spot syndrome virus (WSSV), yellow head virus (YHV), gill-associated virus (GAV), Taura syndrome virus (TSV), infectious myonecrosis virus (IMNV), *Baculoviruspenaei* (BP), monodon baculovirus (MBV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), hepatopancreatic parvovirus (HPV), Mourilyan virus (MoV), Laem–Singh virus (LSNV), spawner-isolated mortality virus (SMV), baculoviral midgut gland necrosis virus (BMNV), and lymphoid organ vacuolization virus (LOVV), have been reported to infect marine shrimp species [4.41]. With the exception of WSSV, YHV, GAV, MBV, IHHNV, and HPV, the genera of many viruses have not yet been assigned due to the poor level of current virus genome characterization [4.41].

4.7.1 RNA Viruses in Shrimp

Seven ssRNA viruses and one dsRNA shrimp virus have been reported to date (Table 4.3). Among the RNA viruses, YHV and TSV are major threats to shrimp aquaculture, with the YHV virus producing 100% shrimp mortality within 3–7 days of infection. YHV is an enveloped, rod-shaped virus measuring 170 nm × 38 nm that belongs to the *Rhabdoviridae* family; it has a genome size of about 22 kb [4.42].

TSV is a highly infectious disease-producing virus which belongs to the *Picornaviridae* family. TSV is a nonenveloped icosahedron with a diameter of 30–32 nm and an ssRNA genome of approximately 10 kb. Infection with this virus is always associated with large economic losses. TSV has a linear positive-sense ssRNA genome of about 10 kb, excluding the 3′-poly (A) tail, which contains two large open reading frames (ORFs). The viral capsid is made up of one minor and three major structural proteins [4.44].

4.7.2 DNA Viruses of Shrimp

Shrimp-infecting DNA viruses are listed in Table 4.4. Two occluded baculoviruses, BP and MBV, have been reported as serious disease-causing viruses and are responsible for major economic losses in the shrimp farming industry. These are both double-stranded viruses belonging to the *Baculoviridae* family. The BP virion is a rod-shaped nucleocapsid surrounded by a trilaminar envelope. The enveloped virion is 312–320 × 75–87 nm with a nucleocapsid of approximately 306–312 × 62–68 nm containing a circular dsDNA genome. MBV is a highly infectious virus that spreads rapidly and causes high mortality rates in juvenile and larval stages. The virus has an enveloped rod-shaped particle 265–282 × 68–77 nm with a nucleocapsid of 250–269 × 62–68 nm containing a genome of approximately 200 kb [4.44].

4.7.3 White Spot Syndrome Virus (WSSV)

WSSV is also a DNA virus that has been responsible for massive mortality rates among penaeid shrimp cultured on the southern and western coasts of Korea [4.45]. WSSV, which belongs to the *Whispovirus* genus of the *Nimaviridae* family [4.46], is a rapidly replicating virulent pathogen. WSSV consists of an enveloped, rod-

Table 4.3 Known shrimp RNA viruses (after [4.41, 43])

RNA Viruses	Abbreviation	Genome	Taxonomic classification	Known geographic distribution
Yellow head virus	YHV	(+) ssRNA	*Reoiviridae/Okavirus*	Asia, Central America
Taura syndrome virus	TSV	(+) ssRNA	*Picornavirale/Dicistroviridae*	Asia, Americas
Infectious myonecrosis virus	IMNV	(+) ssRNA	Totivirus/Unassigned	Asia, South America
Macrobrachium rosenbergii nodavirus	MrNV	(+) ssRNA	Nodavirus/Unassigned	India, China, Taiwan, Thailand, Australia, Caribbean
Laem-Singh virus	LSNV	(+) dsRNA	Luteovirus-like/Unassigned	South and Southeast Asia
Mourilyan virus	MoV	(−)ssRNA	Bunavirus/Unassigned	Australia, Asia
Gill-associated virus	GAV	(+) ssRNA	*Roniviridae/Okavirus*	Australia, Asia, Pacific
Lymphoid organ vacuolization virus	LOVV	(+) ssRNA?	Togavirus-like?/Unassigned	Americas

Table 4.4 Known shrimp DNA viruses (after [4.41, 43])

DNA Virus	Abbreviation	Genome	Taxonomic classification	Known geographic distribution
Monodonbaculovirus	MBV	dsDNA	Baculoviridae/ Nucleopolyhedrosis virus	Asia, Australia, Americas, Africa
Baculoyiral midgut gland necrosis virus	BMNV	dsDNA	Baciloviridae/Unassigned	Asia, Australia
Whit spot syndrome virus	WSSV	dsDNA	*Nimaviridae/Whispovirus*,	Asia, Americas
Baculoviruspenaei	BP	dsDNA	*Baculoviridae*/Unassigned	Asia
Spawner-isolated mortality virus	SMV	ssDNA	*Parvoviridae*/Unassigned	Australia, Asia
Infectious hypodermal and haematopoietic necrosis virus	IHHNV	ssDNA	*Parvoviridae/ Brevidensovirus*	Asia, Australia, Africa, Americas, Pacific
Hepatopancreatic parvovirus	HPV	ssDNA	Densovirus	Asia, Australia, Africa, Americas

shaped nucleocapsid enclosing a large circular dsDNA genome of approximately 293–300 kb, encompassing 181–184 major ORFs [4.47]. The virion contains five major structural proteins, including two envelope proteins and three nucleocapsid proteins [4.48]. White spot disease commonly causes 80–100% mortality within 1 week of the appearance of clinical signs [4.45, 49].

4.7.4 Shrimp Parvoviruses

Two emerging parvoviruses and one parvo-like virus have been reported in shrimp. Of the parvoviruses in crustaceans, HPV and IHHNV have been studied in detail. A third parvo-like virus, SMV, has been reported from *P. monodon* but has not yet been characterized in detail [4.43]. Another virus, lymphoidalparvo-like virus (LPV), consisting of intranuclear particles 18–20 mm in diameter, has been found only in Australia [4.50]. IHHNV and HPV are nonenveloped icosahedral viruses 22–25 nm in diameter, with ssDNA genomes. The morphology, genome structure, and genome organization of both IHHNV and HPV share similarities with members of the *Parvoviridae* family. These two viruses possess three ORFs and belong to the *Densovirinae* subfamily. However, genome analysis of IHHNV indicated that it is closely related to the mosquito densovirus. Therefore, it has recently been classified as belonging to the genus *Brevidensovirus* and the species' name has been changed to *Penaeus stylirosis densovirus* [4.43]. These two viruses also differ in their infection target organ – HPV infects epithelial cells of the hepatopancreas and midgut, while IHHNV infects multiple organs of ectodermal and mesodermal origin [4.43]. However, the classification of HPV is still uncertain due to the wide range of variation in the VP (viral protein) genes and the existence of many subtypes [4.51].

General Characteristics of HPV

HPV is a small (22–24 nm in diameter) nonenveloped icosahedral shrimp-infecting parvovirus, which was recently proposed as a new member of the *Densovirinae* subfamily of ssDNA viruses in the family *Parvoviridae* [4.52]. The genome of HPV is a linear ssDNA molecule about 6 kb in length, the 5′ and 3′ ends of which have a loop-like self-priming hairpin struc-

ture [4.51]. The genome contains three long ORFs: ORF1 (or left ORF), ORF2 (or mid ORF), and ORF3 (or right ORF). ORF1 encodes a putative nonstructural protein-2 (NS2) of 428 amino acids, the function of which is not yet known. ORF2 encodes nonstructural protein-1 of 579 amino acids, which possesses the most conserved region that includes replication initiator motifs, NTP-binding, and helicase domains. ORF3 encodes a polypeptide of 818 amino acids, which is the major capsid protein (VP) [4.51]. The purified viral proteins were detected as a major band at 57 kDa and a minor band at 54 kDa due to posttranslational modification of the deduced 92-kDa polypeptide. However, the function of the minor band is still unknown [4.51].

Hosts and Geographical Distribution of HPV

HPV was first reported in 1984 in the wild shrimp species *F. chinensis* from Singapore [4.53]. HPV was detected in South Korea in 1985 in *F. chinensis* [4.54] and was subsequently observed in wild and farmed shrimp species in many geographical locations, including Australia, China, Thailand, India, Madagascar, Tanzania, New Caledonia, the Philippines, Indonesia, Malaysia, Kenya, Kuwait, Israel, and South and North America [4.43].

HPV infects epithelial cells of the hepatopancreas and shows basophilic inclusions within enlarged nuclei of tubular epithelial cells. HPV infects a wide range of shrimp species including wild and farmed *P. monodon*, *Penaeusesculentus*, *Penaeusjaponicus*, *F. chinensis*, *Penaeussemisulcatus*, *Penaeusindicus*, *Penaeuspenicillatus*, *Penaeusschmitti*, *L. vannamei*, and *Penaeusstylirostris* [4.43, 44].

HPV Genotypes

Viruses are obligate parasites that are normally present in the environment in stable ecological associations with one or more hosts [4.55]. Viruses should have efficient replication and transmission of infection in the environment or host. The environment is constantly changing because of individual differences in host genetics and the immune response of the host. These environmental variables select for mutants or suitable variant phenotypes through the inherent capacity for evasive behavior [4.41].

The HPV genome has greater genetic diversity than those of other shrimp viruses, such as IHHNV and TSV. In total, eight HPV genomes are currently available in GenBank, and among these, complete genome sequence data are available for only four isolates, including the Korean isolates [4.51, 56–58]. However, incomplete or partial genome sequences are available in GenBank for many isolates. The sequencing results of different isolates suggest that HPV isolates from different shrimp species and/or different geographical regions are genetically different [4.59]. Of the three ORFs of HPV, ORF3 (or right ORF), which encodes the major capsid protein, shows greater variability than the other ORFs. Based on the amino acid sequence of the capsid protein gene, it has been divided into three genotypes: genotype I from Korea, Tanzania, and Madagascar; genotype II from Thailand and Indonesia; and genotype III from Australia and New Caledonia. The Korean strains HPVchin and *Fc*DNV were isolated from the shrimp species *F. chinensis*, Australian (*Pmerg*DNV) and New Caledonian isolates were from the host *P. merguiensis*, and Thai isolate (*Pm*DNV) was from *P. monodon*.

4.7.5 Diagnostic Methods for Shrimp Virus Diseases

Microscopy

Most diagnostic methods applied to shrimp have been adopted from other fields of pathology and are slightly different from those used in fish, veterinary, and human pathology. Diagnostic methods for shrimp pathogens include the traditional methods of morphological pathology (direct light microscopy, histopathology, and electron microscopy), traditional microbiology, and the application of serological methods. Initially, diagnosis of shrimp diseases was dependent on microscopic observations, and characterization viruses isolated from infected tissues. Transmission electron microscopy (TEM) was added to the diagnostic toolkit in the mid-1970s and was used to discover the first *Baculovirus-penaei* in shrimp [4.60]. However, electron microscopic methods are not in common use due to their limited sensitivity as well as the long preparation time, specialized equipment, and highly trained personnel requirements.

Hematology and Clinical Chemistry

Hematology and clinical chemistry are principal diagnostic tools for human and veterinary medicine, but they have also been applied to shrimp pathology by examining the changes in hemolymph parameters (hemocyte count, hemolymph clotting time, glucose, nonprotein nitrogen, ammonia, alkaline phosphatase, and total serum protein levels). However, with the exception of hemocyte count and hemolymph clotting time, these tests have not yet been adopted for routine diagnostic purposes [4.61].

Serological Methods

Several serology-based diagnostic methods have been applied for shrimp virus disease diagnosis. Polyclonal antibodies (pAbs) and monoclonal antibodies (mAbs) have also been virus diagnosis. A fluorescent pAb test for BMNV, the ELISA-based pAb test for BP, and pAb for rhabdovirus of penaeid shrimp (RPS) have been reported, and many attempts have been made to develop mAbs against many other viruses, such as HPV, YHV, WSSV, BP, and IHHNV. However, the development of mAbs involves problems related to specificity. Although mAbs may be specific for a particular virus, under some conditions, they may react nonspecifically to shrimp tissue due to the nature of IgM (immunoglobulin M) antibodies [4.62]. Specific mAb-based diagnostic tools are likely to be used because of their good sensitivity, versatility, low cost, speed, and simplicity.

Western blotting is a specific method for detecting antigens using mAb or pAb developed against viral capsid protein. This method requires the separation and transfer of the antigens prior to antibody treatment. Therefore, the component of the pathogen at a particular molecular weight can be identified with a high degree of accuracy. However, this is a time-consuming process that requires specialized equipment for protein separation and transformation. Therefore, this method is not commonly used for pathogen diagnosis [4.63, 64].

Recently, diagnostic lateral flow strip-based kits have been developed for WSSV and YHV using mAbs to VP28 and structural protein p20 [4.65, 66]. An mAb was raised to *Pm*DNV recombinant VP protein, which was applied to detect HPV infection in Thailand. However, the detection sensitivity of this method is not comparable to sensitive molecular diagnostic tools [4.67]. In this method, a pre-coated mouse antiviral protein antibody is used as a capture antibody at the test line (T), an anti-mouse IgG (immunoglobulin G) is used as the capture antibody at the control line (C), and a colloidal gold-conjugated monoclonal antibody pad is located adjacent to the sample pad. The sample is applied to the sample pad located at one end of the strip and allowed to flow by chromatography through the nitrocellulose membrane from one end to the other. Virus particles in test samples bind to the colloidal gold-conjugated monoclonal antibody and the resulting complex is captured by the antiviral antibody at the test line, yielding a reddish-purple band. Any unbound monoclonal antibody conjugated with colloidal gold moves across the test line and is captured by the IgG to form a band at the control line (C). However, this method is about 500 times less sensitive than a one-step real-time polymerase chain reaction, but is slightly more sensitive than dot blotting.

Molecular Diagnostic Methods

Molecular diagnostic methods include radioactive and nonradioactive labeled genomic probes and DNA amplification methods based on polymerase chain reaction (PCR), and additional PCR-based methods are being applied for the development of efficient diagnostic techniques. The first gene probe introduced into shrimp viral detection involved using a radioactive tag for IHHNV. However, it could only be applied in well-equipped laboratories due to the use of radioactive tags. Therefore, an alternative nonradioactive gene probe was developed using digoxigenin 11-dUTP (DIG). This technology allowed the development of many DIG-labeled probes for viral pathogens, such as IHHNV, HPV, TSV, WSSV, MBV, BP, and the ssRNA virus YHV [4.68]. A commercial kit for making DIG-labeled probes is available for IHHNV [4.68].

Dot blot hybridization is a simplification of the Northern, Southern, and Western blotting methods. In dot blot hybridization, the biomolecules to be detected are not first separated by chromatography. Instead, a mixture containing the molecule to be detected or a homogenized tissue sample is applied directly onto a negatively charged nitrocellulose membrane or a positively charged nylon membrane as a dot.

Dot blotting is a quick, convenient, and relatively cheap method to detect pathogens in hemolymph or tissue homogenate. The nitrocellulose membrane is the most common solid matrix used for sample adsorption. However, the major disadvantage of this method is that the sensitivity is limited to 1000 times less than that of PCR, and in most cases, the sensitivity is insufficient for detecting asymptomatic infection and it is usually associated with high background staining due to nonspecific binding of antibodies to the high concentration of shrimp proteins [4.63]. The background staining resulting from the nonspecific binding of antibodies to various shrimp components usually causes difficulty of interpretation in the case of light infection.

In situ hybridization (ISH) is a unique and powerful hybridization technique that uses a labeled complementary DNA or RNA probe to localize a specific DNA or RNA sequence in a portion or section of tissue. This method has been developed to detect shrimp pathogens using nonradioactive DIG-labeled gene probes [4.61].

Several DNA amplification methods based on PCR have been developed, and PCR has been applied in numerous methodologies for pathogen detection. Small,

often otherwise undetectable amounts of DNA can be amplified by PCR to produce detectable quantities of the target DNA. This is accomplished using specific oligonucleotide primers designed for the target DNA sequence [4.68]. Additional PCR-based methods are being applied for the development of efficient diagnostic methodologies. Multiple viral diseases can be simultaneously detected in a single reaction using more than one set of primers. Real-time PCR is especially attractive as it allows real-time analysis, high specificity, quantitative results, and the detection of low copy numbers (even as low as a single copy of a viral genome).

4.7.6 Factors Responsible for Shrimp Viral Diseases

Poor management in the shrimp farming industry leads to severe pollution in shrimp culture ponds, thereby creating a suitable environment for the development of viral diseases. The emergence of disease in shrimp aquaculture has been attributed to three major factors:

1. High density of cultured shrimp in aquaculture ponds
2. Importation or transportation of brood stock of shrimp from one place to another for culture
3. The introduction of wild brood stock into culture ponds.

The introduction of wild brooders carrying pathogens and inadequate information about disease symptoms have been responsible for the emergence of new diseases in the farming system. For example, HPV originated in the Indo-Pacific region but was later spread to America via importation of live Asian shrimp for aquaculture [4.69]. Similarly, TSV was found in Ecuador and spread to America and Southeast Asia [4.70, 71].

Environmental factors and hosts also play important roles in the emergence of shrimp diseases. Poor sanitation conditions, such as high salinity, pH, and nitrogen levels, in culture ponds can cause stress in shrimp, leading to an increased susceptibility to disease. For example, some viruses such as WSSV are highly lethal stress-related viruses. The temperature also plays an important role in virus infection; e.g., WSSV enters the target cell and replicates at 22 °C and thus leads to 100% mortality within 3 days. However, at a reduced temperature of 16–20 °C, 20–35 days were required to reach 100% mortality in experimentally infected crayfish [4.72]. However, high water temperatures from 27–33 °C inhibit the effects of WSSV in shrimp (*L. vannamei*) at the acute infection stage. However, at the chronic stage, increases in water temperature result in rapid disease progression and mortality in WSSV-infected shrimp [4.73].

4.7.7 Control of Shrimp Viral Disease

The most effective means of control or prevention of viral disease is to destroy the infected animals, decontaminate the ponds, and start again with virus-free brood stocks. Viruses, bacteria, protozoa, and fungi have emerged as major causes of disease in farmed shrimp. Bacterial, fungal, and protozoan diseases are manageable by improving culture practices, routine sanitation, and using probiotics and chemotherapeutic agents. However, management of viral diseases is problematic and has been responsible for the most costly epizootic outbreaks reported to date.

Probiotics

Live bacterial cells, referred to as *probiotics*, have been applied in aquaculture farms to improve water quality or prevent disease. The potential benefits of probiotics in aquaculture ponds include enhanced decomposition of organic matter, reduction of nitrogen and phosphorus concentrations, improved control of algal growth, greater availability of dissolved oxygen, less cyanobacteria (blue-green algae), control of ammonia, nitrite, and hydrogen sulfide, lower incidence of disease and greater survival, and improved levels of shrimp and fish production [4.74]. Few detailed studies on disease control using probiotics have been performed [4.75, 76]. However, properly controlled field tests on probiotics revealed no significant effect on measured water quality parameters [4.74]. The use of probiotics in commercial shrimp farming would be beneficial for control of disease only if positive evidence of efficacy with cost benefit analysis is acquired.

Shrimp–Virus Interactions

Shrimp–virus interactions at the molecular and genetic levels are interesting phenomena that may be useful in disease control. Many new shrimp genes have been discovered, some of which may lead to new products for disease control. A study performed in Japan indicated that Kuruma shrimp (*P. japonicus*) survived in a pond after WSSV injection; it was not protected from infection, but showed resistance to disease. This mechanism was designated as a *quasi-immune* response (immune-like system). Recently, a factor was found in shrimp hemolymph that could prevent shrimp from dying upon

injection of WSSV [4.77]. Another phenomenon has been reported that describes protection against WSD (white spot disease) by persistent IHHNV infection in *P. stylirostris*. However, infection was not prevented, although disease severity was reduced [4.78]. We still know very little about the interaction of shrimp with viral pathogens, and a better understanding of shrimp–virus interactions may lead to the development of better methods for viral disease control.

Shrimp Viral Vaccines

The term *vaccine* is applicable to vertebrates because the vertebrate process involves antibodies. However, antibodies do not occur in shrimp. In addition, *vaccinated* shrimp generally become infected but do not develop disease as a result. Therefore, the term *tolerines* has been recommended to describe agents that could be used in the same way as vaccines in shrimp [4.79].

Two types of tolerine have been studied in shrimp; the first type was developed in Thailand in the mid-1990s and is still commercially available under the brand name SEMBVAC, while the second consists of inactivated whole particles of WSSV. Shrimp acquires some degree of tolerance to WSSV and suffer less from the disease after infection due to ingestion of these products [4.80]. Other types of tolerance have also been reported consisting of individual or mixed protein subunits of viral particles that are administered either by injection or by mixing with shrimp feed [4.81, 82].

RNA Interference (RNAi)

RNA interference (RNAi) is a gene silencing technology and the process by which a gene is post transcriptionally suppressed using dsRNA with sequences that match those of viral genes to destroy their homologous mRNA in a sequence-specific manner [4.83]. This technology was recently used in the laboratory to protect shrimp from viral diseases [4.84, 85]. RNAi was used to suppress the replication of YHV and *Pm*DNV [4.83, 86]. However, application of this technology has disadvantages associated with issues of cost, safety, and public acceptance of genetic engineering techniques.

4.8 Conclusion

Viruses, one of the most rapidly evolving genetic agents among all biological entities are involved noncyclic changes in their genetic characteristics. Furthermore, the abundance of both DNA and RNA viruses in the marine environment, viral diversity, and the interaction between their hosts are ecologically important. A disease caused by marine viruses can cause a huge impact on aquaculture practice and aquatic organisms. In some circumstances, the environment pressure or high density of cultured organisms also allows for stress to the organisms, which facilitate virus infections. Moreover, it is challenging to control virus infection in wild marine organisms and viral host shift events in the ocean. Molecular biology-based methods are rapidly advancing the study of virus genomes and their molecular mechanisms. These advanced studies enable the development of rapid diagnostic tools, which could be useful for identifying viral pathogens in marine organisms and marine water samples. The advantages of molecular diagnostic methods are that they are sensitive and beneficial to preventing the spread of viral diseases to other organisms, due to the diagnosis at an early stage of infections in farmed aquaculture systems. Furthermore, therapeutic studies such as virus–host interactions, phage therapy, and RNAi are tools to control viral diseases. Although it is possible to control viral diseases in farmed aquaculture systems by using modern technology, it is still a big question as to how to control viral disease in the ocean. In addition, we must consider the cost of applying new technologies. Despite the fact that the diagnosis and control of known viral pathogens are quite possible, it is an unresolved problem to diagnose an unidentified or unknown virus population in the marine ecosystem. Even though there are a few drawbacks of viral diagnosis or control of virus impacts in aquaculture organisms, it is necessary to characterize the viruses associated with marine organisms for further studies in the future. Moreover, only a tiny fraction of viruses has been discovered and the potential discovery still seems as vast as the ocean itself. Therefore, further research is still needed to analyze the presence of viruses in the marine environment and the new potential hosts of marine viruses yet to be discovered.

References

4.1 C.A. Suttle: Marine viruses – major players in the global ecosystem, Nat. Rev. Microbiol. **5**, 801–812 (2007)

4.2 B.M. Davis, M.K. Waldor: Filamentous phages linked to virulence of *Vibrio cholerae*, Curr. Opin. Microbiol. **6**, 35–42 (2003)

4.3 T.W. Flegel, T. Pasharawipas, L. Owens, H.J. Oakey: *Evidence for Phage-Induced Virulence in the Shrimp Pathogen* (Asian Fisheries Society, Manila 2005)

4.4 C.A. Suttle: Viruses in the sea, Nature **437**, 356–361 (2005)

4.5 C.B. Munn: Viruses as pathogens of marine organisms – from bacteria to whales, J. Mar. Biol. Assoc. UK. **86**, 453–467 (2006)

4.6 J. Carter, V. Saunders: *Virology: Principles and Applications* (Wiley, England 2007)

4.7 D. Raoult, S. Audic, C. Robert, C. Abergel, P. Renesto, H. Ogata, B. La Scola, M. Suzan, J.M. Claverie: The 1.2-megabase genome sequence of Mimivirus, Science **306**, 1344–1350 (2004)

4.8 R. Webby, E. Hoffmann, R. Webster: Molecular constraints to interspecies transmission of viral pathogens, Nature Med. **10**, S77–S81 (2004)

4.9 G. Rogers, J. Paulson, R. Daniels, J. Skehel, I. Wilson, D. Wiley: Single amino acid substitutions in influenza haemagglutinin change receptor binding specificity, Nature **304**, 76–78 (1983)

4.10 A. Vines, K. Wells, M. Matrosovich, M.R. Castrucci, T. Ito, Y. Kawaoka: The role of influenza A virus hemagglutinin residues 226 and 228 in receptor specificity and host range restriction, J. Virology **72**, 7626–7631 (1998)

4.11 K.L. Brentlinger, S. Hafenstein, C.R. Novak, B.A. Fane, R. Borgon, R. McKenna, M. Agbandje-McKenna: Microviridae, a family divided: isolation, characterization, and genome sequence of φMH2K, a bacteriophage of the obligate intracellular parasitic bacterium Bdellovibrio bacteriovorus, J. Bacteriology **184**, 1089–1094 (2002)

4.12 J.L. Van Etten, M.V. Graves, D.G. Müller, W. Boland, N. Delaroque: *Phycodnaviridae*– large DNA algal viruses, Arch. Virology **147**, 1479–1516 (2002)

4.13 M. Breitbart: Marine viruses: truth or dare, Annu. Rev. Mar. Sci. **4**, 425–448 (2012)

4.14 T. Flegel, T. Pasharawipas, L. Owens, H. Oakey: *Evidence for Phage-Induced Virulence in the Shrimp Pathogen Vibrio harveyi, Diseases in Asian Aquaculture V Fish Health Section, Asian Fisheries Society* (Asian Fisheries Society, Manila 2005) pp. 329–337

4.15 P. Hyman, S. Abedon: *Bacteriophage (overview), Encyclopedia of Microbiology* (Elsevier, Oxford 2009) pp. 322–338

4.16 S. Jiang, J. Paul: Significance of lysogeny in the marine environment: studies with isolates and a model of lysogenic phage production, Microbial Ecol. **35**, 235–243 (1998)

4.17 W. Wilson, N. Mann: Lysogenic and lytic viral production in marine microbial communities, Aquat. Microb. Ecol. **13**, 95–100 (1997)

4.18 M. Łoś, G. Węgrzyn: Pseudolysogeny. In: *Bacteriophages. Part A*, Advances in Virus Research, Vol. 82, ed. by Ł. Małgorzata, W.T. Szybalski (Academic, Amsterdam 2012) pp. 339–349

4.19 F. Rohwer, R.V. Thurber: Viruses manipulate the marine environment, Nature **459**, 207–212 (2009)

4.20 L.M. Proctor, J.A. Fuhrman: Viral mortality of marine bacteria and cyanobacteria, Nature **343**, 60–62 (1990)

4.21 W.H. Wilson, I.R. Joint, N.G. Carr, N.H. Mann: Isolation and molecular characterization of five marine cyanophages propagated on Synechococcus sp. strain WH7803, Appl. Environ. Microbiol. **59**, 3736–3743 (1993)

4.22 F.E. Angly, B. Felts, M. Breitbart, P. Salamon, R.A. Edwards, C. Carlson, A.M. Chan, M. Haynes, S. Kelley, H. Liu: The marine viromes of four oceanic regions, PLoS Biology **4**, e368 (2006)

4.23 S.T. Fitz-Gibbon, H. Ladner, U.-J. Kim, K.O. Stetter, M.I. Simon, J.H. Miller: Genome sequence of the hyperthermophilic crenarchaeon *Pyrobaculum aerophilum*, Proc. Natl. Acad. Sci. USA **99**, 984–989 (2002)

4.24 A. Millard, M.R.J. Clokie, D.A. Shub, N.H. Mann: Genetic organization of the psbAD region in phages infecting marine Synechococcus strains, Proc. Natl. Acad. Sci. USA. **101**, 11007–11012 (2004)

4.25 C. Suttle: *Cyanophages and their Role in the Ecology of Cyanobacteria* (Springer, Netherlands 2002)

4.26 F.G. Flores: *The Cyanobacteria: Molecular Biology, Genomics, and Evolution* (Horizon Scientific, Spain 2008)

4.27 A. Sulakvelidze, Z. Alavidze, J.G. Morris: Bacteriophage therapy, Antimicrob. Agents Chemotherap. **45**, 649–659 (2001)

4.28 D.H. Duckworth: Who discovered bacteriophage?, Bacteriol. Reviews **40**, 793 (1976)

4.29 R.M. Carlton: Phage therapy: Past history and future prospects, Archivum Immunologiae et Therapiae Experimentalis **47**, 267–274 (1999)

4.30 I. Karunasagar, M. Vinod, B. Kennedy, A. Vijay, K. Deepanjali, K. Umesha: *Biocontrol of Bacterial Pathogens in Aquaculture with Emphasis on Phage Therapy* (Asian Fisheries Society, Manila 2005)

4.31 T. Renault, B. Novoa: Viruses infecting bivalve molluscs, Aquat. Living Resour. **17**, 397–409 (2004)

4.32 I. Arzul, J.-L. Nicolas, A.J. Davison, T. Renault: French Scallops: A New Host for Ostreid Herpesvirus-1, Virology **290**, 342–349 (2001)

4.33 A.J. Davison, B.L. Trus, N. Cheng, A.C. Steven, M.S. Watson, C. Cunningham, R.M. Le Deuff, T. Renault: A novel class of herpesvirus with bivalve hosts, J. Gen. Virol. **86**, 41–53 (2005)

4.34 S. Oshima, M. Imajoh, T. Hirayama: Interaction mechanism of marine birnavirus (MABV) in fish cell lines, Uirusu **55**, 133 (2005)

4.35 S.-I. Kitamura, S.-J. Jung, S. Suzuki: Seasonal change of infective state of marine birnavirus in Japanese pearl oyster *Pinctada fucata*, Arch. Virology **145**, 2003–2014 (2000)

4.36 S.I. Kitamura, S.I. Kamata, S.I. Nakano, S. Suzuki: Detection of marine birnavirus genome in zooplankton collected from the Uwa Sea, Japan, Dis. Aquat, Org. **54**, 69–72 (2003)

4.37 T.W. Flegel: Historic emergence, impact and current status of shrimp pathogens in Asia, J. Invertebr. Pathol. **110**, 166–173 (2012)

4.38 M. Haq, R. Vignesh, M. Srinivasan: Deep insight into white spot syndrome virus vaccines: A review, Asian Pac. J. Trop. Med. **2**, 73–77 (2012)

4.39 B. Rosenberry: *World shrimp farming 2002* (Shrimp News International, San Diego, California, 2002)

4.40 S.M. Moss, D.R. Moss, S.M. Arce, D.V. Lightner, J.M. Lotz: The role of selective breeding and biosecurity in the prevention of disease in penaeid shrimp aquaculture, J. Invertebr. Pathol. **110**, 247–250 (2012)

4.41 P.J. Walker, C. Mohan: Viral disease emergence in shrimp aquaculture: origins, impact and the effectiveness of health management strategies, Rev. Aquacult. **1**, 125–154 (2009)

4.42 L.M. Nunan, B.T. Poulos, D.V. Lightner: The detection of White Spot Syndrome Virus (WSSV) and Yellow Head Virus (YHV) in imported commodity shrimp, Aquaculture **160**, 19–30 (1998)

4.43 J.R. Bonami, B.W.J. Mahy, M.H.V.V. Regenmortel (Eds.): *Shrimp Viruses* (Academic, Oxford 2008)

4.44 P.C. Loh: Shrimp viruses. In: *Encyclopedia of Virology*, 2nd edn., ed. by R.G. Webster, A. Granoff (Elsevier, Oxford 1999)

4.45 J. Park, Y. Lee, S. Lee, Y. Lee: An infectious viral disease of penaeid shrimp newly found in Korea, Dis. Aquat. Organ. **34**, 71–75 (1998)

4.46 J.-H. Leu, F. Yang, X. Zhang, X. Xu, G.-H. Kou, C.-F. Lo: Whispovirus, Lesser Known Large dsDNA Viruses. **328**, 197–227 (2009)

4.47 M.C.W. van Hulten, J. Witteveldt, S. Peters, N. Kloosterboer, R. Tarchini, M. Fiers, H. Sandbrink, R.K. Lankhorst, J.M. Vlak: The white spot syndrome virus DNA genome sequence, Virology **286**, 7–22 (2001)

4.48 M.C.W. Van Hulten, M. Reijns, A.M.G. Vermeesch, F. Zandbergen, J.M. Vlak: Identification of VP19 and VP15 of white spot syndrome virus (WSSV) and glycosylation status of the WSSV major structural proteins, J. Gen. Virol. **83**, 257–265 (2002)

4.49 H.C. Chiang, C.F. Lo: Pathogenicity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan, Dis. Aquat. Organ. **23**, 165–173 (1995)

4.50 L. Owens, S. De Beer, J. Smith: Lymphoidal parvo-virus-like particles in Australian penaeid prawns, Dis. Aquat. Organ. **11**, 129–134 (1991)

4.51 W. Sukhumsirichart, P. Attasart, V. Boonsaeng, S. Panyim: Complete nucleotide sequence and genomic organization of hepatopancreatic parvovirus (HPV) of *Penaeus monodon*, Virology **346**, 266–277 (2006)

4.52 J.R. Bonami, J. Mari, B.T. Poulos, D.V. Lightner: Characterization of hepatopancreatic parvo-like virus, a second unusual parvovirus pathogenic for penaeid shrimps, J. Gen. Virol. **76**, 813–817(1995)

4.53 Y. Chong, H. Loh: Hepatopancreas chlamydial and parvoviral infections of farmed marine prawns in Singapore, Singap. Vet. J. **9**, 51–56 (1984)

4.54 D. Lightner, R. Redman: A parvo-like virus disease of penaeid shrimp, J. Invertebr. Pathol. **45**, 47–53 (1985)

4.55 B.A. Wilcox, D.J. Gubler: Disease ecology and the global emergence of zoonotic pathogens, Environ. Health Prev. Med. **10**, 263–272 (2005)

4.56 S. Jeeva, S.-W. Kang, Y.-S. Lee, I.K. Jang, H.C. Seo, T.-J. Choi: Complete nucleotide sequence analysis of a Korean strain of hepatopancreatic parvovirus (HPV) from *Fenneropenaeus chinensis*, Virus Genes **44**, 89–97 (2012)

4.57 K.A. La Fauce, J. Elliman, L. Owens: Molecular characterisation of hepatopancreatic parvovirus (*Pmerg* DNV) from Australian *Penaeus merguiensis*, Virology **362**, 397–403 (2007)

4.58 M.P. Safeena, A. Tyagi, P. Rai, I. Karunasagar, I. Karunasagar: Complete nucleic acid sequence of *Penaeus monodon* densovirus (*Pm*DNV) from India, Virus Res. **150**, 1–11 (2010)

4.59 K.A. La Fauce, J. Elliman, L. Owens: Molecular characterisation of hepatopancreatic parvovirus (PmergDNV) from Australian Penaeus merguiensis, Virology **362**, 397–403 (2007)

4.60 J.A. Couch: An enzootic nuclear polyhedrosis virus of pink shrimp: Ultrastructure, prevalence, and enhancement, J. Invertebr. Pathol. **24**, 311–331 (1974)

4.61 D.V. Lightner, K.W. Hasson, B.L. White, R.M. Redman: Chronic toxicity and histopathological studies with Benlate, a commercial grade of benomyl, in *Penaeus vannamei* (Crustacea: Decapoda), Aquat. Toxicol. **34**, 105–118 (1996)

4.62 B.T. Poulos, D.V. Lightner, B. Trumper, J.R. Bonami: Monoclonal Antibodies to a Penaeid Shrimp Parvovirus, Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), J. Aquat. Anim. Health. **6**, 149–154 (1994)

4.63 H.S. Erickson, M. Zarain-Herzberg, D.V. Lightner: Detection of Taura syndrome virus (TSV) strain differences using selected diagnostic methods: diagnostic implications in penaeid shrimp, Dis. Aquat. Org. **52**, 1–10 (2002)

4.64 A. Sahul Hameed, M. Anilkumar, M. Stephen Raj, K. Jayaraman: Studies on the pathogenicity of systemic ectodermal and mesodermal baculovirus and its detection in shrimp by immunological methods, Aquaculture **160**, 31–45 (1998)

4.65 W. Sithigorngul, S. Rukpratanporn, N. Sittidilokratna, N. Pecharaburanin, S. Longyant,

P. Chaivisuthangkura, P. Sithigorngul: A convenient immunochromatographic test strip for rapid diagnosis of yellow head virus infection in shrimp, J. Virol. Methods **140**, 193–199 (2007)

4.66 Y. Takahashi, K. Fukuda, M. Kondo, A. Chongthaleong, K. Nishi, M. Nishimura, K. Ogata, I. Shinya, K. Takise, Y. Fujishima: Detection and prevention of WSSV infection in cultured shrimp, Asian Aquacult. Mag., 25–27 (2003)

4.67 C. Srisuk, P. Chaivisuthangkura, W. Sukhumsirichart, P. Sridulyakul, S. Longyant, S. Rukpratanporn, P. Sithigorngul: Improved immunodetection of *Penaeus monodon* densovirus with monoclonal antibodies raised against recombinant capsid protein, Aquaculture **311**, 19–24 (2011)

4.68 D. Lightner, R. Redman: Shrimp diseases and current diagnostic methods, Aquaculture **164**, 201–220 (1998)

4.69 D.V. Lightner: *A Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp* (World Aquaculture Society, Baton Rouge 1996)

4.70 K. Hasson, D. Lightner, B. Poulos, R. Redman, B. White, J. Brock, J. Bonami: Taura syndrome in *Penaeus vannamei*: Demonstration of a viral etiology, Dis. Aquat. Organ. **23**, 115–115 (1995)

4.71 D. Lightner, R. Redman, K. Hasson, C. Pantoja: Taura syndrome in *Penaeus vannamei* (Crustacea: Decapoda): gross signs, histopathology and ultrastructure, Dis. Aquat. Organ. **21**, 53–53 (1995)

4.72 P. Jiravanichpaisal, K. Söderhäll, I. Söderhäll: Characterization of white spot syndrome virus replication in in vitro-cultured haematopoietic stem cells of freshwater crayfish, *Pacifastacus leniusculus*, J. Gen. Virol. **87**, 847–854 (2006)

4.73 M.M. Rahman, M. Corteel, M. Wille, V. Alday-Sanz, M.B. Pensaert, P. Sorgeloos, H.J. Nauwynck: The effect of raising water temperature to 33 °C in *Penaeus vannamei* juveniles at different stages of infection with white spot syndrome virus (WSSV), Aquaculture **272**, 240–245 (2007)

4.74 C.E. Boyd, A. Gross: Use of probiotics for improving soil and water quality in aquaculture ponds, Advances in Shrimp Biotechnology, ed. by T.W. Flegel (1998) pp. 101–106

4.75 S. Rengpipat, W. Phianphak, S. Piyatiratitivorakul, P. Menasveta: Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth, Aquaculture **167**, 301–313 (1998)

4.76 S. Rengpipat, A. Tunyanun, A.W. Fast, S. Piyatiratitivorakul, P. Menasveta: Enhanced growth and resistance to Vibrio challenge in pond-reared black tiger shrimp *Penaeus monodon* fed a Bacillus probiotic, Dis. Aquat. Organ. **55**, 169–173 (2003)

4.77 C. Venegas, L. Nonaka, K. Mushiake, T. Nishizawa, K. Muroga: Quasi-immune response of *Penaeus japonicus* to penaeid rod-shaped DNA virus (PRDV), Dis. Aquat. Organ. **42**, 83–89 (2000)

4.78 K.F.J. Tang, S.V. Durand, B.L. White, R.M. Redman, L.L. Mohney, D.V. Lightner: Induced resistance to white spot syndrome virus infection in *Penaeus stylirostris* through pre-infection with infectious hypodermal and hematopoietic necrosis virus – a preliminary study, Aquaculture **216**, 19–29 (2003)

4.79 T.W. Flegel, T. Pasharawipas: Active viral accommodation: A new concept for crustacean response to viral pathogens, Advances in Shrimp Biotechnology, ed. by T.W. Flegel (1998) pp. 245–250

4.80 I.B. Singh, M. Manjusha, S.S. Pai, R. Philip: *Fenneropenaeus indicus* is protected from white spot disease by oral administration of inactivated white spot syndrome virus, Dis. Aquat. Org. **66**, 265–270 (2005)

4.81 A. Namikoshi, J.L. Wu, T. Yamashita, T. Nishizawa, T. Nishioka, M. Arimoto, K. Muroga: Vaccination trials with *Penaeus japonicus* to induce resistance to white spot syndrome virus, Aquaculture **229**, 25–35 (2004)

4.82 B. Vaseeharan, T. Prem Anand, T. Murugan, J. Chen: Shrimp vaccination trials with the VP292 protein of white spot syndrome virus, Lett. Appl. Microbiol. **43**, 137–142 (2006)

4.83 T. Tuschl, P.D. Zamore, R. Lehmann, D.P. Bartel, P.A. Sharp: Targeted mRNA degradation by double-stranded RNA in vitro, Gene Dev. **13**, 3191–3197 (1999)

4.84 J. Robalino, T. Bartlett, E. Shepard, S. Prior, G. Jaramillo, E. Scura, R.W. Chapman, P.S. Gross, C.L. Browdy, G.W. Warr: Double-stranded RNA induces sequence-specific antiviral silencing in addition to nonspecific immunity in a marine shrimp: convergence of RNA interference and innate immunity in the invertebrate antiviral response?, J. Virol. **79**, 13561–13571 (2005)

4.85 J. Robalino, C.L. Browdy, S. Prior, A. Metz, P. Parnell, P. Gross, G. Warr: Induction of antiviral immunity by double-stranded RNA in a marine invertebrate, J. Virol. **78**, 10442–10448 (2004)

4.86 P. Attasart, R. Kaewkhaw, C. Chimwai, U. Kongphom, S. Panyim: Clearance of *Penaeus monodon* densovirus in naturally pre-infected shrimp by combined ns1 and vp dsRNAs, Virus Res. **159**, 79–82 (2011)

5. Marine Microalgae

Tsuyoshi Tanaka, Masaki Muto, Yue Liang, Tomoko Yoshino, Tadashi Matsunaga

Marine microalgae, the largest primary producer in the marine system, have been attracting wide attention as potential resources of new metabolites and biofuels. Whole genome sequencing and genetic modifications of microalgae have been rapidly advancing during the last few decades. In this chapter, the diversity of marine microalgae, the microalgal natural components, and the biotechnologies associated with microalgae are reviewed.

5.1 Overview

Algae are the primary producers of oxygen in the aquatic environment. These microorganisms are widely distributed in marine systems and have a great diversity with respect to size, morphology, life cycle, pigments, and metabolism. About half of the global oxygen production is accomplished by marine microalgae. They play an important role in CO_2 recycling through photosynthesis, which is similar to higher plants in O_2-evolved systems (PSI (photosystem I) and PSII (photosystem II)). In addition to having a long history of use as food and as live feed in aquaculture, microalgae have also been considered as a promising source for high-value added products for pharmaceuticals, cosmetics, and other industrial applications, such as β-carotene, astaxanthin, polyunsaturated fatty acids (PUFAs). The utilization of microalgae as a suitable feedstock for sustainable biofuel production has gained worldwide attention over the last 20 years. In general, microalgal triacylglycerols, hydrocarbons, and polysaccharides are considered as biofuel precursors. Alkanes and short chain fatty acid methyl esters (FAMEs), ranging from C9 to C17, have been considered as the potential alternative jet fuel, while those ranging from C9 to C23 have been mainly recognized as biodiesel. FAMEs are normally generated from the methyl esterification of triacylglycerols. On the other hand, with the degradation and fermentation of microalgal polysaccharides, the generated ethanol can be used as an alternative fuel to gasoline. Compared with higher plants, biofuel from microalgae has two advantages: 1) a relatively higher productivity, and 2) no competition to agriculture.

On the other hand, increased reports of the microalgal whole genome sequence data have been significantly facilitating the better understanding of their evolutionary lineage and the species specificity of the microalgal metabolic pathway. In addition, gene transformation has been achieved in 18 microalgal genera.

In this chapter, we reviewed the fundamental characteristics of marine microalgae, the useful microalgal natural products, as well as the biotechnological aspects of marine microalgae.

5.2 Marine Microalgae

Algae are a very diverse group of photosynthetic organisms other than land plants, which have been classified into many classes, such as *Cyanophyceae*, *Chlorophyceae*, *Rhodophyceae*, *Cryptophyceae*, *Dinophyceae*, *Bacillariophyceae*, *Haptophyceae*, *Euglenophyceae* or *Prasinophyceae*. For convenience, they are referred to as blue-green algae, green algae, brown algae, or red algae due to the difference in composition of photosynthetic pigments. However, it is difficult to make a clear definition of algae because even multicellular eukaryotic microalgae (what is called seaweed) are also included. In this section, some representative marine microalgae are summarized to introduce their biotechnological applications.

Cyanophyceae (cyanobacteria, blue-green algae) are oxygenic photosynthetic prokaryotes that comprise a single taxonomic and phylogenetic group. Chloroplasts in eukaryotes evolved from endosymbiotic cyanobacteria. They show a large diversity in their morphology, physiology, ecology, biochemistry, and other characteristics. Typically, cyanobacteria contain chlorophyll a and phycocyanin. Three genera, i. e., *Prochlorococcus*, *Prochloron*, and *Prochlorothrix*, lack phycocyanin and possess chlorophyll a and b [5.1]. A unicellular cyanobacterium that synthesizes chlorophyll d has also been discovered [5.2]. Marine *Synechococcus* and *Prochlorococcus* contribute largely to global oxygen production. Cyanobacteria have gained attention as a source of bioactive compounds and biopolymers (polyhydroxyalkanoates (PHA)s) [5.3]. Bioactive compounds isolated from marine cyanobacteria were summarized by *Burja* et al. [5.4] and *Takeyama* and *Matsunaga* [5.5]. Several strains of cyanobacteria (*Synechococcus elongates* and *Anabaena variabilis*) have been reported to produce long-chain alkanes and alkenes [5.6]. These findings make cyanobacterial alkane and alkenes a promising source of biofuels [5.7]. Two enzyme families that are responsible for straight-chain hydrocarbon production in cyanobacteria have recently been identified as an acyl–acyl carrier protein reductase (AAR) and an aldehyde-deformylating oxygenase (ADO). These enzymes convert fatty acid intermediates to alkanes and alkenes. This discovery of the cyanobacterial alkane biosynthesis indicates possibilities for optimizing the biodiesel production in cyanobacterial strains with modest gains in alkanes [5.8].

Chlorophyceae, which are one of the classes of green algae, possess chlorophyll a and b, the same predominant photosynthetic pigments as those of land plants. *Chlorophyceae* form starch in the chloroplast as a storage product of photosynthesis. Especially, *Chlamydomonas reinhardtii* has been used as a representative eukaryotic microalgae for biology and molecular biology studies. Chloroplast transformation was firstly achieved in *C. reinhardtii* [5.9]. Some species of *Chlorophyceae* are found in the marine environment. A marine species of *Chlorophyceae*, *Dunaliella* has been cultivated commercially for food supplements and β-carotene production [5.10]. *Chlorella*, which is a genus of single-cell and chlorophyll a/b-containing algae, belongs to the phylum *Chlorophyta*. It has been known as a potential food resource because of its high content of protein and other nutrients. Miura and others [5.11] reported that *Chlorella* sp. NKG 042 401 contains 10% γ-linolenic acid (C18:3), which is present in the cells mainly in the form of galactolipids. In *Euglenophyceae*, the genus *Euglena* is well known. The chloroplast of *Euglena* originated from the eukaryotic green algae and contains chlorophyll a and b. Although most species are found in freshwater environments, some species also occur in marine environments.

Bacillariophyceae (diatoms) possess chlorophyll a and c, and fucoxanthin as the major carotenoid. Diatoms are widely used as feed in mariculture/aquaculture [5.12, 13]. *Chaetoceros calcitrans*, *Chaetoceros gracilis*, *Chaetoceros muelleri*, *Skeletonema costatum*, and *Thalassiosira pseudonana* are commonly used as live feed for all growth stages of bivalve molluscs (e.g., oysters, scallops, clams, and mussels), for crustacean larvae, and for zooplankton used as feed for larvae. The genera *Navicula*, *Nitzschia*, *Cocconeis*, and *Amphora* also are used to feed juvenile abalone. They store energy either as lipids or as chrysolaminarin. Most diatoms have a high content of eicosapentaenoic acid (EPA) 20 : 5 (n-3). *Phaeodactylum tricornutum* and *Nitzschia laevis* have been especially investigated for EPA production. In addition, EPA production by diatoms was reviewed recently by *Lebeau* and *Robert* [5.14, 15]. Recent advances in heterotrophic production of EPA by microalgae were also reviewed by *Wen* and *Chen* [5.16].

The cells of *Haptophyceae* are brownish or yellowish-green and contain chlorophylls a/c and carotenoids such as β-carotene, fucoxanthin, diadinoxanthin, and diatoxanthin. The cells are commonly covered with scales made mainly by carbohydrates or calcium bicarbonate. Many species known as coccol-

ithophorids produce calcified scales called coccoliths. Most are primarily marine species inhabiting tropical seawater. Microalgal biomass of *Haptophyceae* is commonly used as living feed in aquaculture [5.17]. *Isochrysis galbana* and *Pavlova lutheri*, especially, are used as living feed for bivalve molluscs, crustacean larvae, and zooplanktons that in turn are used for crustacean and fish larvae. Some cells can produce PUFAs such as docosahexaenoic acid (DHA), or EPA. In addition, the DHA content in *I. galbana* has been shown to be enhanced by low temperature or incubation of the culture in the dark after reaching the plateau phase growth [5.18]. Furthermore, it was shown that these algae are useful for DHA enrichment of feed such as rotifers for the larvae of several marine fish species [5.19].

In *Dinophyceae*, a genus *Symbiodinium* (dinoflagellate) has been well described. Various marine invertebrates, such as reef-building corals, jellyfish, sea anemones, and bivalves form symbiotic associations with *Symbiodinium*, commonly known as zooxanthellae. *Symbiodinium* strains have been classified into more than three clades using restriction fragment length polymorphism based on 18S rRNA sequence analysis [5.20]. The composition of *Symbiodinium* populations may also play an important role in the tolerance or sensitivity of corals towards bleaching.

5.3 Microalgal Genomes

Sequencing of microbial genomes has become a routine procedure for gene discovery and genetic engineering of microalgae. *Synechocystis* sp. PCC 6803 was the first photosynthetic organism for which the entire genome sequence was determined. Currently, 72 finished cyanobacterial genome sequences are listed in GenBank, and many additional genome analyses are currently in progress. Most cyanobacteria possess a circular chromosome and a small number of additional plasmids. Genome sizes range from a minimum of 1.44 Mb for the marine cyanobacterium UCYN-A [5.21] to a maximum of 11.58 Mb for the *Calothrix* sp. PCC7103 [5.22]. Prokaryotes typically contain a single copy of their chromosome such as *Escherichia coli*, while large differences between cyanobacteria and other prokaryotes have been reported for chromosomal copy numbers. Some cyanobacteria are oligoploid, for example, *Synechocystis* sp. PCC 6803 are highly polyploid, and the motile wild-type strain contains 218 genome copies in exponential phase and 58 genome copies in linear and stationary phases [5.23].

Recently, a comparative genomics-based approach was used to screen cyanobacteria for the direct production of alkanes, the primary hydrocarbon components of gasoline, diesel, and jet fuel [5.8]. Eleven different cyanobacteria with available genome sequences were grown, and their culture extracts were evaluated for hydrocarbon production. Indeed, ten of these strains produced alkanes. The comparison of predicted proteins from these ten genomes against the eleventh finally led to the discovery of two hypothetical proteins as candidates for alkane biosynthesis. This discovery is the first description of genes responsible for alkane biosynthesis and the first example of a single-step conversion of sugar to fuel-grade alkanes by an engineered microorganism. A comparison of the genome sequences of producing and non-producing organisms led to the identification of the responsible genes.

In eukaryotic microalgae genomics, large-scale sequencing has been demonstrated by next-generation sequencing technologies. These have drastically increased the number of bases obtained per sequencing run while at the same time decreasing the costs per base. The first whole genome sequence of *C. merolae* was determined in 2004 [5.26]; this was the first identi-

Table 5.1 Sequenced whole genomes of microalgal strains

Microalgae species	Genome length (Mbp)	References
Ochrophyta		
Phaeodactylum tricornutum	27.4	[5.24]
Thalassiosira pseudonana	32.4	[5.25]
Rhodophyta		
Cyanidioschyzon merolae	16.5	[5.26]
Chlorophyta		
Chlamydomonas reinhardtii	121	[5.27]
Chlorella variabilis	46.2	[5.28]
Micromonas pusilla	21.9	[5.29]
Micromonas sp.	20.9	[5.29]
Volvox carteri	138	[5.30]
Ostreococcus lucimarinus	13.2	[5.31]
Ostreococcus tauri	12.6	[5.32]
Coccomyxa subellipsoidea	48.8	[5.33]

fied eukaryotic microalgal genome. Up until November 2012, the whole genome sequence of 11 strains of microalgae had been sequenced, including 2 diatoms (*Thalassiosira pseudonana* [5.24] and *Phaeodactylum tricornutum* [5.25]), a red alga (*Cyanidioschyzon merolae* [5.26]), and 8 green algae (*Chlamydomonas reinhardtii* [5.27], *Ostreococcus lucimarinus* [5.31], *Ostreococcus tauri* [5.32], *Chlorella variabilis* [5.28], *Volvox carteri* [5.30], *Coccomyxa subellipsoidea* [5.33], *Micromonas pusilla* [5.29], and *Micromonas* sp. [5.29]), see Table 5.1. In addition, the draft genome sequences of 17 strains of microalgae can been found in the NCBI GenBank databases [5.34]. With next generation technology, the draft genome sequence of the biodiesel producing microalga *Nannochloropsis gaditana* strain CCMP526 were also identified recently [5.35]. The identified microalgal whole genome sequences provide a powerful tool for the discovery of genes and metabolic pathways. Even though most of the predicted microalgal pathways have been proved to be similar to corresponding pathways in higher plants, the urea cycle identified from genomes of diatoms is absent in higher plants but present in animals [5.24]. The existence of an animal metabolic pathway in microalgal cells further highlights the importance of genome analysis for microalgae.

5.4 Genetic Engineering of Microalgae

5.4.1 Genetic Transformation Methods

Genetic studies on microalgae have been redirected mainly toward analysis of photosynthesis and metabolic pathways. A limited number of microalgae such as cyanobacteria have been used in biotechnological applications. The development of molecular techniques for physiological analysis and enhancement of biotechnological applications is necessary. Many attempts at gene transfer have been made in eukaryotic and prokaryotic microalgae. Genetic manipulation in prokaryotic microalgae cyanobacteria was studied extensively after several transformable unicellular strains were discovered. First, the freshwater cyanobacterium *Synechococcus* PCC7942 was reported to have the ability to take up DNA. Subsequently, several other naturally transformable freshwater strains were found. Gene transfer has been developed mainly in the freshwater strains *Synechococcus, Synechocystis, Anabaeba*, and *Nostoc* [5.69]. Several marine cyanobacterial strains of the genus *Synechococcus* have been also used for heterogeneous gene expression and other genetic applications [5.70, 71]. There are two commonly used gene transfer procedures: transformation using naturally occurring or artificially competent cells, e.g., conjugation with *Escherichia coli*, or physical methods for gene introduction, e.g., electroporation and particle bombardment. Natural transformation has been reported for *Synechococcus* sp. PCC7002 [5.72]. Other strains have been transformed successfully by electroporation or conjugation. Further, plasmids harvested from several marine microalgal species have been used as vector DNA for gene transfer. Marine plasmids have been found in *Synechococcus* sp. NKBG042902, which has a high phycocyanin content and a rapid growth rate. This strain contains more than three cryptic endogenous plasmids, and one of these, the plasmid pSY10 has the unique replication characteristic that its copy number increases under high salinity conditions [5.73]. Plasmids are maintained at a high copy number in cyanobacteria, which suggests the possibility that they act as a shuttle vector between cyanobacteria and *E. coli*. In fact, a shuttle vector with *E. coli* has been constructed using pSY10. Conjugative gene transfer using a broad-host range vector pKT230 was successful for the marine cyanobacterium *Synechococcus* sp. NKBG 15041C [5.74]. It has been demonstrated that this plasmid is stably maintained in cyanobacterial cells [5.75]. In marine cyanobacteria, in addition to the plasmid vector system, the construction of a phage vector system is also required to enable the cloning of large DNA fragments in specific cyanobacterial hosts. Since cyanophages were first reported by *Safferman* and *Morris* [5.76], various types of cyanophages have been found in seawater [5.77, 78] and characterized according to their genetic diversity and phylogenetic affiliations [5.79].

Due to the advance of genome, proteome, and metabolome analyses of microalgae, many attempts at gene transfer to eukaryotic microalgae have been made to enhance the production of useful compounds and biomass. However, because of the stiff cell wall of microalgae, the introduction of exogenous genes into microalgal cells could be challenging. The additional frustules and coccoliths surrounding some species of microalgae cells further increases the difficulty. Thus,

Table 5.2 Microalgal strains achieved for the stable transformation

Phylum species	Organelle	Transformation methods	Gene knock-down	References
Diatom				
Cyclotella cryptica	Nucleus	Biolistic		[5.36]
Cylindrotheca fusiformis	Nucleus	Biolistic		[5.37]
Chaetoceros sp.	Nucleus	Biolistic		[5.38]
Navicula saprophila	Nucleus	Biolistic		[5.36]
Phaeodactylum tricornutum	Nucleus	Biolistic	✓	[5.39, 40]
Thalassiosira pseudonana	Nucleus	Biolistic		[5.41]
Fistulifera sp.	Nucleus	Biolistic	✓	[5.42]
Chlorophyta				
Chlamydomonas reinhardtii	Nucleus	Biolistic, Electroporation, Glass beads, Agrobacterium	✓	[5.43–48]
	Chloroplast	Biolistic		[5.9]
	Mitochondoria	Biolistic		[5.33]
Chlorella spp.	Nucleus	Biolistic, Electroporation, Agrobacterium		[5.49–53]
Dunaliella spp.	Nucleus	Biolistic, Electroporation , Glass beads	✓	[5.54–58]
Haematococcus pluvialis	Nucleus	Biolistic, Agrobacterium		[5.59, 60]
Volvox carteri	Nucleus	Biolistic		[5.61]
Dinoflagellate				
Amphidinium sp.	Nucleus	Glass beads		[5.62]
Symbiodinium microadriaticum	Nucleus	Glass beads		[5.62]
Rhodophyta				
Cyanidioschyzon merolae	Nucleus	Glass beads	✓	[5.63, 64]
Porphyridium spp.	Chloroplast	Biolistic, Agrobacterium		[5.65, 66]
Euglenophyta				
Euglena gracilis	Chloroplast	Biolistic		[5.67]
Eustigmatophyte				
Nannochloropsis spp.	Nucleus	Electroporation		[5.35, 68]

the optimization of the gene transformation method for each specific species turns out to be important. Depending on the physiological characteristics of microalgal cells, electroporation, glass beads-mediated transformation, agrobacterium-mediated transformation, and biolistics have frequently been used. Moreover, the level of target protein varied due to multiple insertion, random integration, and (or) gene silencing [5.80]. Stable transformants that have already been reported are summarized in Table 5.2.

Biolistics, also referred to as a gene gun that was originally designed for the delivery of nucleic acid through the cell wall of intact plant cells, has been mostly applied for microalgae gene transformation. The payload in this system is a plasmid DNA-coated tungsten particle (particle size: 0.6−1.6 μm), which can be shot with helium gas. After bombardment, the tungsten particles were shot down to the plant organism or the cell culture on the petri dish. Some cells that are not disrupted by the firing may envelope the DNA-coated tungsten particles and the DNA can then migrate to and integrate into the plant chromosome [5.81]. The transformation efficiency of this methodology is not related to the physical property of the host cell but is highly controlled by the gas pressure at the point of firing. Therefore, theoretically, despite the hard cell wall and frustules, gene transformation can be achieved when the gas pressure is high enough.

Electroporation is a phenomenon when the electrical conductivity and permeability of the cell membrane increase by the externally applied electrical field. If the host cells and plasmids are mixed together, the plasmids can be transferred into the host cells through the transient holes in the cell membrane generated by the electronic shock. Electroporation-based gene transformation methodology has been commonly used to

transform mammalian cells with plasmids. The transformation efficiency was significantly decreased for plant cells due to their thick cell walls. Electroporation-based gene transformation has only been achieved with the *Chlamydomonas reinhardtii* cell wall-deficient mutant and *Dunaliella salina* cells, which have no cell wall [5.46, 57, 82]. However, in the studies mentioned above, the transformation efficiency was tenfold higher than the gene gun method applied to the corresponding strains [5.83].

The glass beads method is a relatively simple transformation procedure that has a higher transformation efficiency than biolistics but it is only capable of transforming cells without cell walls. Both the cell-wall deficient *C. reinhardtii* mutant and *D. salina* have been reported to have been successfully transformed by the glass bead method with a higher efficiency than with the gene gun method [5.56].

Agrobacterium-mediated transformation is based on the characteristic of the soil bacterium *Agrobacterium tumefaciens* that it naturally transfers and inserts its genes into plant chromosomes. Exogenous genes can be transferred into plant cells through *Agrobacterium* transformation using target gene inserted agrobacterium transfer DNA (T-DNA). Although reports of microalgae transferred by agrobacterium transformation are few, *Kathiresan* et al. achieved a twofold transformation efficiency with *Haematococcus pluvialis* over the gene gun method [5.60].

In the transformants generated by the methods mentioned above, it is not rare to find the continuous expression of the target genes in the chloroplast and (or) mitochondria due to the insertion of the target genes into their organelle genome. By using a specific vector containing a homologous sequence in the organelle genome, stable chloroplast and (or) mitochondria transformation can be expected. On the other hand, the target genes are usually found to be randomly inserted into the nucleic genome and even homologous recombination occurs. Thus, it is hardly possible to control the insertion site and the number of the target genes inserted into the nucleic genome, which has made gene functional analysis via gene knock-out difficult. With further consideration of the dual nature of the microalgal life cycle as either haploid or diploid, the possibility of complete knock-out dwindled significantly in diploid cells. The homologous recombination has been applied to the transformation of *C. reinhardtii* and *Volvox carteri*, which maintain an asexual haploid zoospore during the life cycle; their recombination efficiency, however, was inferior [5.84, 85]. Recently, highly efficient homologous recombination was reported in *Nannochloropsis* sp., which suggested the possible use in microalgal gene functional analysis [5.68]. For those diploid microalgae, the knock-down of the target gene via RNAi has been reported and considered as the substitute for knock-out [5.40, 44, 55, 64].

So far, six microalgae including *Phaeodactylum tricornutum*, *Thalassiosira pseudonana*, *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *Volvox carteri*, and *Cyanidioschyzon merolae* have not only obtained stable transformants but also the whole genome sequence. *P. tricornutum* has been widely used for the studies of metabolic engineering towards enhanced lipid production. Yet, most research in this field has focused on the established stable transformant rather than on high oil-producing strains whose transformation method have not been determined.

5.4.2 Metabolic Engineering

Enhanced production of valuable primary or secondary metabolites in microalgae can be rendered possible by high density cultivation and/or application of genetic manipulation. Recent pharmaceutical interest in unsaturated fatty acids has triggered the search for sources of these valuable compounds. Several eukaryotic microalgae are known to produce highly unsaturated fatty acids such as EPA and DHA, which are valuable dietary components [5.16, 19]. Genetic engineering has been applied to produce EPA in the marine cyanobacterium *Synechococcus* sp. [5.71]. Cyanobacteria do not have the biosynthetic pathway to produce them. The EPA synthesis gene cluster (ca. 38 kbp) isolated from a marine bacterium *Shewanella putrefaciens* SCRC-2738 was cloned to the marine cyanobacterium using a broad-host cosmid vector. The content of EPA grown at 2 °C increased to 0.64 mg g^{-1} dry cells after 24 h incubation at 17 °C. Furthermore, EPA production was improved by partial deletion of the EPA gene cluster to stabilize its expression and maintenance in host cyanobacterial cells [5.86].

Genetic engineering of microalgae for industrial purposes has also been performed in freshwater cyanobacteria where the ketocarotenoid astaxanthin, an extremely efficient antioxidant, was synthesized by the introduction of the β-c-4-oxygenase gene (*crt*O) from the green alga *Haematococcus* [5.87]. Ethylene production was also demonstrated in the cyanobacterium *Synechococcus elongates* PCC7942 by chromosomal insertion of an ethylene forming enzyme [5.88]. How-

ever, the reaction catalyzed by the ethylene forming enzyme induced metabolic stress, which was detrimental to the host cell.

Microalgal biodiesel production is expected to be improved though metabolic engineering. Several transformants have been established for the increased oil content of the microalgal cell, enhanced biomass productivity, and improved quality of the lipids. Acetyl CoA carboxylase (ACCase), which catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, the primary substrate of fatty acids synthesis, has been overexpressed in diatom cells to elevate the cell lipid content [5.36]. The vector containing the ACCase gene and its 5′ UTR (untranslated region) from the diatom *Cyclotella cryptica* was constructed and introduced into the diatoms *C. cryptica* and *Navicula saprophila*. Stable high ACCase expression transformants were obtained successfully, yet the predicted increase of neutral lipid content was not achieved, which indicates that TAG (triacylglycerol) accumulation in the microalgal cell is much more complex than previously assumed. With the expression of the hexose transporter that transfers the monohexose from the culture medium into the cell, the transformants of green algae *C. reinhardtii* and *V. carteri* as well as diatom *P. tricornutum* have been demonstrated to be capable to grow in the dark in a medium containing glucose [5.89–91]. Especially, the glucose transporter (Glut1) gene transformant of *P. tricornutum* cultured in dark conditions showed an almost threefold higher biomass production than in light conditions [5.91]. On the other hand, the de-regulation of the light-harvesting proteins in *C. reinhardtii* has been demonstrated to be able to elevate the solar energy conversion efficiencies in photosynthesis when the light-harvesting chlorophyll antenna size is minimized [5.92]. This permits a greater photosynthetic productivity under high cell density conditions as well as the possibility of culturing cells under high sunlight conditions. The transformation of *Cinnamomum camphora* (C12-TE) and *Umbellularia californica* (C14-TE) Acyl-ACP thioesterases genes into diatom *P. tricornutum* resulted in an increased lauric (C12:0) and myristic acid (C14:0) accumulation mutant [5.93]. Levels of lauric acid of up to 6.2% of total fatty acids and myristic acid of up to 15% by weight were achieved. Moreover, 75–90% of the shorter chain length fatty acids produced were demonstrated to be incorporated into triacylglycerols.

5.5 Photobioreactors for Marine Microalgae

Microalgae mass cultivation for the production of useful compounds has been widely discussed since the 1950s. Even though large-scale production of astaxanthin, DHA, and EPA from microalgae have been achieved, the industrial production of microalgal biofuel is still under development. Lower cost and higher productivity and efficiency than current bioreactors are necessary due to the extremely low final price of biodiesel (1 dollar L^{-1}) compared with those high value-added microalgal products.

Both the biology and the economics of microalgae mass cultivation are strongly influenced by photobioreactor design. Photosynthetic microalgae can be cultured in photobioreactors as either an open culture system or a closed system. Based on their localization, these photobioreactors can be divided into outdoor culture systems or indoor culture systems.

Outdoor open culture systems are the simplest method of algal cultivation due to the low construction cost and effortless operation. However, the productivity of these systems can be easily affected by several environmental factors such as contamination of other microorganisms, changes of weather conditions, and the disability of transgenic microalgae cultivation. The need to achieve higher productivity and to maintain monoculture of algae led to the development of closed photobioreactors. Despite higher biomass concentration and better control of culture parameters, CO_2 recycling efficiency, energy profit ratio, energy payback time, and cost of production in these enclosed photobioreactors are not better than those achievable in open systems.

The growth rate and maximum biomass yield of microalgal strains are affected by culture parameters (light, temperature, and pH) and nutritional status (CO_2, nitrogen, and phosphate concentration). On the other hand, increasing the density of cultures decreases photon availability to individual cells. Light penetration of microalgal cultures is poor, especially at high cell densities, and such poor photon availability decreases specific growth rates. Higher biomass yields can be expected if sufficient photons are provided in high density cultures of microalgae. Two major types of bioreactors (tubular [5.94, 95] or flat plate [5.96, 97]) are generally applied for the enclosed system (Fig. 5.1).