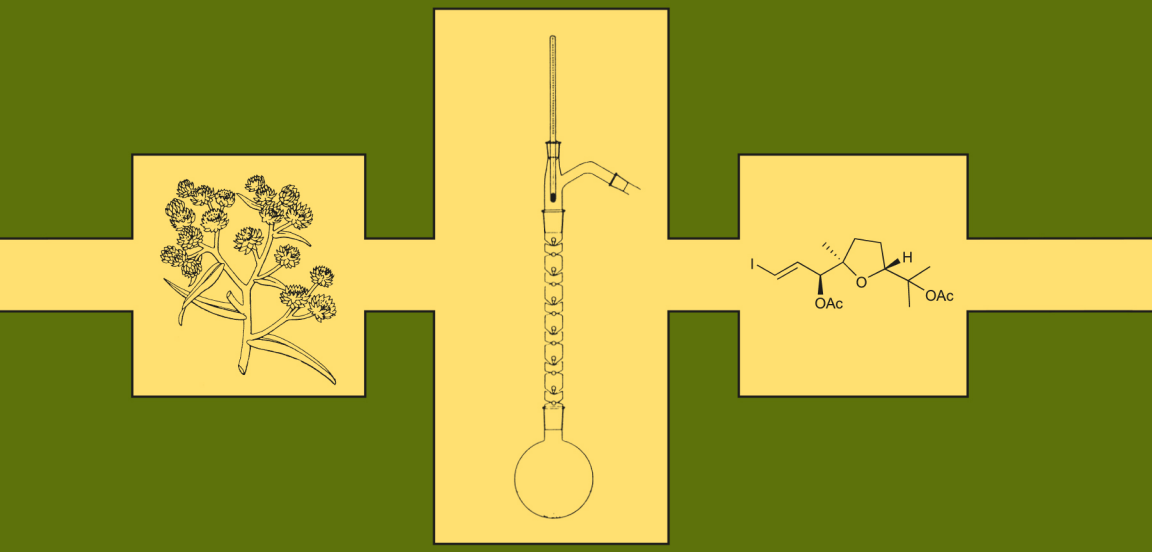
Studies in Natural Products Chemistry

Atta-ur-Rahman, FRS
Editor



Volume 67

Bioactive Natural Products

ELSEVIER

Studies in Natural Products Chemistry

Bioactive Natural Products

Volume 67

Studies in Natural Products Chemistry

Edited by Atta-ur-Rahman

Vol. 1 Stereoselective Synthesis (Part A)
Vol. 2 Structure Elucidation (Part A)
Vol. 3 Stereoselective Synthesis (Part B)
Vol. 4 Stereoselective Synthesis (Part C)
Vol. 5 Structure Elucidation (Part B)
Vol. 6 Stereoselective Synthesis (Part D)
Vol. 7 Structure and Chemistry (Part A)
Vol. 8 Stereoselective Synthesis (Part E)
Vol. 9 Structure and Chemistry (Part B)
Vol. 10 Stereoselective Synthesis (Part F)
Vol. 11 Stereoselective Synthesis (Part G)
Vol. 12 Stereoselective Synthesis (Part H)
Vol. 13 Bioactive Natural Products (Part A)
Vol. 14 Stereoselective Synthesis (Part I)
Vol. 15 Structure and Chemistry (Part C)
Vol. 16 Stereoselective Synthesis (Part J)
Vol. 17 Structure and Chemistry (Part D)
Vol. 18 Stereoselective Synthesis (Part K)
Vol. 19 Structure and Chemistry (Part E)
Vol. 20 Structure and Chemistry (Part F)
Vol. 21 Bioactive Natural Products (Part B)
Vol. 22 Bioactive Natural Products (Part C)
Vol. 23 Bioactive Natural Products (Part D)
Vol. 24 Bioactive Natural Products (Part E)
Vol. 25 Bioactive Natural Products (Part F)
Vol. 26 Bioactive Natural Products (Part G)
Vol. 27 Bioactive Natural Products (Part H)
Vol. 28 Bioactive Natural Products (Part I)
Vol. 29 Bioactive Natural Products (Part J)
Vol. 30 Bioactive Natural Products (Part K)
Vol. 31 Studies in Natural Products Chemistry: Cumulative Indices Vol. 1-30
Vol. 32 Bioactive Natural Products (Part L)
Vol. 33 Bioactive Natural Products (Part M)
Vol. 34 Bioactive Natural Products (Part N)
Vol. 35 Bioactive Natural Products (Part O)
Vol. 36 Bioactive Natural Products
Vol. 37 Bioactive Natural Products
Vol. 38 Bioactive Natural Products
Vol. 39 Bioactive Natural Products
Vol. 40 Bioactive Natural Products
Vol. 41 Bioactive Natural Products
Vol. 42 Bioactive Natural Products
Vol. 43 Bioactive Natural Products
Vol. 44 Bioactive Natural Products
Vol. 45 Bioactive Natural Products
Vol. 46 Bioactive Natural Products
Vol. 47 Bioactive Natural Products
Vol. 48 Bioactive Natural Products
Vol. 49 Bioactive Natural Products
Vol. 50 Bioactive Natural Products
Vol. 51 Bioactive Natural Products
Vol. 52 Bioactive Natural Products
Vol. 53 Bioactive Natural Products
Vol. 54 Bioactive Natural Products
Vol. 55 Bioactive Natural Products
Vol. 56 Bioactive Natural Products
Vol. 57 Bioactive Natural Products
Vol. 58 Bioactive Natural Products
Vol. 59 Bioactive Natural Products
Vol. 60 Bioactive Natural Products
Vol. 61 Bioactive Natural Products
Vol. 62 Bioactive Natural Products
Vol. 63 Bioactive Natural Products
Vol. 64 Bioactive Natural Products
Vol. 65 Bioactive Natural Products
Vol. 66 Bioactive Natural Products

Studies in Natural Products Chemistry

Bioactive Natural Products

Volume 67

Edited by

Atta-ur-Rahman, FRS
International Center for Chemical and Biological Sciences
H.E.J. Research Institute of Chemistry
University of Karachi
Karachi, Pakistan

ELSEVIER

Elsevier
Radarweg 29, PO Box 211, 1000 AE Amsterdam, Netherlands
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

ISBN: 978-0-12-819483-6
ISSN: 1572-5995

Publisher: Susan Dennis
Acquisitions Editor: Emily M. McCloskey
Editorial Project Manager: Sara Valentino
Production Project Manager:
Joy Christel Neumarin Honest Thangiah
Cover Designer: Mark Rogers
Typeset by SPi Global, India

Contents

Contributors

Numbers in Parentheses indicate the pages on which the author's contributions begin.

Manashi Aditya (81), Plant Physiology & Biochemistry Research Laboratory, UGC Centre for Advanced Study, Department of Botany, The University of Burdwan, Burdwan, West Bengal, India

Iffat Zareen Ahmad (345), Department of Bioengineering, Integral University, Lucknow, Uttar Pradesh, India

Carlos Areche (1), Departamento de Química, Facultad de Ciencias, Universidad de Chile, Ñuñoa, Santiago, Chile

Soumen Bhattacharjee (81), Plant Physiology & Biochemistry Research Laboratory, UGC Centre for Advanced Study, Department of Botany, The University of Burdwan, Burdwan, West Bengal, India

Jasna Čanadanovć-Brunet (307), University of Novi Sad, Faculty of Technology, Novi Sad, Serbia

Valentina Caro (1), Escuela de Tecnología Médica, Laboratorio Catem V primer piso, Facultad de Medicina, Universidad Andrés Bello, Santiago, Chile

Gordana Ćetković (307), University of Novi Sad, Faculty of Technology, Novi Sad, Serbia

Haixia Chen (199), Tianjin Key Laboratory for Modern Drug Delivery & High-Efficiency, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, PR China

Somenath Roy Chowdhury (389), Organic & Medicinal Chemistry Division, CSIR-Indian Institute of Chemical Biology, Kolkata, India

Fernando Morgadinho Santos Coelho (161), Departamento de Neurologia e Neurocirurgia, Universidade Federal de São Paulo, São Paulo, SP, Brazil

Alberto Cornejo (1), Escuela de Tecnología Médica, Laboratorio Catem V primer piso, Facultad de Medicina, Universidad Andrés Bello, Santiago, Chile

Fábio M.L. Dantas (463), Pesquisador associado, Projeto Coral-Sol, Instituto Brasileiro de Biodiversidade-BrBio; Laboratório de Tecnologia de Materiais Poliméricos, Divisão de Processamento e Caracterização de Materiais, Instituto Nacional de Tecnologia, Rio de Janeiro, RJ, Brazil

Abhijit Dey (27), Department of Life Sciences, Presidency University, Kolkata, India

Sugapriya Dhanasekaran (221), Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Prince Sattam Bin Abdulaziz University, Wadi Al Dawaser Campus, Riyadh Province, Kingdom of Saudi Arabia

Yollanda C.S. Ferreira-Vançato (463), Programa de Pós Graduação em Ecologia e Evolução, Universidade do Estado do Rio de Janeiro; Pesquisador associado, Projeto Coral-Sol, Instituto Brasileiro de Biodiversidade-BrBio, Rio de Janeiro, RJ, Brazil

Beatriz G. Fleury (463), Pesquisador associado, Projeto Coral-Sol, Instituto Brasileiro de Biodiversidade-BrBio; Departamento de Ecologia, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Sumit Ghosh (411), Council of Scientific and Industrial Research, Central Institute of Medicinal and Aromatic Plants, Lucknow, India

Qingwen Guo (199), Tianjin Key Laboratory for Modern Drug Delivery & High-Efficiency, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, PR China

Vivek Kumar Gupta (389), Laboratory of Molecular Parasitology, Infectious Diseases & Immunology Division, CSIR-Indian Institute of Chemical Biology, Kolkata, India

Camila Guzmán (1), Escuela de Tecnología Médica, Laboratorio Catem V primer piso, Facultad de Medicina, Universidad Andrés Bello, Santiago, Chile

Ana Claudia Losinskas Hachul (161), Departamento de Fisiologia, Universidade Federal de São Paulo, São Paulo, SP, Brazil

Yanan Jia (199), Tianjin Key Laboratory for Modern Drug Delivery & High-Efficiency, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, PR China

Irena Kolouchová (123), Department of Biotechnology, University of Chemistry and Technology, Prague, Czech Republic

Hemanta K. Majumder (389), Laboratory of Molecular Parasitology, Infectious Diseases & Immunology Division, CSIR-Indian Institute of Chemical Biology, Kolkata, India

Olga Mat'átková (123), Department of Biotechnology, University of Chemistry and Technology, Prague, Czech Republic

Anuradha Mukherjee (27), MMHS, South 24Pgs, West Bengal, India

Banadipa Nanda (27), Department of Biological Sciences, Presidency University (Erstwhile Presidency College), Kolkata, India

Samapika Nandy (27), Department of Biological Sciences, Presidency University (Erstwhile Presidency College), Kolkata, India

Radhakrishnan Narayanaswamy (269), Department of Biochemistry, St. Peter's Institute of Higher Education and Research (Deemed to be University), Chennai, Tamil Nadu, India

Maria Fernanda Naufel (161), Departamento de Fisiologia, Universidade Federal de São Paulo, São Paulo, SP, Brazil

Lila Missae Oyama (161), Departamento de Fisiologia, Universidade Federal de São Paulo, São Paulo, SP, Brazil

Devendra Kumar Pandey (27), Department of Biotechnology, Lovely Faculty of Technology and Sciences, Lovely Professional University, Phagwara, Punjab, India

Sidrah Parvez (345), Department of Bioengineering, Integral University, Lucknow, Uttar Pradesh, India

Amanda Paula Pedroso (161), Departamento de Fisiologia, Universidade Federal de São Paulo, São Paulo, SP, Brazil

George Perry (1), Department of Biology, The University of Texas at San Antonio, San Antonio, TX, United States

Tomáš Řezanka (123), Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Eliane Beraldi Ribeiro (161), Departamento de Fisiologia, Universidade Federal de São Paulo, São Paulo, SP, Brazil

Sandeep (411), Council of Scientific and Industrial Research, Central Institute of Medicinal and Aromatic Plants, Lucknow, India

Aline Boveto Santamarina (161), Departamento de Fisiologia, Universidade Federal de São Paulo, São Paulo, SP, Brazil

Vesna Tumbas Šaponjac (307), University of Novi Sad, Faculty of Technology, Novi Sad, Serbia

Debasmita Sen (81), Plant Physiology & Biochemistry Research Laboratory, UGC Centre for Advanced Study, Department of Botany, The University of Burdwan, Burdwan, West Bengal, India

Vanja Šeregelj (307), University of Novi Sad, Faculty of Technology, Novi Sad, Serbia

Matías Silva (1), Escuela de Tecnología Médica, Laboratorio Catem V primer piso, Facultad de Medicina, Universidad Andrés Bello, Santiago, Chile

Slađana Stajčić (307), University of Novi Sad, Faculty of Technology, Novi Sad, Serbia

Heena Tabassum (345), Department of Bioengineering, Integral University, Lucknow, Uttar Pradesh, India

Mônica Marques Telles (161), Departamento de Fisiologia, Universidade Federal de São Paulo, São Paulo, SP, Brazil

Vijayakumar Veeraragavan (269), Department of Chemistry, Anna University, Chennai, Tamil Nadu, India

Divya Venugopal (221), Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Prince Sattam Bin Abdulaziz University, Wadi-Al Dawaser Campus, Riyadh Province, Kingdom of Saudi Arabia

Jelena Vulić (307), University of Novi Sad, Faculty of Technology, Novi Sad, Serbia

This page intentionally left blank

Preface

The present 67th volume of this series, authored by leading experts in the field of bioactive natural products, brings together 13 comprehensive reviews on the most recent developments.

In Chapter 1, Cornejo Mora and coworkers provide a review about the two major targets of dementia that include tau and α-synuclein involved in the formation of toxic oligomers and intracellular aggregates essential for the development of neurodegeneration. Huntington disease (HD) is a genetic neurodegenerative disorder for which currently there is no cure. In Chapter 2, Pandey et al. have reviewed a number of plant-derived natural products as disease-modifying agents that are found to be active against HD pathology in various neurotoxic and transgenic models.

According to several traditional beliefs, *Amaranthus hypochondriacus* is a tropical plant which has antioxidant potential for combating degenerative diseases. In Chapter 3, Bhattacharjee et al. have provided a review on the effectiveness of this plant including RP-HPLC and GC–MS analysis of structure and composition of its constituents. Omega-3 fatty acids are essential nutrients that are important for preventing and managing heart diseases. In Chapter 4, Rezanka and his colleagues give a detailed account on the potent microbial sources for the production of omega-3 polyunsaturated fatty acids. Chapter 5 written by Naufel et al. focuses on one of the most prevalent diseases of the world, i.e., diabetes mellitus. The chapter covers the use of bioactive natural products for the prevention and treatment of diabetes mellitus. Similarly, in Chapter 6, Chen et al. discuss antidiabetic properties and antihyperglycemic effects of polysaccharides and polysaccharide complexes from animals, plants, bacteria, and fungi. Chapter 7 is a detailed review by Dhanasekaran and Venugopal focused on the phytochemical constituents of bitter gourd possessing the ability to address metabolic abnormalities and treat various malignancies.

Many natural products have been found to be associated with antiinflammatory properties. Chapter 8 by Narayanaswamy and Veeraragavan presents a comprehensive review on the antiinflammatory agents extracted from natural products. Carrot waste and carotenoids are beneficial antioxidants that can protect from diseases and enhance the immune system. In Chapter 9, Šeregelj and his colleagues have provided a review on food application and health benefits associated with the use of natural bioactive compounds in

carrot waste. Chapter 10 discusses the structural and functional analysis of peptides produced by cyanobacterial strains. Ahmad et al. have presented a review on anticancer activity of cyanobacterial peptides. It is always a top priority to identify drug targets, with minimal adverse effects. Chowdhury et al. in Chapter 11 have provided a review on the bioactive compounds that target protozoal DNA topoisomerase to prevent parasitic infections. Chapter 12 by Sandeep and Ghosh provides details about structural diversity and pharmaceutical potential of triterpenoids.

In Chapter 13, Ferreira et al. have presented a comprehensive review on the use of nanobiocides and nontechnology against unwanted accumulation of organisms, termed as biofouling.

I would like to thank all the authors for their excellent contributions. I would also like to express my thanks to Dr. Taqdees Malik for her help in the preparation of this volume. I am also grateful to Mr. Mahmood Alam for his special editorial assistance.

Atta-ur-Rahman
Patron-in-Chief
International Centre for Chemical & Biological Sciences
University of Karachi
Karachi, Pakistan

Chapter 4

Alternative sources of omega-3 polyunsaturated fatty acids

Tomáš Řezanka[a,*], Irena Kolouchová[b] and Olga Mat'átková[b]
[a]*Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic*
[b]*Department of Biotechnology, University of Chemistry and Technology, Prague, Czech Republic*
[*]*Corresponding author: e-mail: rezanka@biomed.cas.cz*

Chapter Outline

Introduction

The rapid development of society after World War II, especially Western civilization, has led to a change in the human diet. Primarily due to the high consumption of vegetable oils, a change in the type of consumed fatty acids has occurred, especially in the amounts of ω-6 and ω-3 polyunsaturated fatty acids (PUFAs) that are consumed. The excessive use of vegetable oils has shifted the ratio of ω-6/ω-3 from the ideal value of 1:1 to 1:0.25 recommended by the WHO to an almost unbelievable 1:0.1 to 1:0.03, values which are typical of European and US diets [1]. The change in this ratio carries a number of complications, including an increased incidence of cardiovascular disease, an increased risk of cancer and autoimmune diseases, mental disorders, worsening memory in the form of Alzheimer's disease, developmental retardation, visual disturbances, muscle weakness and tremor, dyslipidemia, insulin resistance, high blood pressure, thrombosis, diabetes, asthma, and obesity [1].

Studies in Natural Products Chemistry, Vol. 67. https://doi.org/10.1016/B978-0-12-819483-6.00004-7

The most common ω-3 PUFA is α-linolenic acid (ALA), which is present in some vegetable oils, nuts, flaxseeds, and soy products. ALA production by bacteria is uninteresting from a scientific point of view, as ALA production is very low in bacteria, where it is essentially a minor biosynthetic product of biosynthesis as well as from an economic perspective because ALA production is sufficiently obtained from vegetable oils such as linseed oil [2–4]. Humans have the ability to biosynthesize eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from α-linolenic acid (ALA), but the production is too low to provide sufficient amounts of ω-3 PUFAs for the needs of humans, especially with regard to the high prevalence of obesity and hepatic steatosis in the population, which leads to a lower bioavailability of these PUFAs needed under normal physiological conditions [5–11]. The current production of these ω-3 PUFAs (EPA and DHA) from natural sources is insufficient for commercial use, the primary source for which is currently sea fish, which is not a truly effective source. Feeding wheat or corn to fish on fish farms or freshwater ponds may lead to lower contents of EPA and DHA in the fish and shift the ω-6/ω-3 ratio toward the ω-3 PUFAs [12]. Furthermore, environmental pollution by heavy metals, toxic organic molecules, and the recently appreciated microplastics [13] may lead to serious contamination problems in sea fish. Currently, microbial-derived ω-3 PUFA-rich oils are being implemented as an alternative to fish oils in the food industry [14].

Commonly used vegetable oils are obtained from agricultural crops such as sunflower, soy, canola, and palm oil. These oils are relatively inexpensive and are not contaminated with pollutants. However, the PUFAs present in commercially available vegetable oils are typically limited to linoleic acid (LA; 18:2ω-6) and α-linolenic acid.

Other important sources of PUFA include algae, which are an advantageous source due to their favorable ratios of ω-6 and ω-3 PUFAs. Here, we give examples of this ratio in some biotechnologically cultivated algae published by Lang et al. 2011 [15]. For commonly grown algae, such as *Chlorella*, *Parachlorella kessleri* (formerly *Chlorella kessleri*), and *Scenedesmus,* the ω-6/ω-3 ratios are 1:0.40, 1:0.19, and 1:0.45, respectively. In the case of cyanobacteria, the ratio of these fatty acids ranges from 1:0.30 for *Arthrospira* (*Spirulina*) to instances where the cyanobacterium does not produce any ω-3 PUFAs. Thus, this ratio is much worse and is almost similar to that observed in common vegetable oils, such as soybean (1:0.14), olive (1:0.07), or sunflower oils, the latter of which does not contain ω-3 PUFAs.

In conventional PUFA biosynthesis, saturated long-chain fatty acids produced by fatty acid synthase (FAS) are modified by a series of elongation and desaturation reactions (Fig. 4.1). A number of separate desaturase and elongase enzymes are required to produce unsaturated and longer chain ω-3 PUFA, typically EPA and/or DHA. Genetically untreated flowering plants do not synthetize these PUFAs. The classical pathway of PUFA biosynthesis using acetyl-CoA as the primary precursor for EPA and/or DHA biosynthesis

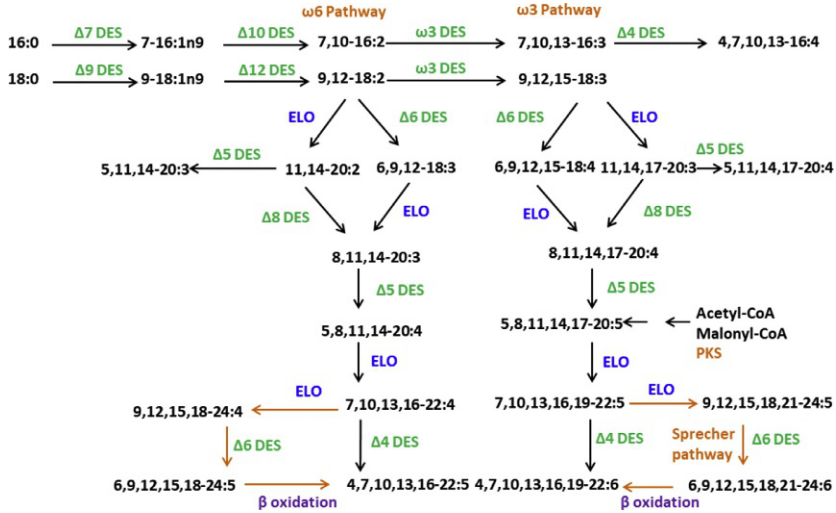


FIG. 4.1 Scheme for the biosynthesis of PUFAs. FA description legend: the first number corresponds to the number of carbon atoms in the chain; and the second number (number after the colon) is the number of double bonds; the number(s) before the hyphen defines the position(s) of double bond(s); *DES*, desaturation; *ELO*, elongation.

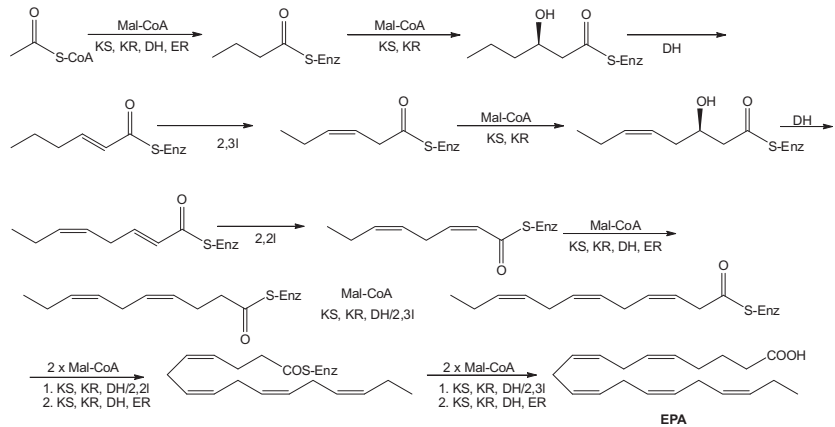


FIG. 4.2 Probable pathway for EPA biosynthesis by the *Shewanella* PKS I. Abbreviations: *DH*, dehydratase; *ER*, enoyl reductase; *KR*, 3-ketoacyl reductase; *KS*, 3-ketoacyl synthase; *2,3I*, 2-trans, 3-cis isomerase; *2,2I*, 2-trans, 2-cis isomerization; *Mal-CoA*, malonyl-CoA.

requires approximately 30 different enzymes and 70 reactions [16]. A completely different and much simpler pathway (Fig. 4.2) catalyzed by type I polyketide synthase (PKS I) has been identified in a number of deep-sea bacteria, such as *Shewanella, Aetherobacter* [17], *Photobacterium profundum* [18], *Moritella marina* (also known as *Vibrio marinus*) [19], and *Colwellia*

psychrerythraea [20], as well as marine dinoflagellates, including *Schizochytrium* and *Thraustochytrium* [16]. However, it should be emphasized that dinoflagellates are known to be capable of PUFA biosynthesis via both conventional (aerobic) and PKS I (anaerobic) pathways.

The traditional polyketide synthases described in the literature belong to one of the three basic types that are typically referred to as type I (modular or iterative), type II, and type III. As an example of the variety of products that can be biosynthesized by PKSs, we present two bacterial metabolites in Fig. 4.3 that are industrially produced, biologically active substances; see reviews [21,22].

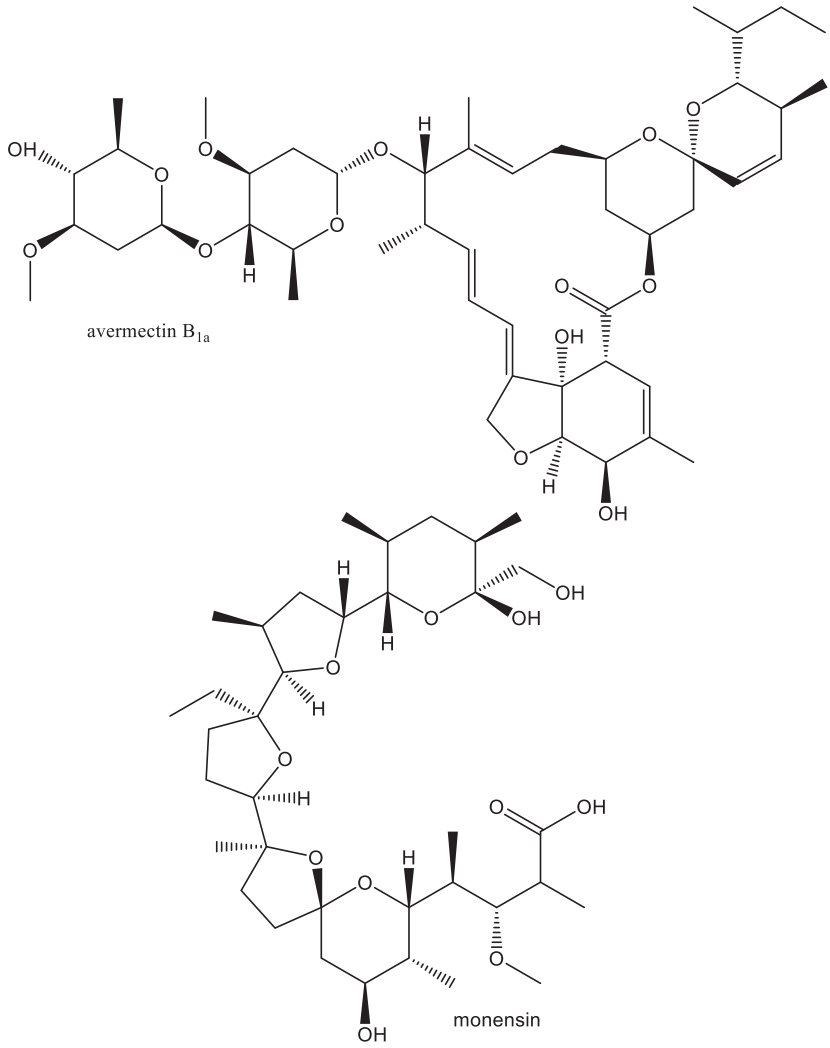


FIG. 4.3 Industrially produced and biologically active microbial metabolites.

Type I PKSs are present in both bacteria and fungi and include a single large polypeptide chain that contains several different domains capable of catalyzing different reactions. Type II PKSs are typically present in bacteria and are composed of separate dissociating proteins, each of which have a different function in polyketide synthesis and are primarily involved in the production of aromatic metabolites [23]. Type III PKSs also produce aromatic metabolites and are primarily distributed in the plant kingdom but are occasionally observed in bacteria [24]. This division is not entirely accurate, as transient biosynthetic reactions occur. However, in this chapter, we will only deal with type I PKSs, which remain only applicable for polyunsaturated fatty acid biosynthesis.

Two groups of type I PKSs are present in both fungi and bacteria, including iterative and/or noninteractive enzymes [25–29]. Fungal iterative PKSs are typically single-module proteins, and since they are largely restricted to fungi, they will not be mentioned further in this chapter. In contrast, bacterial type I PKSs contain multiple sequential modules.

Type I PKSs have been predominantly identified in *Actinobacteria*, mycobacteria, *Pseudomonas*, and cyanobacteria [30] and are multifunctional enzymes that are arranged in modules. A significant correlation has been observed between the size of the genome and the presence of type I PKSs, showing that small genomes (<2 MB) generally lack these genes [31]. Furthermore, bacteria having genomes <3 MB do not tend to produce secondary metabolites, suggesting that natural product formation requires energy costs that only bacteria with large genomes can afford [32]. The results of studies by Hayashi et al. [33–35] show the domain organizations of bacterial PUFA synthases and the proposed biosynthetic pathway of EPA and DHA synthases.

It was assumed that PKS enzymes only exist in plants, but genome sequencing has shown that these enzymes are also widely present in bacteria and fungi. Polyketide synthase I is an enzyme system similar to FAS. Fatty acid synthase is present in all groups of organisms (excluding archaea), and FA biosynthesis is probably one of the oldest metabolic pathways. Both FAS and PKS utilize the same biosynthetic strategy. FAS and PKS are similar with respect to their domain organization, the reactions they catalyze, the use of precursors and overall architecture, suggesting an evolutionary link between these two biochemical pathways.

Bacteria biosynthesize FA in three different ways. Most bacteria produce fatty acids via type II FASs and dominant cellular fatty acids typically contain 14–18 carbon atoms. An alternative type I FAS system is present in some corynebacteria, i.e., members of the order *Actinomycetales*, and consists of a polyfunctional complex of enzymes harboring catalytic activity in separate functional domains [36]. The third bacterial biosynthesis pathway involved in de novo fatty acids occurs in a select group of predominantly marine bacteria.

PUFA biosynthesis utilizes the acyl carrier protein for hydrocarbon chain synthesis and continues the iteration cycles with the addition of C2 units

and double bonds. Unlike the classical biosynthesis of PUFAs, double bonds are retained in the PUFA molecules after they are introduced during the fatty acid biosynthetic process and are not reduced during the following processes. Thus, aerobic desaturation is not required to introduce double bonds into the existing acyl chain. This pathway is sometimes called the "anaerobic" pathway although it occurs in aerobic organisms, such as microorganisms of the dinoflagellate group. As the hydrocarbon chain extends, it is believed that the oxo (keto) groups are reduced to hydroxyl groups and then dehydrated to form double bond(s). Eight proteins are required for this biosynthetic pathway: malonyl-CoA:ACP acyltransferase (MAT), acyl carrier protein (ACP), 3-ketoacyl synthase (KS), 3-ketoacyl-ACP reductase (KR), acyltransferase (AT), chain length factor (CLF), enoyl reductase (ER), and 3-hydroxyacyl-ACP dehydratase/isomerase (DH/IS). The genes for PKS are widespread based on gene database searches. However, the synthesis of ω-3 PUFAs via the PKS pathway has only been demonstrated in a few studies, as the identification of genes encoding specific enzymes in a given microorganism does not necessarily indicate that these PUFAs are produced. For example, although genes encoding a type I PKS were identified in the cyanobacterium *Microcystis aeruginosa* [37], no synthetized products (EPA or DHA) have yet been identified in the species [15,38]. To further complicate the situation, Shelest et al. [39] reported the presence of type I PKS genes in many algae, including known green algae such as *Chlorella variabilis*, *Coccomyxa subellipsoidea*, and *Monoraphidium neglectum*, as well as other algae such as *Nannochloropsis gaditana* or *N. oceanica*. Among 32 algal species analyzed, the type I PKS was identified in 15 (nearly one half).

Type I PKS enzymes produce a limited set of fatty acids as primary products, including DHA, EPA, and docosapentaenoic acid (DPA, 22:5ω-6), as opposed to classical (aerobic) biosynthesis, where dozens of other PUFAs are produced. This set FAs have been recently extended to include arachidonic acid (ARA, 20:4ω-6), which was identified in the marine bacterium *Aureispira marina* [40], and linoleic acid, which is present in myxobacteria [41]. Depending on the specific PKS I, the major product may be a single PUFA, such as DHA, EPA, or ARA, or a mixture of PUFAs such as DHA and DPA or DHA and EPA. All of these fatty acids are 18–22 carbons in length and have between 2 and 6 carbon–carbon double bonds interrupted by methylene. Fig. 4.4 shows the structural formulas of the three most important PUFAs, i.e., ARA, EPA, and DHA. The double bonds in these PUFAs are always in the E (*cis*) configuration, and the last double bond occupies either the ω-3 or ω-6 position. Why these particular PUFAs are created and accumulate in microorganisms remains an open question.

Microbial diversity represents an enormous genetic pool that can be used to identify novel genes and metabolic pathways for novel products. In addition, the characterization of a complex community of cultivable and noncultivable microorganisms can also provide new information for understanding the

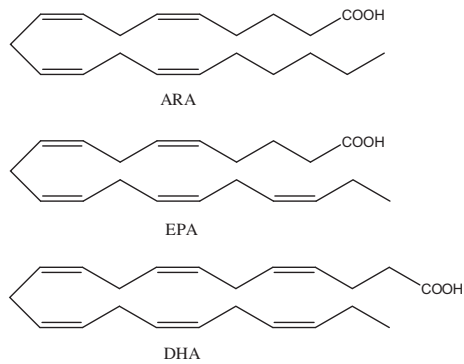


FIG. 4.4 Structures of ω-6 (ARA) and ω-3 (EPA and DHA) PUFAs.

role of individual microorganisms in various ecosystems. As a result, new insights into microbial physiology could help to cultivate microorganisms that are currently noncultivable.

PUFA influence on humans

Studies have been performed involving data reported in the literature for nearly 70 years and their processing using meta-analysis of randomized controlled trials to estimate the effect of ω-3 PUFA, EPA, and DHA on coronary heart disease (CHD) to estimate the association between EPA and/or DHA and CHD risk [42]. The results showed that higher EPA and/or DHA intake may reduce coronary heart disease.

In one study, a meta-analysis of 10 studies involving 77,917 individuals and monitoring the effect of ω-3 PUFAs on cardiovascular disease was performed [43]. Similarly, additional research has focused on assessing the efficacy of dietary ARA administered as triacylglycerols or phospholipids as substrates for brain activation in baboon neonates [44].

Another medical-focused article investigated ω-3 and ω-6 PUFAs, i.e., EPA, DHA, and ARA, and their transformation to bioactive lipid mediators [1]. Omega-6 PUFAs, i.e., ARA, have been shown to be overrepresented in most cancer tissues compared to normal tissues, whereas the contents of ω-3 PUFA, i.e., EPA and DHA, are reduced. The consumption of ω-6/ω-3 PUFAs in a 2–4:1 ratio is associated with a reduced risk of breast, prostate, colon, and kidney cancers. In addition, ω-3 PUFAs have cardioprotective functions, such as reducing platelet aggregation and inflammation, especially in the liver and brain, which is crucial for optimal brain function. Since DHA is the primary ω-3 PUFA present in the cortical gray matter, the importance of DHA and its lipid uptake has recently been reported in patients with significant depressive and bipolar disorders, Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis.

The American Heart Association recommends that individuals consume fish in such a quantity that approximately 0.5 g of ω-3 PUFA is received, especially EPA and DHA [45]. This recommendation was based on a study involving 24,621 people, where more than 90% of people consumed less than the recommended dose (median = 0.11 g/day, mean = 0.17 g/day). Among the 15% of individuals who were the most significant consumers of ω-3 PUFAs, fish was the largest dietary contributor (71.2%). The effects were highest among men aged 20 and over and lowest among children as well as women who are or may be pregnant or breastfeeding. Only 6.2% of the total population used food supplements.

The issue of DHA and EPA deficiency is essentially based on their inadequate biosynthesis in humans. Although α-linolenic acid is commonly present in vegetable oils, unfortunately, only trace amounts are converted to DHA in humans, with the same problem applying to EPA. An extensive study has been published analyzing the PUFA content in dozens of different sources, such as fish, crustaceans, and molluscs, as well as common types of meat, eggs and milk, plant sources such as fruits, vegetables, and seeds; including cyanobacteria, bacteria, fungi, and microalgae [46].

A similar review describing the typical EPA, DPA, and DHA contents of selected seafood and meats, including the recommended consumption of ω-3 PUFA intake, has been reported [47]. Amusingly, the recalculated amount of meat an adult would need to consume to meet the recommended daily dose of ω-3 PUFAs (500 mg) was 50 kg of beef per day. From this calculation, it is clear that only fatty sea fish, e.g., mackerel, can fulfill the daily consumption needs.

Omega-3 PUFA-producing bacteria

In the following subchapters, we describe the primary producers of ω-3 PUFAs, which are biosynthesized using type I PKSs.

Aureispira

Aureispira is a Gram-positive and aerobic genus of bacteria in the class *Sphingobacteria*, order *Sphingobacteriales*, family *Saprospiraceae*. The colonies are yellow or yellow-orange, with the bacteria being chemoorganotrophic and aerobic. The predominant PUFA produced by *Aureispira* is ARA, with the other characteristic FAs being 16:0 and i17:0. The optimum growth temperature of these bacteria is between 25°C and 30°C, and the optimum pH is approximately 7.0.

The marine bacterium *Aureispira marina* has been shown to produce ARA [40]. The expression of a specific gene cluster in *E. coli* was shown to induce ARA production, suggesting that the genes in this cluster encode the PKS required for ARA biosynthesis.

The marine bacterium *Aureispira* sp. CCB-QB1 was collected from the Penang coast (Malaysia), and the genome sequence of this strain was determined [48]. The genome of this *Aureispira* sp. CCB-QB1 consists of 7,370,077 bases and contains 5911 protein-coding genes and 76 RNA genes. A number of these genes have been previously characterized, e.g., linoleoyl-CoA desaturase, the key gene in arachidonic acid biosynthesis, and the gene encoding the enzyme responsible for the synthesis of geranylgeranyl diphosphate, which is a precursor of diterpenoids and is used to synthesize menaquinones.

A. marina, which was isolated from marine sponges and algae on the south coast of Thailand, has been studied by GC–MS. In addition to the commonly described FAs, including i15:0, i16:0, 16:0, i17:0, etc., ARA was also identified and accounted for more than 1/3 of the total FA content [49]. The bacterium was cultivated at 25°C for 48 h.

A bacterium with a similar name, *A. maritima*, was isolated from marine barnacle debris [50] and exhibited an ARA content of up to 43.6% of the total FA content.

Colwellia

Colwellia is a Gram-negative bacterium, class *Gammaproteobacteria* and family *Colwelliaceae*, they are facultatively anaerobic and chemoorganotrophic. *C. psychrerythraea* produces a red pigment. It is psychrophilic bacterium and is able to grow from 0°C to 20°C, with a typical optimum of 15°C. This genus can even grow at −5°C in the liquid medium. Some genera are barophilic and require high hydrostatic pressure for growth, with these strains being isolated from permanently cold and often deep-sea habitats (Atlantic and Pacific Oceans). These strains have been shown to produce DHA and EPA, in addition to the common FAs that are characteristic of the order *Alteromonadales*, e.g., 14:0, 14:1, 16:0, 16:1ω7, 18:1, etc.

Eight strains of *Colwellia*, a genus of deep-sea psychrophilic, barophilic, and facultative anaerobic bacteria were identified by 16S rRNA gene sequence analysis [51]. Based on the sequence similarities of these eight strains, they were observed to comprise five different species of *Colwellia*, one that had previously been described (*C. psychrerythraea*) and four new species (*C. demingiae*, *C. psychrotropica*, *C. rossensis*, and *C. hornerae*). DHA production in these strains ranged from 1.2% to 4.1% of the total FA content, culturing proceeded at 10°C for 2–7 days.

Kocuria

Bacteria of the genus *Kocuria* form Gram-positive coccoid cells that are aerobic or slightly facultatively anaerobic. Polar lipids detected in these bacteria include cardiolipin (CL), phosphatidylglycerol (PG), and phosphatidylinositol (PI), with the major fatty acid being ai15:0.

Most *Kocuria* strains grow between 20°C and 37°C, with *K. aegyptia* and *K. marina* being able to grow at up to 40°C and 43°C, respectively. In contrast, *K. polaris* is also able to grow at 5°C. Salt tolerance is among members of this genus is variable, if indicated, with most strains tolerating 5% NaCl. Equally variable is the occurrence of *Kocuria* species, which have been isolated from a wide range of habitats, including meat, milk, mammalian skin, or freshwater. In addition, they have been isolated from cold, temperate, and desert soils; cyanobacterial mats; seawater, including deep sediments of the Mariana Trench; cold habitats such as glaciers and Antarctica; or deepwater radioactive springs.

Four PUFA-producing bacterial isolates were obtained from water samples of radioactive springs (Agricola, Behounek, C1, and Curie) collected from a Jáchymov spa [52]. Jáchymov (Sankt Joachimsthal) is a city in northwestern Bohemia (Czech Republic) where Marie and Pierre Curie isolated radium in 1898 from the mineral uraninite. To date, four springs have been used for spa purposes, i.e., for the treatment of nervous and rheumatic disorders with water containing the radioactive gas radon (^{222}Rn), which is continually dissolved in the water. The radioactivity reaches 24 kBq/L. Through 16S RNA gene sequence analysis, all four isolates were identified as members of the genus *Kocuria*, with two isolates, designated *Kocuria* sp. 208 and 401, being affiliated with *Kocuria kristinae*, while isolates *Kocuria* sp. 101 and 301 are most likely *K. rhizophila*. The fatty acid content in polar lipids was determined by GC–MS, resulting in the identification of two polyunsaturated fatty acids, i.e., arachidonic and eicosapentaenoic acids. The position of double bonds was confirmed by GC–MS for 3-pyridylcarbinol (formerly picolinyl) esters. We presume that all four isolates of *Kocuria* produce PUFAs to increase the stability of cell membranes, which may be impaired by the production of reactive oxygen species which can arise from the production of α radiation during ^{222}Rn decay.

Moritella

The genus *Moritella* is a group of Gram-negative bacteria belonging to the class *Gammaproteobacteria* and family *Moritellaceae*. These bacteria are chemoorganotrophic, facultatively anaerobic, and halophilic (optimum growth in ~3% NaCl). All *Moritella* species have been detected in marine environments. *M. marina* was isolated from fish skin, seawater, and marine sediment, while *M. japonica*, which is barotolerant, was obtained from oceanic sediments, as was *M. yayanosii*. The latter species grows at a pressure of 50 MPa or greater. Members of this genus are psychrophilic and barophilic, which is not surprising given their isolation sites. The majority of FAs detected in these species are 14:0, 16:0, 16:1, and DHA.

DHA, as well as medium-chain fatty acids (MCFAs), were shown to be produced by *M. marina* [53]. When the bacterium was cultivated in a medium

in the presence of cerulenine, an inhibitor of FA biosynthesis, the proportion of MCFA decreased, while an increase in DHA production was observed. These results suggest that medium-chain fatty acid synthesis is independent of DHA synthesis.

The effect of changing culture conditions on DHA production was investigated in another study of *M. marina* [54]. The production of DHA was optimized by changing carbon sources (glucose and glycerol), nitrogen sources (tryptone and yeast extract), salts (NaCl and $MgCl_2$), pH (5, 6, or 8), and temperature (10°C, 15°C, and 20°C). By evaluating all of the above combinations of variables, the highest DHA production was observed in a culture medium containing marine broth, glycerol, yeast extract, and tryptone, at 15°C and pH 6.0 for 96 h with orbital shaking at 150 rpm. The yield was 11.1% of the total FA content, and the DHA titer was 82 mg/L.

Myxobacteria

A completely separate subchapter is the presence of PUFAs in myxobacteria, a coherent group of *Deltaproteobacteria*. Myxobacteria are one of the most fascinating Gram-negative prokaryotic groups of microorganisms. They exhibit unique and complex life cycles and also form multicellular fruiting bodies, which are more commonly observed in eukaryotic fungi. Myxobacteria have been isolated from deep-sea vents, hydrothermal springs, marine sediments, alkaline peat, deserts, Antarctica, and freshwater. The primary reason for the unsuccessful cultivation of many myxobacterial strains is their still unexplained nutritional requirements and metabolism. Myxobacteria are the source of dozens, if not hundreds, of secondary metabolites, with one study identifying 32 major classes of cytotoxic compounds [55]. Many myxobacteria produce PUFAs, as discussed below.

A total of 47 aerobic myxobacterial strains comprising 19 genera and mostly obtained from different collections were analyzed for the presence of FAs, with an emphasis on PUFAs. Of the above strains, PUFAs were identified in 30 strains. For instance, in the genus *Aetherobacter*, EPA can reach 10.9% of the total FA content [56].

Two myxobacteria were isolated from dried soil samples containing decaying plant material [17]. Through GC–MS analysis, the PUFA content was observed to be 10.3% EPA and 12.7% DHA of the total FA content. An analysis of their 16S rRNA gene sequences indicated that the two strains are *Aetherobacter fasciculatus* and *A. rufus*. Strains used in the fatty acid analysis were grown in a medium containing Bacto casitone, $CaCl_2$, $MgSO_4$, glucose, and HEPES, adjusted to pH 7.0 and shaken at 200 rpm, for 5 days, with optimum culture temperatures observed at room temperature and 30°C, respectively.

In addition, a patent [56] disclosed a method for producing PUFAs by culturing specific myxobacteria, including *Aetherobacter* sp., *A. fasciculatus* and

A. rufus, where the amount of EPA and DHA ranged from approximately 5% to 19% of the total FA content.

The halophilous genus *Plesiocystis* sp. is a producer of ARA [57], which was later detected in other genera of marine myxobacteria [58,59] and in some novel strains of myxobacteria [60].

Two strains of the novel myxobacterium species *Plesiocystis pacifica* were isolated from the Japanese coast. These strains were shown to be strictly aerobic and have cell FA profiles characterized by a predominance of i15:0, ai16:0, and ai17:0 and ARA in amounts of 17.5% and 14.1% of the total FA content, respectively [57].

Another myxobacterium, *Phaselicystis flava*, was isolated from forest soil in the Philippines. This strain is mesophilic, strictly aerobic and was shown to have 15:0, 2-OH-17:1 and ARA as major FAs [61].

Two different types of clusters of PUFA biosynthetic genes [41] responsible for the production of linoleic acid as well as EPA and DHA were identified in myxobacteria of the genera *Sorangium* and *Aetherobacter*. The relevant biosynthetic pathways differ from the biosynthesis described in marine bacteria (myxobacteria are of terrestrial origin), primarily with respect to the organization of genes, the arrangement of the catalytic domain, and the sequence identity of PUFA synthetases, with 1-acylglycerol-3-phosphate *O*-acyltransferase being identified as a unique domain.

One of the many patents involving myxobacterial PUFAs describes a method of producing PUFAs by heterologous expression of gene clusters encoding enzymes involved in their corresponding biosynthetic pathways derived from specific myxobacteria. These gene clusters can be cloned into a wide variety of microorganisms suitable for the production of increased amounts of ω-3 PUFAs [62].

Photobacterium

The genus *Photobacterium* belongs to the Gram-negative family *Vibrionaceae* and the class *Gammaproteobacteria*. Bacteria in this genus are widespread and have been isolated from seawater, sediments, and the gastrointestinal tract of some marine animals. Some strains have been shown to function as symbionts in specialized light organs in marine fish, as suggested by a part of the name of this genus, i.e., "photo." Optimal growth of these bacteria occurs in the temperature range of 18–25°C, with the exception being psychrophilic species such as *P. profundum*. In particular, 16:1ω7 and 16:0, 12:0, 18:1ω7, and to a lesser extent 14:0 or 14:1 have been identified in various *Photobacterium* species, with hydroxy-FA being sometimes detected. The FA profile of *P. profundum* is very different from that of other photobacteria and is particularly characterized by a substantial amount of i16:0 and EPA, as discussed below.

A new, psychrotolerant, barophilic deep-sea bacterium (*P. profundum*) that can be cultivated at various temperatures and pressures has been shown to produce FAs [63]. *P. profundum* isolated from an amphipod homogeneous enrichment in the Sulu Sea (near Philippines) at a depth of 2551 m produces multiple monounsaturated FAs (MUFAs) and PUFAs when grown at reduced temperatures or elevated pressure. After the addition of the inhibitor cerulenine, MUFA biosynthesis was inhibited, but PUFA biosynthesis was not. Several mutants were prepared, of which the mutant designated EA2 (low MUFA production but increased EPA production) produced EPA at 32.7% of the total FA content at 4°C and 28 MPa. Furthermore, the authors discuss the influence of culture conditions on understanding the role of unsaturated FA production during growth at low temperatures and high pressures.

Furthermore, a barophilic bacterium was isolated from sediment obtained from a depth of 5110 m from the Ryukyu Trench (between Taiwan and Japan) [64]. Based on 16S rRNA gene sequence analysis, the sequence was determined to be that of *P. profundum*. The optimum growth and production of EPA by this bacterium was observed at a temperature of 10°C and a pressure of 10 MPa, reaching 13% of the total FA content.

As mentioned many times, deep-sea marine bacteria living in low-temperature and high-pressure environments produce PUFAs [18], including *P. profundum*, in which four of the five genes needed for EPA biosynthesis have been detected. Cultivation at elevated hydrostatic pressure or reduced temperature did not increase the expression of the *pfa* gene required for PUFA production. Nevertheless, the percentage of EPA increased. However, a regulatory mutant has been characterized that has been shown to exhibit increased *Pfa* expression and an increase in the percentage of EPA. This result suggests that there is a regulatory factor that coordinates the transcription of the *pfa* genes.

Gram-negative, psychrophilic, and slightly halophilic *P. frigidiphilum* strains were isolated from deep sediments (1450 m depth) of the Edison Seamount in the western Pacific Ocean [65]. A phylogenetic analysis of these strains was performed using the 16S rRNA gene. Under optimal culture conditions, i.e., the salinity of 1.5% NaCl, pH 6, and 14°C, EPA production was 6% of the total FA content.

Shewanella

Shewanella species were the first to be described as a source of PUFAs [16]. These bacteria belong to the marine family *Shewanellaceae*, order *Alteromonadales* and class *Gammaproteobacteria*, with the genus containing nearly 70 species. *Shewanella* are facultatively anaerobic Gram-negative rods that primarily occur in extreme deep-ocean habitats where the water temperature

is approximately 4°C and the pressure is tens of MPa. *Shewanella* species are also typical microorganisms that live on fish skin and in the fish digestive tract. The major fatty acids in *Shewanella* species are 13:0, 14:0, 15:0, 15:0, 16:1ω7, 16:0, and 17:1ω8. *Shewanella* contains both aerobic and anaerobic desaturase biosynthetic pathways for FA synthesis. When cultivated anaerobically, *Shewanella* species have different FA profiles compared to when they are cultured aerobically [66]. Psychrotolerant and psychrophilic *Shewanella* species have an unusual ability to synthesize EPA, with levels ranging from 0% to 23.1%. An example of the rich diversity of FAs in *Shewanella* species is presented in Table 4.1. PG and phosphatidylethanolamine (PE) have been identified as major polar lipids in *S. putrefaciens*, *S. baltica*, and *S. algae*, which are typical phospholipids of Gram-negative bacteria [76].

A marine bacterium related to *S. putrefaciens* was isolated from the intestines of Pacific mackerel [77]. In particular, the effect of cultivation temperature on EPA production was observed, with the highest production (up to 24%–40% of the total FA content) being detected at 20°C. Other PUFAs, such as linoleic acid or ARA were not identified. In addition, 90%–95% of the EPA was shown to be present in phospholipids, with PE and PG being the major ones. Furthermore, EPA was almost exclusively bound in the *sn*-2 position as determined by using snake venom phospholipase. In summary, *S. putrefaciens* can produce 15 g of dry matter per liter and a yield of 2% EPA based on dry matter, making it an excellent EPA producer.

Psychrophilic bacteria of the genus *Shewanella* [78] were obtained from samples of frozen sea ice in eastern Antarctica. Phylogenetic analysis performed with the 16S rRNA gene sequence identified two new species among the isolates, *S. frigidimarina* and *S. gelidimarina*. For the former strain, the optimum growth was between 20°C and 22°C and an EPA production ranged from 1.7% to 6.8% based on the total FA content. For the latter, optimum growth was between 15°C and 17°C and EPA production ranged from 11.7% to 16.0% based on the total FA content.

In *S. gelidimarina*, the production of EPA was assessed under culture temperature and carbon source conditions [66]. The EPA yield remained was observed to remain essentially constant in the optimum temperature range (−0.3 to 14.9°C) but dropped sharply at 19.7°C to 0.7 mg/g of dry matter. The presence of FAs was influenced by the presence of potential starter units as the sole carbon sources in the culture medium. Most EPA (23.7% of the total FA content) was produced using L-proline as the sole carbon source. Further analysis showed that acyl groups containing monounsaturated FAs and EPA were concentrated in PG, while branched FAs were detected in PE. This result corresponds to the composition of the molecular species, where 16:1/EPA or 16:0/EPA were identified as the major constituents in PG by negative-ion fast atom bombardment tandem mass spectra, whereas in PE they were i15:0/EPA and 16:1/EPA.

TABLE 4.1 Fatty acid content (%) of *Shewanella* strains from published papers.

Strain	Ref.	Cult. Temp.	11:0	12:0	i13:0	13:0	i14:0	14:0	14:1	i15:0	ai15:0	15:0	ai15:1	15:1ω8	15:1ω6
S. violacea strain DSS12	[67]	8	3.0	4.0	8.0			6.0	1.0	14.0		7.0			
S. benthica ATCC 43992	[67]	4	2.0	5.0	11.0			17.0		5.0					
S. pealeana ANG-sq1	[67]	25		4.0	9.0			5.0		13.0		1.0			
S. hanedai IAM12641^T	[67]	15		5.0	12.0	1.0		10.0		7.0		4.0			
S. algae IAM14159^T	[67]	25		2.0	5.0			1.0		27.0		3.0			
S. putrefaciens IAM12079	[67]	25		5.0	11.0	1.0		3.0	1.0	13.0		6.0			1.0
S. gelidimarina ACAM 456^T	[66]	14,9		2.6	11.3	1.7		7.6		7.0		8.8			2.3
S. japonica sp.	[68]	12		4.0	6.3	0.5		4.8	0.3	12.5	2.5	1.1	0.1	0.1	0.9
S. waksmanii KMM 3823^T	[69]	22–24		2.0	10.0			1.7		32.5		5.3			
S. fidelis KMM 3582^T	[70]	25		1.5	7.7			2.0		16.6		10.6			
S. pealeana ATCC 700345^T	[69]	25		0.2	2.6			2.6		8.4		1.2			
S. gelidimarina ACAM 456^T	[69]	25			8.0			5.2		13.2		3.8			
S. woodyi ATCC 51908^T	[6]	25		4.6	10.5			6.4		14.3		4.8			
S. hanedai CIP103207^T	[69]	25		0.3	5.8			9.1		4.6		5.0			

Continued

TABLE 4.1 Fatty acid content (%) of *Shewanella* strains from published papers.—Cont'd

Strain	Ref.	Cult. Temp.	11:0	12:0	i13:0	13:0	i14:0	14:0	14:1	i15:0	ai15:0	15:0	ai15:1	15:1ω8	15:1ω6
S. algae ATCC 51192^T	[71]	30			0.5		1.4	1.3		27.4		6.5			0.2
S. amazonensis ATCC 700329	[71]	30			4.7		1.5	1.4		26.7		9.2			0.8
S. benthica ($n=2$)[a]	[71]	10			7.3		1.1	14.3		6.6		0.8			0.1
S. frigidimarina ($n=8$)[a]	[71]	10			6.3		0.6	3.7		9.0		2.5			1.2
S. hanedai ($n=2$)[a]	[71]	10			8.3		0.3	11.5		10.4		4.2			0.3
S. gelidimarina ($n=2$)[a]	[71]	10			13.1		0.4	5.1		12.3		5.1			0.1
S. oneidensis ATCC 700550	[71]	30			2.5		2.3	2.6		25.4		4.7			0.3
S. pealeana ATCC 700345	[71]	30			15.1		1.2	6.1		17.5		2.8			
S. putrefaciens ATCC 8071	[71]	30			2.5		0.3	2.3		21.1		3.2			0.2
S. olleyana sp.	[72]	4		2.0	7.3	0.7	0.9	3.4		8.7	2.0	2.6	2.0		
S. halifaxensis HAW-EB4	[73]	10			3.0			6.0	1.0	12.0	1.0	1.0			
S. psychrophila JCM 13876	[70]	10		5.9	7.3			7.9		4.0					
S. piezotolerans JCM 13877	[70]	15		2.6	8.0	0.6		2.6		10.3	0.4	3.0			
Shewanella sp. 717	[74]	20				2.5		3.7				1.1			
Shewanella sp. NB72	[75]	20		3.2	3.5	0.2	0.3	5.5	0.4	13.8	0.5	1.1			

Strain	i16:0	16:0	16:1ω9	16:1ω7	i17:0	ai17:0	17:0	17:1ω8	17:1ω6	18:0	18:1ω9	18:1ω7c	18:1ω7t
S. violacea strain DSS12		16.0		19.0			2.0		2.0			5.0	
S. benthica ATCC 43992		15.0		37.0	2.0							1.0	
S. pealeana ANG-sq1		21.0	1.0	20.0			2.0		1.0	3.0	2.0	4.0	
S. hanedai IAM12641^T		24.0	1.0	19.0			1.0		2.0			1.0	
S. algae IAM14159^T	1.0	13.0	1.0	18.0	3.0		3.0		12.0		4.0	8.0	
S. putrefaciens IAM12079		10.0	3.0	28.0			1.0		13.0		1.0	2.0	
S. gelidimarina ACAM 456^T		13.4	1.6	21.0					3.6		0.5	0.6	
S. japonica sp.	0.2	15.5		25.9	0.8	0.4	0.4	0.2	1.7	0.4	1.6	9.1	
S. waksmanii KMM 3823^T		6.2		9.8						0.3		2.0	
S. fidelis KMM 3582^T		9.9		20.0								4.5	
S. pealeana ATCC 700345^T		24.9		19.4					1.2	4.1		6.3	
S. gelidimarina ACAM 456^T		13.9		27.9					4.2	0.3		6.8	
S. woodyi ATCC 51908^T		26.1		16.2					1.6				
S. hanedai CIP103207^T		21.0		18.6					2.4	0.5		1.4	
S. algae ATCC 51192^T	0.5	16.8	2.8	15.3	1.4		4.1	10.9	0.9	0.4	4.9	5.2	
S. amazonensis ATCC 700329	1.4	6.1	0.7	14.7	1.8		3.9	23.4	2.4	0.1	1.4	4.5	
S. benthica ($n=2$)[a]		13.8		42.3	1.0		5.4	5.1	0.7	0.3	0.1	1.1	
S. frigidimarina ($n=8$)[a]		11.8	2.2	51.1	0.3		1.2	3.0		0.1	1.7	5.3	
S. hanedai ($n=2$)[a]		17.6		37.7	2.1		1.0	1.3		0.3	0.6	3.8	
S. gelidimarina ($n=2$)[a]		10.9	0.4	39.9	0.3		0.6	3.8	1.1	0.2	0.7	5.8	

Continued

TABLE 4.1 Fatty acid content (%) of *Shewanella* strains from published papers.—Cont'd

Strain	i16:0	16:0	16:1ω9	16:1ω7	i17:0	ai17:0	17:0	17:1ω8	17:1ω6	18:0	18:1ω9	18:1ω7c	18:1ω7t
S. oneidensis ATCC 700550	1.4	14.8	2.1	23.3	1.7		2.8	8.0	1.5	1.1	2.9	5.7	
S. pealeana ATCC 700345		20.3	1.1	22.0	1.1		1.6	3.2	1.0	1.1	2.6	3.2	
S. putrefaciens ATCC 8071	0.1	19.1	3.5	29.6	1.7		1.5	6.7	0.9	2.1	3.8	6.0	
S. olleyana sp.	0.2	10.8		23.0	0.6		1.2	1.5	0.3	0.7	0.6	8.7	
S. halifaxensis HAW-EB4		20.0	1.0	37.0						2.0		7.0	
S. psychrophila JCM 13876		12.7		38.2				1.6		2.0			9.4
S. piezotolerans JCM 13877		10.0		23.5			2.2	5.6		1.7		2.5	9.3
Shewanella sp. 717		27.9		37.1			5.8	8.4		3.1	1.9		
Shewanella sp. NB72	0.5	20.6		31.5	0.4		0.2	1.0		1.8	2.8	5.5	

Strain	18:4ω3	20:1ω7	ARA	EPA	DHA
S. violacea strain DSS12				15.0	
S. benthica ATCC 43992				8.0	
S. pealeana ANG-sq1				11.0	
S. hanedai IAM12641^T				15.0	
S. algae IAM14159^T					
S. putrefaciens IAM12079					
S. gelidimarina ACAM 456^T				12.8	
S. japonica sp.	0.1		0.2	8.3	
S. waksmanii KMM 3823^T				6.7	
S. fidelis KMM 3582^T					

S. pealeana ATCC 700345^T			1.6	19.1	
S. gelidimarina ACAM 456^T			0.7	14.0	
S. woodyi ATCC 51908^T					
S. hanedai CIP103207^T			1.4	23.1	
S. algae ATCC 51192^T					
S. amazonensis ATCC 700329					
S. benthica ($n=2$)[a]					
S. frigidimarina ($n=8$)[a]					
S. hanedai ($n=2$)[a]					
S. gelidimarina ($n=2$)[a]					
S. oneidensis ATCC 700550					
S. pealeana ATCC 700345					
S. putrefaciens ATCC 8071					
S. olleyana sp.	1.1		2.3	19.2	0.5
*S. halifaxensis*HAW-EB4			1.0	7.0	
S. psychrophila JCM 13876				7.1	
S. piezotolerans JCM 13877				13.4	
Shewanella sp. 717				7.6	0.3
Shewanella sp. NB72		1.0		4.0	

[a]n *indicates the number of strains examined.*

A new *Shewanella* species was isolated from seawater and a mussel (*Protothaca jedoensis*) taken from the Sea of Japan [72]. Based on 16S rRNA gene sequencing, the species was designated as *S. japonica*. In addition to producing EPA (8.3% of the total FA content) at 12°C for 4 days, 18:4ω-3 (0.1%) and ARA (0.2%) were also identified as minor FAs. The EPA content at a cultivation temperature 28°C was 2.0%, making this a mesophilic *Shewanella* species.

Two identical strains, later designated as *S. olleyana*, were isolated from freshwater sediments in Tasmania (Australia) [72]. These strains were able to utilize aromatic compounds such as tannic acid and derivatives of anthraquinone as sole carbon sources. PUFA production was studied at 4°C, 10°C, and 24°C and was shown to decrease as the temperature increase. Nevertheless, even at 24°C, PUFAs accounted for 8.8% of the total FA content.

Two of the studied bacteria isolated from sipuncula (*Phascolosoma japonicum*) from the Gulf of Peter the Great (Sea of Japan) and taxonomically identified by 16S rRNA gene sequencing were shown to represent a new species (*S. waksmanii*) [69]. Bacterial culturing was performed at 28°C, resulting in an EPA content that was 6.7% of the total FA content.

Six marine bacteria were isolated from the Sea of Japan as part of the Pacific Ocean and were identified as a new *S. pacifica* species based on 16S rRNA gene sequence analysis [79]. The bacterium was characterized as mesophilic, as the production of EPA at 28°C in medium with a culture medium salinity of up to 6% reached up to 5.3% of the total FA content.

Four bacterial strains isolated from hydrocoral species (hydrozoan) and/or from *Sipuncula* (i.e., marine worms) collected from the Gulf of Peter the Great (Sea of Japan) and Kuril Islands (North-West Pacific Ocean) [80]. Using 16S rRNA gene they were taxonomically determined as *S. affinis*. The strains were mesophilic and grew between 10°C and 34°C, with EPA production of approximately 2.1% of the total FA content at 28°C.

The 16S rRNA gene sequence analysis identified a bacterium named *S. halifaxensis* that was isolated from the Atlantic Ocean near Nova Scotia, Canada [73]. EPA produced by aerobically cultivated bacteria using the culture medium marine broth 2216 (Difco) was 7% of the total FA content at 10°C.

The genes encoding PKS of *S. oneidensis* were cloned into *E. coli*, and the optimal synthesis of EPA in four strains of *E. coli* was shown to occur at 20°C, whereas production at 30°C or higher was negligible [81]. In addition, the production depended on or was inversely proportional to the glucose concentration in the culture medium.

Two strains of *Shewanella* (*S. psychrophila* and *S. piezotolerans*) were isolated from deep-sea sediment collected from the west Pacific [70]. Taxonomic assignments were performed based on 16S RNA gene sequences. Both strains were psychrophilic (psychrotolerant) and optimal cultivation occurred at approximately 10–15°C. Both strains grew at a pressure of 0.1–50 MPa, with an optimum pressure of 20 MPa. The produced EPA

accounted for 7.1% of the total FA content in the *S. psychrophila* strain and 13.4% in the *S. piezotolerans* strain.

The psychrotrophic bacterium *S. livingstonensis* was isolated from Antarctica produced and shown to use EPA as part of phospholipids [82]. After the addition of synthetic phospholipids to a culture of a mutant strain disrupted in an EPA biosynthetic gene, these phospholipids were shown to be incorporated into the membrane. These results suggest that intact EPA is best suited to allowing this bacterium to adapt to the cold.

Shewanella and other bacteria

Based on 16S rRNA gene sequence analysis, two extremely barophilic bacterial strains of *Shewanella benthica* and *Moritella yaynanosii* were obtained from a depth of nearly 11 km in the Mariana Trench at Challenger Deep (western Pacific Ocean) [83]. Both strains grew optimally at 10°C and a pressure of 70 MPa. However, none of the strains grew at atmospheric pressure, i.e., 0.1 MPa, at any temperature. The *S. benthica* and *M. yaynanosii* strains produced 9% DHA based on the total FA content.

Three bacterial isolates of *Shewanella colwelliana*, *Vibrio splendidus*, and *Photobacterium lipolyticum* collected from German North Sea coast [75] and identified via 16S rRNA gene sequence analysis were shown to exhibit decreased EPA production with increasing cultivation temperature (10°C, 20°C, or 30°C), ranging from 0.3% to 13.9% of the total FA content. Unlike in most previous studies, a phospholipid analysis was performed and three classes of phospholipids (PE, PG, and CL) and their molecular species were identified. In the culture at 20°C, the major phospholipids were those containing a glycerol backbone and palmitic, palmitoleic, or oleic acids in one position and EPA always in the second position. Furthermore, based on a sediment analysis and depending on the depth at which the sample was taken, the content of PG-containing EPA differed. These molecular species taken at a depth of 11–15 cm may indicate the presence of bacteria from the class Gammaproteobacteria. Thus, these bacteria may be an important source of EPA in buried, anoxic deposits under layers that carry a significant population of benthic eukaryotic organisms. Differences in FA composition were noted as a function of the phase of growth and the pressure used during the culturing process.

In two studies, Abd Elrazak et al. [74,84] described the optimization of *Vibrio cyclitrophicus* and *Shewanella* sp. using mathematical models. In *Shewanella* sp., a yield of 45 mg of EPA 1 g of biomass was achieved under optimal pH, temperature, and dissolved oxygen conditions. In *V. cyclitrophicus*, maximum EPA production of 7.5 mg/g dry weight was observed that represented 10% of the total FA content when cultured in a bioreactor. In this study, the concentration of sodium chloride, yeast extract, temperature, glucose, peptone, dissolved oxygen, and pH was monitored. The ability of the organism to produce EPA in the complete absence of oxygen was tested,

and although oxygen was dispensable for EPA biosynthesis, total PUFA production was improved in the presence of oxygen. The stability of the produced oil and the complete absence of heavy metals in the bacterial biomass are considered to be further benefits of bacterial PUFAs compared to other PUFA sources, such as seafood or algae, which always contain trace amounts of heavy metals in the culture medium, e.g., Mn, Co, Cu, Zn, Mo, etc.

Omega-3 PUFA-producing *E. coli* or marine bacteria cannot be used directly as PUFA sources because they are not approved as safe microorganisms for food supplements [85] which is not the case for lactic bacteria, which were therefore used by the authors as a ω-3 PUFA producer. The 35-kb EPA/DHA gene cluster was isolated from the marine bacterium *S. baltica* and transferred to *E. coli*. Interestingly, recombinant *E. coli* carrying a 20-kb gene cluster was shown to produce 3.5–6.1-fold more EPA than one carrying a 35-kb DNA fragment harboring genes needed for EPA/DHA synthesis. Therefore, the 20-kb EPA/DHA gene cluster was cloned into *Lactococcus lactis*. Recombinant *L. lactis* produced DHA (1.35 mg/g dry weight) and EPA (0.12 mg/g dry weight). The most interesting finding of the study was that with increasing temperature, i.e., at 10°C, 15°C, and 30°C, the production of both DHA and EPA increased. This finding is unique since all previously published articles reported that PUFA content decreases with increasing temperature.

In another study, 40 Antarctic marine bacterial isolates identified by 16S rRNA gene sequence analysis were shown to have produced ω-3 PUFAs. Strains of *Cellulophaga*, *Pibocella,* and *Polaribacter* produced either EPA, DHA or both [86]. The production was initially assessed using 2,3,5-triphenyltetrazolium chloride and verified by GC–MS. *Shewanella* sp. produced up to 2% EPA (1.8 mg of EPA per g of cell dry weight) based on the total FA content.

Table 4.2 summarizes the most significant published results of engineered microbial strains. Although yield figures may appear to be inconsistent at first glance, specific conclusions can be drawn from a given ω-3 PUFA being present. In particular, the yield of ω-3 PUFAs is not overwhelming, but when culturing non-GMO (genetically modified organism) bacteria, the values are often higher. In addition, the use of *E. coli* is not suitable because the bacterium is not a safe microorganism for food supplements. Furthermore, the cultivation period is very diverse from hardly 1 to 8 days. Based on these values and taking into account that although some progress has been made in the field of genetically modified organisms, the commercial use of genetically manipulated products in food and feed is subject to many scientific and social debates, with a great deal of work remaining to be performed.

Vibrio

The genus *Vibrio* belongs to the class *Gammaproteobacteria* and the family *Vibrionaceae*, which also includes the genus *Photobacterium*. Members of this genus are facultatively anaerobic Gram-negative bacteria, with cells that

TABLE 4.2 Literature summary of ω-3 PUFA yields achieved by engineered microbial strains.

Host strain	Genes (enzymes)	Omega-3 LC-PUFA	Yield (% of total fatty acids)	Culture temperature (°C)	Time (h)	Culture type	Refs.
E. coli DH5α	DHA synthesis gene cluster from *Moritella marina* MP-1	DHA	5.2	15	96	Batch	[87]
E. coli XL1-Blue	EPA synthesis gene cluster from *Shewanella oneidensis* MR-1	EPA	0.689	20	34	Batch	[81]
E. coli JM109	EPA synthesis gene cluster from *Shewanella* sp. strain SCRC-2738	EPA	22	15	N.D.[a]	Batch	[88]
E. coli EPI 300T1	EPA synthesis gene cluster from *Shewanella baltica* MAC1	EPA	14	15	120	Batch	[89]
E. coli EPI 300T1	DHA synthesis gene cluster from *Shewanella baltica* MAC1	DHA	0.4	15	120	Batch	[89]
Lactococcus lactis subsp. Cremoris	EPA synthesis gene cluster from *Shewanella baltica* MAC1	EPA	0.12 mg/g dcw[b]	15	24	Batch	[85]
Lactococcus lactis subsp. Cremoris	DHA synthesis gene cluster from *Shewanella baltica* MAC1	DHA	1.35 mg/g dcw	15	24	Batch	[85]
Synechococcus sp. NKBG042902	EPA synthesis gene cluster from *Shewanella putrefaciens* SCRC-2738	EPA	0.5	17	24	Batch	[90]
Phaeodactylum tricornutum Pt_Elo5	Overexpressing heterologous genes encoding enzyme activities of the LC-PUFA biosynthetic pathway	DHA	7.5	Ambient	192	Bubble column	[91]
Pseudomonas putida KT2440:: *pfa*dH_KO/pME2 + pPm**Syn*Pfa*Af2a	Replicative plasmid pPm**Syn*Pfa*Af2a (artificial *pfa* gene cluster version 2 a, *trfA**, *Pm***); $Gent^R$, Tet^R, Kan^R Gene source: *Aetherobacter fasciculatus* SBSr002	DHA	1.4 ± 0.3 mg/g dcw	16	24	Batch	[92]

Continued

TABLE 4.2 Literature summary of ω-3 PUFA yields achieved by engineered microbial strains.—Cont'd

Host strain	Genes (enzymes)	Omega-3 LC-PUFA	Yield (% of total fatty acids)	Culture temperature (°C)	Time (h)	Culture type	Refs.
Shewanella baltica MAC1 mutant	Transposon Tn5 mutagenesis	EPA	6 mg/g dcw	4	48–72	Batch	[93]
E. coli DH5α(pEPA (1)[pGBM3::sa1 (*vktA*)])	EPA biosynthesis genes pEPAΔ1, pGBM3::sa1(*vktA*) from *Shewanella* sp. strain SCRC-2738	EPA	12	20	30–40	Batch	[94]
E. coli S17-1	Gene source: *S. putrefaciens* SCRC-2738	EPA	3.3 mg/g dcw	20	ND	Batch	[90]
Synechococcus sp.	Gene source: *S. putrefaciens* SCRC-2738	EPA	7.5 mg/g dcw	18	22	Batch	[95]
E. coli DH5α	Gene source: *M. marina* MP-1 and *S. pneumatophori* SCRC-2738	DHA	0.2	15	96	Batch	[96]
E. coli DH5α	Gene source: *M. marina* MP-1 and *S. pneumatophori* SCRC-2738	EPA	9.2%	15	96	Batch	[96]
E. coli DH5α	Gene source: *C. psychrerythraea* 34H and *S. baltica* MAC1	DHA	2.2 mg/g dcw	15	ND	Batch	[97]
E. coli Nissle1917	Gene source: *S. baltica* MAC1	EPA	31.4 mg/g dcw	15	ND	Batch	[98]

[a] *N.D., not detected.*
[b] *dcw, dry cell weight.*

are curved-rod shaped and nonsporulating. Members of this genus have been isolated from sea fish and seawater and include bacteria that emit bioluminescence that is visible in areas of high cell concentration. Although this genus comprises almost 100 species, only the species *V. splendidus* has been shown to produce PUFAs.

Lipids of *V. pelagius* isolated from turbot (*Scophthalmus maximus*) were analyzed by GC–MS [99]. EPA production was the highest at 4°C (8.7% of the total FA content), but even at 23°C, EPA was still produced at 4.5% of the total FA content. Some psychrophilic marine bacteria from the Vibrionaceae family react in different ways to lowering growth temperature by either increasing the content of branched FAs or odd-numbered FAs [100], such as in *V. marinus* [101]. In this case, the DHA content increased with decreasing culture temperature. This result indicates that there are two different mechanisms affecting the fluidity of membranes in the same genus, depending on whether the bacterium is capable of producing PUFA or not.

Arctic char (charr; *Salvelinus alpinus*), a cold-water fish, were fed an artificial diet containing casein, dextrin, and coconut oil, i.e., a diet lacking PUFAs [99]. Analysis of aerobic microorganisms from their intestines revealed that EPA was present at 3.5% of the total FA content. Four isolates from 15 bacterial strains were tested for EPA production. For the isolate named A7, EPA was observed at 10.7% of the total FA content. All four production isolates belonged to the genus *Vibrio*.

In another study, 22 psychrophilic and psychrotrophic vibrios isolated from deep sediments, i.e., from depths of several hundred to thousands of meters, were analyzed [102]. The bacteria were divided into three groups based on the observed PUFA contents. The first group included isolates that produced DHA and had a maximum growth temperature of 20°C; the second group contained EPA producers and had a maximum growth temperature of 20–25°C; and the third group did not produce PUFAs and grew at 25–37°C. The authors concluded that PUFAs play a role in adapting marine vibrios to low temperatures and further suggested that the presence of PUFAs can be used to distinguish between psychrophilic and psychrotolerant marine bacteria.

The EPA production of a marine bacterium identified as *V. cyclitrophicus* based on 16S rRNA gene sequencing was optimized under various culture conditions [84]. The effects of yeast extract, peptone, glucose, palmitic acid temperature, or pH on EPA production were assessed. The EPA production in the bioreactor, pH, and dissolved oxygen values were further evaluated, and the maximum amount of EPA produced was 7.5 mg/g dry weight, which was 10% the total FA content.

Other bacteria

Bacterial samples from the three different sites, i.e., the Mid-Atlantic Ridge, the Mediterranean Sea, and the Red Sea were analyzed for the presence of PUFAs, especially EPA and DHA [103]. Most strains from the Mid-Atlantic

Ridge tested positive for PUFA production, while fewer of such strains were isolated from the Red Sea and none were obtained from the Mediterranean. Preliminary screening was based on a colorimetric method by adding 2,3,5-triphenyltetrazolium chloride to the culture medium, and if PUFA was present, the medium turned red. Unfortunately, as the authors themselves noted, this colorimetric method for screening for PUFAs has been shown to have a high false positive result. For these reasons, the presence or absence of PUFAs is always verified by GC–MS. Furthermore, the culture conditions were optimized to increase PUFA production, resulting in up to 45 mg/L of EPA, representing 11% of the total FA content, being obtained by adjusting by the culture conditions. The optimal medium contained 10.79 g/L whey and 6.87 g/L glycerol. However, it has always been assumed that the occurrence of PUFA-producing bacteria is temperature-related, with the highest number being obtained in water at approximately 4°C. Furthermore, PUFAs have been shown to be synthesized by bacteria in the Red Sea, which is considered to be warm sea, and these bacteria were collected at a depth of only 25 m. This anomaly is explained by the higher NaCl content observed in this sea and the higher environmental stress.

Psychrophilic bacterial strains, later identified by 16S rRNA gene sequence analysis as *Psychromonas marina*, were isolated from the Okhotsk Sea (44°31′N, 143°39′E) near Hokkaido Island (Japan) [72]. The optimal culture temperature ranged from 14°C to 16°C, and DHA production reached 1.6% of the total FA content.

Dinoflagellates

Dinophyta (also referred to as Dinoflagellata or dinoflagellates) are a group of organisms classified today into the taxa Chromalveolata and Alveolata. They live in freshwater, brackish, and marine water. Dinophyta are mixotrophic organisms (although they are also heterotrophic) that harbor chloroplasts obtained from secondary or tertiary endosymbiosis. Their cells are "armored," as they have walls made of cellulose platelets. In addition, they reproduce both sexually and asexually and have complex cell cycles. Dinophyta are evolutionarily important due to the structure of their nucleus and specific organelles, as well as ecologically as a result of many toxic species among them cause "red tide." Dinoflagellates can also poison shellfish and individuals who consume affected shellfish. The latest estimates suggest that there are 2300 living dinoflagellate species.

The marine dinoflagellate *Schizochytrium* is cultivated as an industrially significant source of PUFAs, primarily DHA and DPA [91]. As previously mentioned, these acids are biosynthesized by type I PKSs. Again, this phenomenon was confirmed by an experiment in which three genes encoding PKS subunits were isolated from genomic DNA and expressed in *E. coli*, where the ratio of DHA and DPA produced remained approximately the same

as that observed in the donor dinoflagellate. The addition of cerulenine to the culture medium resulted in inhibition of MCFA biosynthesis (as detected by ^{14}C acetate incorporation) without affecting PUFA biosynthesis, suggesting the presence of different MCFA and PUFA biosynthetic pathways.

Evidence that PUFAs play an essential role in some of these microorganisms has been provided by observations of the dependence on complementation with PUFAs for the growth of *Schizochytrium* sp. in which the PKS had been inactivated [92]. In addition, the potential roles of PUFA in marine bacteria have been described in detail, including their antioxidant functions and the involvement of PKS in the production of a very-long-chain polyunsaturated hydrocarbon (C31:9).

While marine bacteria produce PUFAs to use as components of their membrane phospholipids, where the overproduction of these substances is limited by membrane size, both marine and freshwater dinoflagellates accumulate large amounts of PUFA in the form of triacylglycerols (TAG), which may in some strains account for >50% of all fatty acids and up to 25% of dry matter [104]. Therefore, PUFA-producing bacteria are not yet commercially used due to their low PUFA productivity and it is for this reason that dinoflagellates are industrially cultivated. Furthermore, dinoflagellates are able to utilize various carbon sources, including industrial waste, sweet sorghum juice, bread crumbs, and glycerol [105–107]. There are three orders of dinoflagellates, *Labyrinthulida*, *Amphitremida*, and *Thraustochytrida*, representing more than 15 of the genera described. Dinoflagellates are ubiquitous in the seas and oceans, where they play an important ecological role in detritus degradation and in the recycling of carbon and nutrients as a result of their extensive repertoire of hydrolytic enzymes, including proteases, amylases, lipases, ureases, phosphatases, chitinases, α-glucosidases, and cellulases. Therefore, they are primarily considered heterotrophic microorganisms.

One of the most common dinoflagellates, the protist *Thraustochytrium*, is used for commercial DHA production [108]. In this microorganism, both PUFA biosynthetic pathways have been demonstrated to be present, including the classical aerobic pathway using a series of desaturation and elongation steps, as well as PUFA-producing type I PKS.

Heterologous expression of PKS from *Thraustochytrium* together with phosphopantetheinyl transferase in *E. coli* showed that the anaerobic pathway is highly active in PUFA biosynthesis [108]. In genetically engineered *E. coli*, DHA and DPA reached up to 18% of the total FA content. As shown in their Fig. 4.3a vs Fig. 4.3b, the ratio of DHA and DPA was different, as observed in chromatograms obtained by GC–MS. Dinoflagellates are cultivated in industrial amounts, and patents exist to protect their manufacture [109,110].

A mutant strain of *Crypthecodinium cohnii* having a deletion of the RuBisCO gene, which is involved in CO_2 fixation under autotrophic conditions, was generated and shown to exhibit increased glucose utilization and have higher yields of lipids [111].

In another study, nearly half-a-year cultivation of *Amphidinium carterae* in a pilot-scale LED-illuminated raceway photobioreactor was performed [112]. Culture conditions were optimized by changing specific parameters, including using different concentrations of medium nutrients, culture modes, or irradiance values.

We would also like to mention here that the longtime denomination of dinoflagellates as algae is used only for commercial purposes since, as stated by Leyland [113], there is no longer any reason for dinoflagellates to be placed among algae.

Recently, type I PKS genes have been discovered in the bloom-forming marine coccolithophore *Emiliania huxleyi* [114]. In this case, all domains of the putative PKS I were encoded on a single large protein. *E. huxleyi* is a major component of the global phytoplankton community and is known to produce significant amounts of PUFAs. As with other eukaryotes that have a PKS system, *E. huxleyi* also contains a set of standard PUFA biosynthesis pathways and elongase enzymes [115]. It remains to be determined what the relative contribution of both pathways to PUFA synthesis in this organism may be.

Conclusion

The history of research on PUFAs in bacteria is full of twists. Importantly, only the broad spread and routine use of the GC–MS method made it possible to identify these FAs in bacteria. In the 1960s and 1970s, the classical method for analyzing FAs from any organism, including bacteria, was gas chromatography. This method uses a comparison of retention times of the standards with the retention times of FAs in the sample to identify the FAs. Although it was known as early as 1962 that bacteria biosynthesize monounsaturated FAs by aerobic biosynthesis [116], some authors interpreted this knowledge as indicating that "bacteria altogether lack polyunsaturated fatty acids" [117]. In the next decade, the doctrine of the absence of PUFAs in bacteria has been questioned [118], but full confirmation of the ability of bacteria to biosynthesize PUFAs has been published more than 25 years later [119].

The production of ω-3 PUFA in commercially available amounts is performed using several sources, each of which has advantages and disadvantages that are discussed above. Here, we will only mention microorganisms used for PUFA production, especially dinoflagellates. PUFA production by algae has been described many times and is commercially successful [120]. Industrial scale dinoflagellate cultivation, specifically including *Crypthecodinium*, *Schizochytrium*, or *Ulkenia*, is predominantly performed to produce DHA. The content of DHA varies in products used as food supplements, whether for humans or livestock, but also for fish [121]. Of course, all industrial scale production is protected by hundreds of patents. For instance, almost 200 patents were found after entering the keywords "dinoflagellate" and "DHA" on the main website of the United States Patent and Trademark Office.

Acknowledgments

The research was supported by Institutional Research Concept RVO61388971 and by the Czech Science Foundation project 18-00036S.

Conflict of interest

The authors declare that there are no conflicts of interest regarding this work.

Abbreviations

ACP	acyl carrier protein
ALA	α-linolenic acid
ARA	arachidonic acid
AT	acyltransferase
CHD	coronary heart disease
CL	cardiolipin
CLF	chain length factor
DES	desaturation
DH	dehydratase
DHA	docosahexaenoic acid
DH/IS	3-hydroxyacyl-ACP dehydratase/isomerase
DPA	docosapentaenoic acid
ELO	elongation
EPA	eicosapentaenoic acid
ER	enoyl reductase
FA	fatty acid
FAS	fatty acid synthase
GMO	genetically modified organism
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
2,2I	2-trans, 2-cis isomerization
2,3I	2-trans, 3-cis isomerase
KR	3-ketoacyl reductase
KS	3-ketoacyl synthase
LA	linoleic acid
Mal-CoA	malonyl-CoA
MAT	malonyl-CoA:ACP acyltransferase
MCFAs	medium-chain fatty acids
MUFAs	monounsaturated fatty acids
PE	phosphatidylethanolamine
PG	phosphatidylglycerol
PI	phosphatidylinositol
PKS I	type I polyketide synthase
PUFAs	polyunsaturated fatty acids
TAG	triacylglycerols

References

[1] R. Zarate, N. El Jaber-Vazdekis, N. Tejera, J.A. Perez, C. Rodriguez, Significance of long chain polyunsaturated fatty acids in human health, Clin. Transl. Med. 6 (2017) 25.

[2] D. González-Mañán, G. Tapia, J.G. Gormaz, A. D'Espessailles, A. Espinosa, L. Masson, P. Varela, A. Valenzuela, R. Valenzuela, Bioconversion of α-linolenic acid to n-3 LCPUFA and expression of PPAR-alpha, acyl coenzyme A oxidase 1 and carnitine acyl transferase I are incremented after feeding rats with α-linolenic acid-rich oils, Food Funct. 3 (2012) 765–772.

[3] M.Á. Rincón-Cervera, R. Valenzuela, M.C. Hernandez-Rodas, C. Barrera, A. Espinosa, M. Marambio, A. Valenzuela, Vegetable oils rich in alpha linolenic acid increment hepatic n-3 LCPUFA, modulating the fatty acid metabolism and antioxidant response in rats, Prostaglandins Leukot. Essent. Fatty Acids 111 (2016) 25–35.

[4] R. Valenzuela, K. Bascuñán, R. Chamorro, C. Barrera, J. Sandoval, C. Puigrredon, G. Parraguez, P. Orellana, V. Gonzalez, A. Valenzuela, Modification of docosahexaenoic acid composition of milk from nursing women who received alpha linolenic acid from chia oil during gestation and nursing, Nutrients 7 (2015) 6405–6424.

[5] I. Khozin-Goldberg, U. Iskandarov, Z. Cohen, LC-PUFA from photosynthetic microalgae: occurrence, biosynthesis, and prospects in biotechnology, Appl. Microbiol. Biotechnol. 91 (2011) 905.

[6] R. Valenzuela, L.A. Videla, The importance of the long-chain polyunsaturated fatty acid n-6/n-3 ratio in development of non-alcoholic fatty liver associated with obesity, Food Funct. 2 (2011) 644–648.

[7] F. Echeverría, M. Ortiz, R. Valenzuela, L.A. Videla, Long-chain polyunsaturated fatty acids regulation of PPARs, signaling: relationship to tissue development and aging, Prostaglandins Leukot. Essent. Fatty Acids 114 (2016) 28–34.

[8] A.F. Domenichiello, A.P. Kitson, R.P. Bazinet, Is docosahexaenoic acid synthesis from α-linolenic acid sufficient to supply the adult brain? Prog. Lipid Res. 59 (2015) 54–66.

[9] F. Echeverría, R. Valenzuela, M.C. Hernandez-Rodas, A. Valenzuela, Docosahexaenoic acid (DHA), a fundamental fatty acid for the brain: new dietary sources, Prostaglandins Leukot. Essent. Fatty Acids 124 (2017) 1–10.

[10] D.S. Siscovick, T.A. Barringer, A.M. Fretts, J.H. Wu, A.H. Lichtenstein, R.B. Costello, P.M. Kris-Etherton, T.A. Jacobson, M.B. Engler, H.M. Alger, Omega-3 polyunsaturated fatty acid (fish oil) supplementation and the prevention of clinical cardiovascular disease: a science advisory from the American Heart Association, Circulation 135 (2017) e867–e884.

[11] R. Valenzuela, M. Ortiz, M.C. Hernández-Rodas, F. Echeverría, L.A. Videla, Targeting n-3 polyunsaturated fatty acids in non-alcoholic fatty liver disease. Curr. Med. Chem. (2019). https://doi.org/10.2174/0929867326666190410121716.

[12] D.R. Tocher, Fatty acid requirements in ontogeny of marine and freshwater fish, Aquacult. Res. 41 (2010) 717–732.

[13] F. Gallo, C. Fossi, R. Weber, D. Santillo, J. Sousa, I. Ingram, A. Nadal, D. Romano, Marine litter plastics and microplastics and their toxic chemicals components: the need for urgent preventive measures, Environ. Sci. Eur. 30 (2018) 1–14.

[14] European Union E, Document 31997R0258 New (novel) Foods and Food Ingredients. Regulation (EC) No 258/97 Concerning Novel Foods and Novel Food Ingredients, 2014).

[15] I. Lang, L. Hodac, T. Friedl, I. Feussner, Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection, BMC Plant Biol. 11 (2011) 124.

[16] J.G. Metz, P. Roessler, D. Facciotti, C. Levering, F. Dittrich, M. Lassner, R. Valentine, K. Lardizabal, F. Domergue, A. Yamada, Production of polyunsaturated fatty acids by polyketide synthases in both prokaryotes and eukaryotes, Science 293 (2001) 290–293.
[17] R. Garcia, M. Stadler, K. Gemperlein, R. Muller, *Aetherobacter fasciculatus* gen. nov., sp. nov. and *Aetherobacter rufus* sp. nov., novel myxobacteria with promising biotechnological applications, Int. J. Syst. Evol. Microbiol. 66 (2016) 928–938.
[18] E.E. Allen, D.H. Bartlett, Structure and regulation of the omega-3 polyunsaturated fatty acid synthase genes from the deep-sea bacterium *Photobacterium profundum* strain SS9, Microbiology 148 (2002) 1903–1913.
[19] M. Tanaka, A. Ueno, K. Kawasaki, I. Yumoto, S. Ohgiya, T. Hoshino, K. Ishizaki, H. Okuyama, N. Morita, Isolation of clustered genes that are notably homologous to the eicosapentaenoic acid biosynthesis gene cluster from the docosahexaenoic acid-producing bacterium *Vibrio marinus* strain MP-1, Biotechnol. Lett. 21 (1999) 939–945.
[20] B.A. Methé, K.E. Nelson, J.W. Deming, B. Momen, E. Melamud, X. Zhang, J. Moult, R. Madupu, W.C. Nelson, R.J. Dodson, The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 10913–10918.
[21] D. Łowicki, A. Huczyński, Structure and antimicrobial properties of monensin A and its derivatives: summary of the achievements, Biomed. Res. Int. 2013 (2013) Article ID 742149.
[22] S. Ōmura, K. Shiomi, Discovery, chemistry, and chemical biology of microbial products, Pure Appl. Chem. 79 (2007) 581–591.
[23] W.J. Zhang, Y. Tang, *In vitro* analysis of type I polyketide synthase, Methods Enzymol. 459 (2009) 367–393.
[24] B.S. Moore, J.N. Hopke, Discovery of a new bacterial polyketide biosynthetic pathway, Chembiochem 2 (2001) 35–38.
[25] S. Smith, S.C. Tsai, The type I fatty acid and polyketide synthases: a tale of two megasynthases, Nat. Prod. Rep. 24 (2007) 1041–1072.
[26] J. Staunton, K.J. Weissman, Polyketide biosynthesis: a millennium review, Nat. Prod. Rep. 18 (2001) 380–416.
[27] R.J. Cox, Polyketides, proteins and genes in fungi: programmed nano-machines begin to reveal their secrets, Org. Biomol. Chem. 5 (2007) 2010–2026.
[28] C. Hertweck, The biosynthetic logic of polyketide diversity, Angew. Chem. Int. Ed. 48 (2009) 4688–4716.
[29] H. Chen, L. Du, Iterative polyketide biosynthesis by modular polyketide synthases in bacteria, Appl. Microbiol. Biotechnol. 100 (2016) 541–557.
[30] H.B. Bode, R. Muller, Possibility of bacterial recruitment of plant genes associated with the biosynthesis of secondary metabolites, Plant Physiol. 132 (2003) 1153–1161.
[31] H. Jenke-Kodama, A. Sandmann, R. Muller, E. Dittmann, Evolutionary implications of bacterial polyketide synthases, Mol. Biol. Evol. 22 (2005) 2027–2039.
[32] S. Donadio, P. Monciardini, M. Sosio, Polyketide synthases and nonribosomal peptide synthetases: the emerging view from bacterial genomics, Nat. Prod. Rep. 24 (2007) 1073–1109.
[33] S. Hayashi, Y. Satoh, T. Ujihara, Y. Takata, T. Dairi, Enhanced production of polyunsaturated fatty acids by enzyme engineering of tandem acyl carrier proteins, Sci. Rep. 6 (2016) 35441.
[34] S. Hayashi, Y. Satoh, Y. Ogasawara, C. Maruyama, Y. Hamano, T. Ujihara, T. Dairi, Control mechanism for *cis* double-bond formation by polyunsaturated fatty-acid synthases, Angew. Chem. Int. Ed. 58 (2019) 2326–2330.

[35] S. Hayashi, M. Naka, K. Ikeuchi, M. Ohtsuka, K. Kobayashi, Y. Satoh, Y. Ogasawara, C. Maruyama, Y. Hamano, T. Ujihara, Control mechanism for carbon-chain length in polyunsaturated fatty-acid synthases, Angew. Chem. Int. Ed. 58 (2019) 6605–6610.
[36] E. Schweizer, J. Hofmann, Microbial type I fatty acid synthases (FAS): major players in a network of cellular FAS systems, Microbiol. Mol. Biol. Rev. 68 (2004) 501–517.
[37] Genbank (WP_061431776.1) Type I polyketide synthase [*Microcystis aeruginosa*], 2020
[38] M. Gugger, Cellular fatty acids as chemotaxonomic markers of the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nostoc* and *Planktothrix* (cyanobacteria), Int. J. Syst. Evol. Microbiol. 52 (2002) 1007–1015.
[39] E. Shelest, N. Heimerl, M. Fichtner, S. Sasso, Multimodular type I polyketide synthases in algae evolve by module duplications and displacement of AT domains in trans, BMC Genomics 16 (2015) 1015.
[40] T. Ujihara, M. Nagano, H. Wada, S. Mitsuhashi, Identification of a novel type of polyunsaturated fatty acid synthase involved in arachidonic acid biosynthesis, FEBS Lett. 588 (2014) 4032–4036.
[41] K. Gemperlein, S. Rachid, R.O. Garcia, S.C. Wenzel, R. Müller, Polyunsaturated fatty acid biosynthesis in myxobacteria: different PUFA synthases and their product diversity, Chem. Sci. 5 (2014) 1733–1741.
[42] D.D. Alexander, P.E. Miller, M.E. Van Elswyk, C.N. Kuratko, L.C. Bylsma, A meta-analysis of randomized controlled trials and prospective cohort studies of eicosapentaenoic and docosahexaenoic long-chain omega-3 fatty acids and coronary heart disease risk, Mayo Clin. Proc. 92 (2017) 15–29.
[43] T. Aung, J. Halsey, D. Kromhout, H.C. Gerstein, R. Marchioli, L. Tavazzi, J.M. Geleijnse, B. Rauch, A. Ness, P. Galan, E.Y. Chew, J. Bosch, R. Collins, S. Lewington, J. Armitage, R. Clarke, Omega-3 Treatment Trialists C, Associations of omega-3 fatty acid supplement use with cardiovascular disease risks: meta-analysis of 10 trials involving 77917 individuals, JAMA Cardiol. 3 (2018) 225–234.
[44] V. Wijendran, M.C. Huang, G.Y. Diau, G. Boehm, P.W. Nathanielsz, J.T. Brenna, Efficacy of dietary arachidonic acid provided as triglyceride or phospholipid as substrates for brain arachidonic acid accretion in baboon neonates, Pediatr. Res. 51 (2002) 265–272.
[45] C.K. Richter, K.J. Bowen, D. Mozaffarian, P.M. Kris-Etherton, A.C. Skulas-Ray, Total long-chain n-3 fatty acid intake and food sources in the United States compared to recommended intakes: NHANES 2003-2008, Lipids 52 (2017) 917–927.
[46] E. Abedi, M.A. Sahari, Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties, Food Sci. Nutr. 2 (2014) 443–463.
[47] P.C. Calder, Very long-chain n-3 fatty acids and human health: fact, fiction and the future, Proc. Nutr. Soc. 77 (2018) 52–72.
[48] G. Furusawa, N.S. Lau, A.C. Shu-Chien, A. Jaya-Ram, A.A. Amirul, Identification of polyunsaturated fatty acid and diterpenoid biosynthesis pathways from draft genome of Aureispira sp. CCB-QB1, Mar. Genomics 19 (2015) 39–44.
[49] S. Hosoya, V. Arunpairojana, C. Suwannachart, A. Kanjana-Opas, A. Yokota, Aureispira marina gen. nov., sp nov., a gliding, arachidonic acid-containing bacterium isolated from the southern coastline of Thailand, Int. J. Syst. Evol. Microbiol. 56 (2006) 2931–2935.
[50] S. Hosoya, V. Arunpairojana, C. Suwannachart, A. Kanjana-Opas, A. Yokota, *Aureispira maritima* sp. nov., isolated from marine barnacle debris, Int. J. Syst. Evol. Microbiol. 57 (2007) 1948–1951.
[51] J.P. Bowman, J.J. Gosink, S.A. McCammon, T.E. Lewis, D.S. Nichols, P.D. Nichols, J.H. Skerratt, J.T. Staley, T.A. McMeekin, *Colwellia demingiae* sp. nov., *Colwellia hornerae*

sp. nov., *Colwellia rossensis* sp. nov. and *Colwellia psychrotropica* sp. nov.: psychrophilic Antarctic species with the ability to synthesize docosahexaenoic acid (22: 6 omega 3), Int. J. Syst. Bacteriol. 48 (1998) 1171–1180.

[52] T. Rezanka, L. Gharwalova, G. Novakova, I. Kolouchova, O. Uhlik, K. Sigler, *Kocuria* bacterial isolates from radioactive springs of Jachymov spa (Joachimsthal) as sources of polyunsaturated fatty acids, Lipids 54 (2019) 177–187.

[53] N. Morita, M. Tanaka, H. Okuyama, Biosynthesis of fatty acids in the docosahexaenoic acid-producing bacterium *Moritella marina* strain MP-I, Biochem. Soc. Trans. 28 (2000) 943–945.

[54] K.B. Kautharapu, J. Rathmacher, L.R. Jarboe, Growth condition optimization for docosahexaenoic acid (DHA) production by *Moritella marina* MP-1, Appl. Microbiol. Biotechnol. 97 (2013) 2859–2866.

[55] K.J. Weissman, R. Müller, Myxobacterial secondary metabolites: bioactivities and modes-of-action, Nat. Prod. Rep. 27 (2010) 1276–1295.

[56] R. Garcia, D. Pistorius, M. Stadler, R. Müller, Fatty acid-related phylogeny of myxobacteria as an approach to discover polyunsaturated omega-3/6 fatty acids, J. Bacteriol. 193 (2011) 1930–1942.

[57] T. Iizuka, Y. Jojima, R. Fudou, A. Hiraishi, J.-W. Ahn, S. Yamanaka, *Plesiocystis pacifica* gen. nov., sp. nov., a marine myxobacterium that contains dihydrogenated menaquinone, isolated from the Pacific coasts of Japan, Int. J. Syst. Evol. Microbiol. 53 (2003) 189–195.

[58] T. Iizuka, Y. Jojima, R. Fudou, M. Tokura, A. Hiraishi, S. Yamanaka, Enhygromyxa salina gen. nov., sp. nov., a slightly halophilic myxobacterium isolated from the coastal areas of Japan, Syst. Appl. Microbiol. 26 (2003) 189–196.

[59] T.F. Schäberle, E. Goralski, E. Neu, Ö. Erol, G. Hölzl, P. Dörmann, G. Bierbaum, G.M. König, Marine myxobacteria as a source of antibiotics-comparison of physiology, polyketide-type genes and antibiotic production of three new isolates of *Enhygromyxa salina*, Mar. Drugs 8 (2010) 2466–2479.

[60] E. Roemer, R. Müller, R.O. Garcia, M. Stadler, D. Pistorius, A. Brachmann, Production of Omega-3 Fatty Acids by Myxobacteria, (2011) WO 2010/063451 A2 Patent.

[61] R.O. Garcia, H. Reichenbach, M.W. Ring, R. Müller, *Phaselicystis flava* gen. nov., sp. nov., an arachidonic acid-containing soil myxobacterium, and the description of *Phaselicystidaceae* fam. nov, Int. J. Syst. Evol. Microbiol. 59 (2009) 1524–1530.

[62] S. Wenzel, S. Rachid, K. Gemperlein, R. Mueller, Production of Fatty Acids by Heterologous Expression of Gene Clusters from Myxobacteria, (2016) EP 2 390 343 A1 patent.

[63] E.E. Allen, D. Facciotti, D.H. Bartlett, Monounsaturated but not polyunsaturated fatty acids are required for growth of the deep-sea bacterium *Photobacterium profundum* SS9 at high pressure and low temperature, Appl. Environ. Microbiol. 65 (1999) 1710–1720.

[64] Y. Nogi, N. Masui, C. Kato, *Photobacterium profundum* sp. nov., a new, moderately barophilic bacterial species isolated from a deep-sea sediment, Extremophiles 2 (1998) 1–7.

[65] H.J. Seo, S.S. Bae, J.H. Lee, S.J. Kim, *Photobacterium frigidiphilum* sp. nov., a psychrophilic, lipolytic bacterium isolated from deep-sea sediments of Edison seamount, Int. J. Syst. Evol. Microbiol. 55 (2005) 1661–1666.

[66] D.S. Nichols, P.D. Nichols, N.J. Russell, N.W. Davies, T.A. McMeekin, Polyunsaturated fatty acids in the psychrophilic bacterium *Shewanella gelidimarina* ACAM 456T: molecular species analysis of major phospholipids and biosynthesis of eicosapentaenoic acid, Biochim. Biophys. Acta 1347 (1997) 164–176.

[67] Y. Nogi, C. Kato, K. Horikoshi, Taxonomic studies of deep-sea barophilic *Shewanella* strains and description of *Shewanella violacea* sp. nov, Arch. Microbiol. 170 (1998) 331–338.

[68] E.P. Ivanova, T. Sawabe, N.M. Gorshkova, V.I. Svetashev, V.V. Mikhailov, D.V. Nicolau, R. Christen, *Shewanella japonica* sp nov, Int. J. Syst. Evol. Microbiol. 51 (2001) 1027–1033.

[69] E.P. Ivanova, O.I. Nedashkovskaya, N.V. Zhukova, D.V. Nicolau, R. Christen, V.V. Mikhailov, *Shewanella waksmanii* sp. nov., isolated from a sipuncula (*Phascolosoma japonicum*), Int. J. Syst. Evol. Microbiol. 53 (2003) 1471–1477.

[70] X. Xiao, P. Wang, X. Zeng, D.H. Bartlett, F. Wang, *Shewanella psychrophila* sp. nov. and *Shewanella piezotolerans* sp. nov., isolated from West Pacific deep-sea sediment, Int. J. Syst. Evol. Microbiol. 57 (2007) 60–65.

[71] K. Venkateswaran, D.P. Moser, M.E. Dollhopf, D.P. Lies, D.A. Saffarini, B.J. MacGregor, D.B. Ringelberg, D.C. White, M. Nishijima, H. Sano, J. Burghardt, E. Stackebrandt, K.H. Nealson, Polyphasic taxonomy of the genus Shewanella and description of Shewanella oneidensis sp. nov, Int. J. Syst. Bacteriol. 49 (1999) 705–724.

[72] J.H. Skerratt, *Shewanella olleyana* sp. nov., a marine species isolated from a temperate estuary which produces high levels of polyunsaturated fatty acids, Int. J. Syst. Evol. Microbiol. 52 (2002) 2101–2106.

[73] J.S. Zhao, D. Manno, C. Leggiadro, D. O'Neil, J. Hawari, *Shewanella halifaxensis* sp. nov., a novel obligately respiratory and denitrifying psychrophile, Int. J. Syst. Evol. Microbiol. 56 (2006) 205–212.

[74] A. Abd Elrazak, A.C. Ward, J. Glassey, Response surface methodology for optimising the culture conditions for eicosapentaenoic acid production by marine bacteria, J. Ind. Microbiol. Biotechnol. 40 (2013) 477–487.

[75] E. Freese, H. Rutters, J. Koster, J. Rullkotter, H. Sass, Gammaproteobacteria as a possible source of eicosapentaenoic acid in anoxic intertidal sediments, Microb. Ecol. 57 (2009) 444–454.

[76] A.L. Moule, S.G. Wilkinson, Polar lipids, fatty-acids, and isoprenoid quinones of *Alteromonas-Putrefaciens* (*Shewanella-Putrefaciens*), Syst. Appl. Microbiol. 9 (1987) 192–198.

[77] K. Yazawa, Production of eicosapentaenoic acid from marine bacteria, Lipids 31 (1996) S297–S300.

[78] J.P. Bowman, S.A. McCammon, D.S. Nichols, J.H. Skerratt, S.M. Rea, P.D. Nichols, T.A. McMeekin, *Shewanella gelidimarina* sp. nov. and *Shewanella frigidimarina* sp. nov., novel Antarctic species with the ability to produce eicosapentaenoic acid (20:5ω3) and grow anaerobically by dissimilatory Fe(III) reduction, Int. J. Syst. Evol. Microbiol. 47 (1997) 1040–1047.

[79] E.P. Ivanova, N.M. Gorshkova, J.P. Bowman, A.M. Lysenko, N.V. Zhukova, A.F. Sergeev, V.V. Mikhailov, D.V. Nicolau, *Shewanella pacifica* sp. nov., a polyunsaturated fatty acid-producing bacterium isolated from sea water, Int. J. Syst. Evol. Microbiol. 54 (2004) 1083–1087.

[80] E.P. Ivanova, O.I. Nedashkovskaya, T. Sawabe, N.V. Zhukova, G.M. Frolova, D.V. Nicolau, V.V. Mikhailov, J.P. Bowman, *Shewanella affinis* sp. nov., isolated from marine invertebrates, Int. J. Syst. Evol. Microbiol. 54 (2004) 1089–1093.

[81] S.J. Lee, Y.S. Jeong, D.U. Kim, J.W. Se, B.K. Hur, Eicosapentaenoic acid (EPA) biosynthetic gene cluster of *Shewanella oneidensis* MR-1: cloning, heterologous expression, and effects of temperature and glucose on the production of EPA in *Escherichia coli*, Biotechnol. Bioprocess Eng. 11 (2006) 510–515.

[82] S. Sato, T. Kurihara, J. Kawamoto, M. Hosokawa, S.B. Sato, N. Esaki, Cold adaptation of eicosapentaenoic acid-less mutant of *Shewanella livingstonensis* Ac10 involving uptake and remodeling of synthetic phospholipids containing various polyunsaturated fatty acids, Extremophiles 12 (2008) 753–761.

[83] Y. Nogi, C. Kato, Taxonomic studies of extremely barophilic bacteria isolated from the Mariana trench and description of *Moritella yayanosii* sp. nov., a new barophilic bacterial isolate, Extremophiles 3 (1999) 71–77.

[84] A. Abd Elrazak, A.C. Ward, J. Glassey, Polyunsaturated fatty acid production by marine bacteria, Bioprocess Biosyst. Eng. 36 (2013) 1641–1652.

[85] M. Amiri-Jami, G. LaPointe, M.W. Griffiths, Engineering of EPA/DHA omega-3 fatty acid production by *Lactococcus lactis* subsp. cremoris MG1363, Appl. Microbiol. Biotechnol. 98 (2014) 3071–3080.

[86] A.C. Bianchi, L. Olazabal, A. Torre, L. Loperena, Antarctic microorganisms as source of the omega-3 polyunsaturated fatty acids, World J. Microbiol. Biotechnol. 30 (2014) 1869–1878.

[87] Y. Orikasa, T. Nishida, A. Yamada, R. Yu, K. Watanabe, A. Hase, N. Morita, H. Okuyama, Recombinant production of docosahexaenoic acid in a polyketide biosynthesis mode in *Escherichia coli*, Biotechnol. Lett. 28 (2006) 1841–1847.

[88] Y. Orikasa, A. Yamada, R. Yu, Y. Ito, T. Nishida, I. Yumoto, K. Watanabe, H. Okuyama, Characterization of the eicosapentaenoic acid biosynthesis gene cluster from *Shewanella* sp strain SCRC-2738, Cell. Mol. Biol. 50 (2004) 625–630.

[89] M. Amiri-Jami, M.W. Griffiths, Recombinant production of omega-3 fatty acids in *Escherichia coli* using a gene cluster isolated from *Shewanella baltica* MAC1, J. Appl. Microbiol. 109 (2010) 1897–1905.

[90] H. Takeyama, D. Takeda, K. Yazawa, A. Yamada, T. Matsunaga, Expression of the eicosapentaenoic acid synthesis gene cluster from *Shewanella* sp. in a transgenic marine cyanobacterium, *Synechococcus* sp, Microbiology 143 (1997) 2725–2731.

[91] M.L. Hamilton, J. Warwick, A. Terry, M.J. Allen, J.A. Napier, O. Sayanova, Towards the industrial production of omega-3 long chain polyunsaturated fatty acids from a genetically modified diatom *Phaeodactylum tricornutum*, PLoS One 10 (2015) e0144054.

[92] K. Gemperlein, G. Zipf, H.S. Bernauer, R. Muller, S.C. Wenzel, Metabolic engineering of *Pseudomonas putida* for production of docosahexaenoic acid based on a myxobacterial PUFA synthase, Metab. Eng. 33 (2016) 98–108.

[93] M. Amiri-Jami, H.F. Wang, Y. Kakuda, M.W. Griffiths, Enhancement of polyunsaturated fatty acid production by Tn5 transposon in *Shewanella baltica*, Biotechnol. Lett. 28 (2006) 1187–1192.

[94] Y. Orikasa, Y. Ito, T. Nishida, K. Watanabe, N. Morita, T. Ohwada, I. Yumoto, H. Okuyama, Enhanced heterologous production of eicosapentaenoic acid in *Escherichia coli* cells that co-express eicosapentaenoic acid biosynthesis *pfa* genes and foreign DNA fragments including a high-performance catalase gene, vktA, Biotechnol. Lett. 29 (2007) 803–812.

[95] R. Yu, A. Yamada, K. Watanabe, K. Yazawa, H. Takeyama, T. Matsunaga, R. Kurane, Production of eicosapentaenoic acid by a recombinant marine cyanobacterium, *Synechococcus* sp, Lipids 35 (2000) 1061–1064.

[96] Y. Orikasa, M. Tanaka, S. Sugihara, R. Hori, T. Nishida, A. Ueno, N. Morita, Y. Yano, K. Yamamoto, A. Shibahara, H. Hayashi, Y. Yamada, A. Yamada, R.K. Yu, K. Watanabe, H. Okuyama, *pfa*B products determine the molecular species produced in bacterial polyunsaturated fatty acid biosynthesis, FEMS Microbiol. Lett. 295 (2009) 170–176.

[97] Y.F. Peng, W.C. Chen, K. Xiao, L. Xu, L. Wang, X. Wan, DHA production in *Escherichia coli* by expressing reconstituted key genes of polyketide synthase pathway from marine bacteria, PLoS One 11 (2016) e0162861.

[98] M. Amiri-Jami, A.G. Abdelhamid, M. Hazaa, Y. Kakuda, M.W. Griffths, Recombinant production of omega-3 fatty acids by probiotic *Escherichia coli* Nissle 1917, FEMS Microbiol. Lett. 362 (2015) fnv166.

[99] E. Ringø, J.P. Jøstensen, R.E. Olsen, Production of eicosapentaenoic acid by freshwater *Vibrio*, Lipids 27 (1992) 564–566.
[100] M. Bhakoo, R.A. Herbert, Effects of temperature on the fatty-acid and phospholipid-composition of 4 obligately psychrophilic *Vibrio* Spp, Arch. Microbiol. 121 (1979) 121–127.
[101] E.F. Delong, A.A. Yayanos, Biochemical function and ecological significance of novel bacterial lipids in deep-sea prokaryotes, Appl. Environ. Microbiol. 51 (1986) 730–737.
[102] T. Hamamoto, N. Takata, T. Kudo, K. Horikoshi, Characteristic presence of polyunsaturated fatty-acids in marine psychrophilic *Vibrios*, FEMS Microbiol. Lett. 129 (1995) 51–56.
[103] A. Abd El Razak, A.C. Ward, J. Glassey, Screening of marine bacterial producers of polyunsaturated fatty acids and optimisation of production, Microb. Ecol. 67 (2014) 454–464.
[104] M.N. Allemann, E.E. Allen, Characterization and application of marine microbial omega-3 polyunsaturated fatty acid synthesis, Methods Enzymol. 605 (2018) 3–32.
[105] Y.N. Liang, N. Sarkany, Y. Cui, J. Yesuf, J. Trushenski, J.W. Blackburn, Use of sweet sorghum juice for lipid production by *Schizochytrium limacinum* SR21, Bioresour. Technol. 101 (2010) 3623–3627.
[106] T. Thyagarajan, M. Puri, J. Vongsvivut, C.J. Barrow, Evaluation of bread crumbs as a potential carbon source for the growth of *Thraustochytrid* species for oil and omega-3 production, Nutrients 6 (2014) 2104–2114.
[107] Z. Chi, D. Pyle, Z. Wen, C. Frear, S. Chen, A laboratory study of producing docosahexaenoic acid from biodiesel-waste glycerol by microalgal fermentation, Process Biochem. 42 (2007) 1537–1545.
[108] D. Meesapyodsuk, X. Qiu, Biosynthetic mechanism of very long chain polyunsaturated fatty acids in *Thraustochytrium* sp. 26185, J. Lipid Res. 57 (2016) 1854–1864.
[109] W.R. Barclay, Process for the Heterotrophic Production of Microbial Products With High Concentrations of Omega-3 Highly Unsaturated Fatty Acids, (1992) EP 1 707 061 A3 and US 2006/0094089 A1 patent.
[110] J.G. Metz, C.A. Weaver, W.R. Barclay, J.H. Flatt, PUFA Polyketide Synthase Systems and Uses Thereof, (2007) US 7.256,023 B2 patent.
[111] J. Diao, X. Song, X. Zhang, L. Chen, W. Zhang, Genetic engineering of *Crypthecodinium cohnii* to increase growth and lipid accumulation, Front. Microbiol. 9 (2018) Article 492.
[112] A. Molina-Miras, L. López-Rosales, A. Sánchez-Mirón, M.C. Cerón-García, S. Seoane-Parra, F. García-Camacho, E. Molina-Grima, Long-term culture of the marine dinoflagellate microalga *Amphidinium carterae* in an indoor LED-lighted raceway photobioreactor: production of carotenoids and fatty acids, Bioresour. Technol. 265 (2018) 257–267.
[113] B. Leyland, S. Leu, S. Boussiba, Are *Thraustochytrids* algae? Fungal Biol. 121 (2017) 835–840.
[114] S. Sasso, G. Pohnert, M. Lohr, M. Mittag, C. Hertweck, Microalgae in the postgenomic era: a blooming reservoir for new natural products, FEMS Microbiol. Rev. 36 (2012) 761–785.
[115] O. Sayanova, R.P. Haslam, M.V. Caleron, N. Ruiz-Lopez, C. Worthy, P. Rooks, M.J. Allen, J.A. Napier, Identification and functional characterisation of genes encoding the omega-3 polyunsaturated fatty acid biosynthetic pathway from the coccolithophore *Emiliania huxleyi*, Phytochemistry 72 (2011) 594–600.
[116] G. Scheuerbrandt, K. Bloch, Unsaturated fatty acids in microorganisms, J. Biol. Chem. 237 (1962) 2064–2068.
[117] J. Erwin, K. Bloch, Biosynthesis of unsaturated fatty acids in microorganisms, Science 143 (1964) 1006–1012.
[118] H. Goldfine, Comparative aspects of bacterial lipids, Adv. Microb. Physiol. 8 (1972) 1–58.

[119] N.J. Russell, D.S. Nichols, Polyunsaturated fatty acids in marine bacteria—a dogma rewritten, Microbiology 145 (1999) 767–779.

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

[121] D.A. Martins, L. Custodio, L. Barreira, H. Pereira, R. Ben-Hamadou, J. Varela, K.M. Abu-Salah, Alternative sources of n-3 long-chain polyunsaturated fatty acids in marine microalgae, Mar. Drugs 11 (2013) 2259–2281.

This page intentionally left blank

Index

Note: Page numbers followed by "*f*" indicate figures and "*t*" indicate tables.

B

C

D

E

F

G

H

I

J

K

L

M

Q

R

S

T

U

V

W

X

Z

This page intentionally left blank

ELSEVIER elsevier.com/books-and-journals

ISBN 978-0-12-819483-6