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Chaitanya G. Joshi *Editors*

Marine Niche: Applications in Pharmaceutical Sciences

Translational Research

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Preface

The ocean embodies a rich bio resource comprising of versatile organisms owing to its diversified environment existing across the different zones. Bioprospecting in terms of biological compounds and diversity of the marine niche has been carried out since ages, and use of fish oil for medication is one of the commonest example. Several marine-derived products from higher animals to microbes have been used as a source of medicine. This subsequently has been increasing the research interests of several biotechnologists and pharmacists towards marine pharmacology studies. Focus has been in the search for genes, natural molecules/ compounds and whole organisms with active pharmacological properties associated with marine flora and fauna. The discovered molecule or organism in its raw/modified form should lead to a product for health improvement in terms of pharmaceutical, cosmeceutical, nutraceutical and other relevant applications. Till date, lakhs of marine species have been discovered, and from them, several thousands of compounds have been extracted and used in medicine.

Nowadays, development of computational algorithms is also allowing advance prediction of drug-like properties and is thus helping in reducing the preliminary screening procedure. Also, modern research is more focused towards marine microbiome exploration for potential pharmaceutical microbial candidates. These are also a potent source of secondary metabolites, and hence can be explored easily without hindering the regular pathways needed for microbial growth and multiplication. More than 50% of drugs in the market are classified as those derived from natural sources. These include huge number of drugs active against microbial infections in humans. Current statistics reveal that the natural drug discovery rate is more than 1000 compounds per year and is increasing every year. OECD (Organization for Economic Cooperation and Development) has been describing the potential of Blue economy in terms of offering service to biotechnology sectors such as pharmaceutical, nutraceutical and cosmetics. In 2019, OECD published a report on the emphasis of the ocean economy and the growing importance of science and technology in the sustainable development of seas and ocean. The report also emphasized the need for improving health of the marine niche along with the increasing use of marine resources to meet global challenges related to food and medicines.

Thus, potential pharmaceutical products and processes from the marine niche include novel medicines, drugs, nutritional supplements, health supplements, biomaterials for medical delivery and diagnosis, adjuvants, and enhancers for efficiency improvement. Environmental changes are also altering the disease patterns, initiating demand for novel treatment molecules and strategies for our enormous global population. In such context, this book is a resource describing drugs developed from the marine niche and the scope of marine flora, fauna and microbes as potential novel drug candidates. We hope this book will generate interest in young minds and make them aware about the avenues for translational research on marine-derived pharmaceutical products.

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Contents

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Neelam M Nathani, Ph.D. is a Post-Doctoral Researcher and visiting faculty at the Department of Life Sciences, Maharaja Krishnakumarsinhji Bhavnagar University. She received the CHARUSAT Gold Medal, presented to her by the former President of India, Dr. A. P. J. Abdul Kalam. She completed her Ph.D. in Microbiology at Saurashtra University in 2016 and has received fellowships including the Department of Science & Technology INSPIRE fellowship, SERB-DST National Post-Doctoral Fellowship and UGC DS Kothari Fellowship.

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Indra R. Gadhvi Dean of the Faculty of Science and Head of the Department of Marine Science, Maharaja Krishnakumarsinhji Bhavnagar University, is a prominent naturalist and ornithologist in Gujarat, and has trained thousands of students in the field of wildlife and motivated them to practice nature conservation. He has also been associated with grassland conservation activities and wildlife research in the region including Blackbuck National Park, Velavadar, Gujarat, India.

Bharat Maitreya Professor of Botany at Gujarat University, completed his undergraduate and graduate degrees at Gujarat University with a specialization in Botany. He has 23 years of teaching experience and is actively engaged in exploring aquatic flora in the region. He has also worked as a Nodal Officer for the All India Survey of Higher Education.

Chaitanya G. Joshi is currently serving as Director, Gujarat Biotechnology Research Centre (GBRC) and formerly Head of Department of Animal Biotechnology, College of Veterinary Sciences & A.H., Anand Agricultural University. He has been involved in extensive research using advanced molecular techniques like next-generation omics for more than a decade and actively engaged in inculcating research aptitude and zeal in the young minds by means of several successful training programs. He has published more than 200 research papers and 2 books, and has contributed significantly to global public scientific databases on molecular biology.

Emerging Trends of Biotechnology in Marine Bioprospecting: A New Vision

1

Ankita Jain and Varsha Tailor

Abstract

The ocean, which is called the "mother of origin of life," is also the source of structurally unique natural products that are mainly accumulated in living organisms. Several of these compounds show pharmacological activities and are helpful for the invention and discovery of bioactive compounds. Marine biotechnology is the science in which marine organisms are used in full or partially to make or modify products, to improve plants or animals, or to develop microorganisms for specific uses. With the help of different molecular and biotechnological techniques, humans have been able to elucidate many biological methods applicable to both aquatic and terrestrial organisms. Marine biotechnology is an innovative field of research in science and technology concerning the support of living organisms with marine products and tools. To understand the *omics* of the living species, it is a novel way to produce genetically modified food, drugs, and energy to overcome global demand. The exploitation of biotechnology for drug discovery, including enzymes, antibiotics, and biopolymers, and chemical compounds from marine sources is deliberated in this Chapter. In addition, well-known and broadly used analytical techniques are derived from marine molecules or enzymes, including green fluorescence protein gene tagging methods and heat-resistant polymerases used in the polymerase chain reaction. Advances in bacterial identification, metabolic profiling, and physical handling of cells are being revolutionized by techniques such as mass spectrometric analysis of bacterial proteins. Advances in instrumentation and a combination of these physical advances with progress in proteomics and bioinformatics are accelerating our ability to harness biology for commercial gain. The objective of this review is to highlight some of the recent developments and findings in the

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area of marine biotechnology with special reference to the biomedical potential of marine natural organisms.

Keywords

Bioprospection · Marine microbes · Omics · Gene targets

1.1 Introduction

As the world population expands and ages, we need to prepare to face new challenges if we hope to live longer and healthier lives while conserving our planet and its natural resources for future generations. Health care is one of the major concerns linked to increased life expectancy and the recent shift in lifestyles. On the one hand, the increase in genetic and lifestyle-related diseases such as cardiovascular disease, ischemic stroke, diabetes, chronic respiratory disease, and some types of cancers is on the rise (Yach et al. 2004), and on the other hand, despite remarkable progress, infectious diseases persist as being the leading causes of mortality in the world. Indeed, even with a supposedly large arsenal of therapeutic methods, infections continue to be a major health concern all over the world including developed countries (Shah et al. 2007). The discovery of new drugs has not been following the curve dictated by the emergence of drug-resistant bacteria: since the golden era of antibiotics in the 1940s, the discovery of novel antibacterial agents has been slowing down, and today the R&D pipeline for such molecules has practically run dry (Spellberg et al. 2013). Unless the rise in antibiotic resistance can be reversed, we can expect to see a substantial rise in incurable infection and fatality in all parts of the world (Guilbert 2003; Wellington et al. 2013). When it comes to lifestyle diseases, although our therapeutic arsenal is very limited, some simple adjustments to our nutrition can considerably reduce the risk of such conditions (Willett et al. 2006), but feeding over seven billion people with healthy and nutritious food is no easy task. In addition to having more and more people to feed, current climate changes are causing reduced yields of some of our most common crops on a global scale (Peng et al. 2004; Lobell et al. 2011), which may have particularly dire effects in developing regions of the world. In fact, it is not just the crops but entire ecosystems that may be remodeled as we continue to burn fossil fuel and release carbon in the atmosphere at a rate one million times faster than it takes the planet to sequester it (Falkowski 2009). The issues of health, nutrition, and energy are all becoming increasingly substantial, but are also strongly intertwined: we must bolster our therapeutic options in spite of the constant race against drug-resistant strains, find new and better ways to feed an increasing population while keeping a healthy diet, and reduce our carbon emissions drastically despite our heavy reliance on oil. Addressing one issue without exacerbating another has proven to be quite a challenge in the past decade. One example is that the 250-fold increase in nitrogen production over the past century (principally driven by its use as fertilizer in agriculture) compares with only a 3-fold increase in the human population over

the same time period. However, the excess nitrogen fertilizer in soil and run-off water feeds certain bacteria which release nitrous oxide into the atmosphere, the third most potent human contributor to global warming today (Cushman 2012).

We believe that novel and less disruptive solutions could be found through bioprospecting the oceans, in particular drawing on the arsenals of molecules, enzymes, and genes found in largely unexplored groups of microscopic marine organisms. Recent advances in DNA technologies and heightened awareness of environmental issues, such as global warming, have come together to potentiate the science of marine microbiology (Bowler et al. 2009). Many international research initiatives have emerged in the past few decades, such as the US-EU marine genomics taskforce on biotechnology research, the Tara-Oceans and Malaspina expedition, the MicroB3 Project, the Global Ocean Sampling expedition, Ocean Sampling Day, and the PharmaSea project. Furthermore, our improved understanding of microbial communities, coupled with new technologies for sampling the ocean and for bioactive screening, has led to the identification of a range of molecules, genes, and strains of interest.

In this chapter, we will discuss how bioprospecting of these organisms can provide us with tools to address key issues facing humanity in the form of novel bioactive molecules and novel high-producing strains and describe screening methodologies that have brought such molecules and/or strains to light.

1.2 What Is Marine Bioprospecting?

Biodiversity prospecting or bioprospecting is the systematic search for biochemical and genetic information in nature in order to develop commercially valuable products for pharmaceutical, agricultural, cosmetic, and other applications.

Marine bioprospecting may be defined as the search for bioactive molecules and compounds from marine sources having new, unique properties and the potential for commercial applications. In other words, it can be described as targeted and systematic search for components, bioactive compounds, or genes within marine organisms (Feist 2008). Among others, applications include medicines, food and feed, textiles, cosmetics, and the process industry. This may include all kinds of organisms; microorganisms like bacteria, fungi, and viruses; and larger organisms such as sea plants, shellfish, and fish. The marine organisms may come from the sea, the coast, the fjord, and the seabed or oil reservoirs beneath the seabed. The result of the bioprospecting could be a purified molecule that is produced biologically or synthetically or the entire organism. Bioprospecting can also be carried out on land and in freshwater organisms. The purpose of marine bioprospecting, from a business perspective, is to find components, compounds, or genes that may be included as components in products or processes. Marine bioprospecting, therefore, may procure different compounds that may be used in many different industries.

Marine bioprospecting is a relatively new endeavor, having its origins in the late 1940s, when Werner Bergman “discovered” arabinoside sugar in marine sponges, a substance which does not occur on land (Munro et al. 1999; Newman and Cragg

2004). This discovery led directly to the development of several anti-viral (ara-A) and anti-cancer (ara-C) compounds (Narsinh 2004). Marine bioprospecting gained momentum in earnest during the 1970s and 1980s due to improved deep sea.

Until recently, the oceans and seas were misjudged as being poor habitats for flourishing biodiversity, due to the voluminous salt content and seemingly infertile environment (Zhang 2005; Simmons et al. 2005). However, advances in technology have made marine exploration possible, and it has become apparent that the oceans are thriving with the greatest diversity of life forms on earth. Life began in the oceans, and therefore it is not surprising that the 2.7 billion years head start on evolutionary process has resulted in the oceans having a more unique and diverse life form than on land (Centre for Marine Biodiversity 2008). Of all of the earth's life forms, 36 out of 38 animal phyla are represented in the marine environment. Marine plants, such as seaweed, phytoplankton, and macroorganisms, are also incredibly diverse, with algae alone having over 100,000 species (UN Atlas of the Ocean n.d.). Novel and undiscovered till today the marine biodiversity is concentrated in coral and temperate reefs, seamounts, hydrothermal vents, abyssal slopes, and plains (Fenical 1983). This marine genetic wealth has resulted in the ocean being aptly described as "blue gold." To date, approximately 300,000 marine species have been documented (Haefner 2003), constituting only a minute fraction of the ocean species. It is anticipated that more than two million species will be discovered in the future (Ruth 2006), as a result of the scientific community's appetite for researching undocumented marine biodiversity and the private sector's relentless search for novel sources for the development of new products.

Bioprospecting can involve the collection of organisms and subsequent screening for a specific molecule or activity of interest. An alternative to prospecting directly for bioactives is to search for DNA sequences encoding activities of interest, either from single organisms or by mining metagenomic sequencing data derived from whole plankton communities collected from the water column (Synnes 2007). Such approaches can help bypass a number of steps required in molecule screening.

1.2.1 Omics-Related Definitions

Systems Biology
Biological research focusing on the systematic study of complex interactions in biological systems using integration models. The ultimate aim is to understand whole systems, e.g., complex cellular pathways, by studying the effect of altered external factors on the genome, transcriptome, proteome, and metabolome simultaneously

Genomics
The study of the structure, function, and expression of all the genes in an organism

Cognitive genomics: Examines the changes in cognitive processes associated with genetic profiles

Comparative genomics: Study of the relationship of genome structure and function across different biological species or strains

Functional genomics: Includes gene and protein functions and interactions (often uses transcriptomics)

Metagenomics: Study of metagenomes, i.e., genetic material recovered directly from environmental samples

Neurogenomics: Study of genetic influences on the development and function of the nervous system

Personal genomics: Branch of genomics concerned with the sequencing and analysis of the genome of an individual. Helps in personalized medicine

Genome
The total DNA of a cell or organism

Polymorphism
Variations in DNA at a specific site

Transcriptomics
The study of the mRNA within a cell or organism

Transcriptome
The total mRNA in a cell or organism

Proteomics
The large-scale study of proteins, including their structure and function, within a cell/system/organism. A name coined as an analogy with the genome

Proteomics: Large-scale study of proteins, particularly their structures and functions. Mass spectrometry techniques are used

Immunoproteomics: Study of large sets of proteins (proteomics) involved in the immune response

Proteogenomics: An emerging field of biological research at the intersection of proteomics and genomics. Proteomics data used for gene annotations

Structural genomics: Study of three-dimensional structure of every protein encoded by a given genome using a combination of experimental and modeling approaches

Epigenomics
The epigenome is the supporting structure of genome, including protein and RNA binders, alternative DNA structures, and chemical modifications on DNA

Epigenomics: Modern technologies include chromosome conformation by Hi-C, various ChIP-seq and other sequencing methods combined with proteomic fractionations, and sequencing methods that find chemical modification of cytosines, like bisulfite sequencing

Proteome
The set of all expressed proteins in a cell, tissue, or organism

Metabolomics
The study of global metabolite profiles in a system (cell, tissue, or organism) under a given set of conditions

Metabolome
The total quantitative collection of low molecular weight compounds (metabolites) present in a cell or organism that participate in metabolic reactions. It also includes those metabolites taken in from external environments or symbiotic relationships

1.3 Diversity in Marine System

The marine environment is the largest habitat on earth and covers more than 70% of the planet. Marine habitats being the dynamic habitat contain a rich variety of distinctive life forms, the majority of them represented by microorganisms. Much of this is in the deep sea at a depth greater than 1000 meters, representing 75% of the oceans' volume. The oceans include the extremes of temperature (350 °C in hydrothermal vents to −35 °C within Ice Sea), light, and pressure (which increases 1 atm/10mt in depth). Microorganisms actually grow and reproduce at the interface between the ice and the seawater. Salinity is the major environmental determinant of microbial community composition, clearly distinguishing marine habitats from terrestrial ones (Lozupone and Knight 2007). The variation in the hydrostatic pressure and organic matter availability have influence on the community structure of marine ecosystem. The sedimentation of organic material and phytodetritus triggers the production of various enzymes and other compounds which have greater importance in medicine and industry by microbial community. Moreover, marine sediments constitute the most phylogenetically diverse environments on earth, in contrast with soil, which bears high species-level diversity but has below-average phylogenetic diversity (Lozupone and Knight 2007). Marine microorganisms are progressively recognized as a promising source of biotechnologically valuable products and capabilities. Over the last years, many biomolecules with unique structural features and unique molecular mode of action have been identified in marine environments (Zotchev 2012).

However, many marine microbial habitats still remain largely unexplored, understudied, and underexploited in comparison with terrestrial ecosystems and organisms. Through billions of years of evolution, marine microorganisms have developed unique metabolic and physiological capabilities to thrive in a variety of marine habitats. In fact, oceans include the greatest extremes of temperature, light, and pressure encountered by life (Munn 2004). In recent years, marine microorganisms living under extreme conditions have been the focus of bioprospecting efforts as novel sources of biomolecules with biotechnological potential (Ferrer et al. 2007; Pettit 2011). For example, hydrothermal vents comprise microorganisms with distinct metabolisms based on chemosynthesis. The high diversity and abundance of these communities are comparable to those found in shallow tropical seas, and thus they are recognized as potentially rich sources of biologically active natural products (Thornburg et al. 2010; Trincone 2011). Piezophilic microorganisms inhabiting deep sea habitats are also of interest, as they can provide enzymes for high-pressure bioreactors, among other applications (Egan et al. 2008). Interestingly, these microorganisms can be either psychrophilic or thermophilic due to the cold temperatures of the deep ocean or to their proximity to hydrothermal vents, respectively.

The oceans contain various different habitats that are suitable for bioprospection. Microorganisms with biotechnological potential are present in pelagic and benthic habitats and also can have symbiotic or epibiotic lifestyle. Competition and defence strategies characteristic of surface-associated microorganisms, such as the production of toxins, signaling molecules, and other secondary metabolites, constitute an unparalleled reservoir from a biotechnological perspective (Penesyan et al. 2010; Schmidt 2005). Bacteria living in symbiotic associations with marine invertebrates often produce complex metabolites as a consequence of coevolution with their host (Wijffels 2008). Sponges and corals are examples of habitats where symbiotic microorganisms with interesting capabilities have been found (Imhoff et al. 2011). In many cases, microorganisms have been found to be the producers of metabolites previously assigned to their hosts (McKew et al. 2011). Microorganisms from intertidal zones must be able to tolerate rapid and repeated fluctuations in environmental conditions. These include temperature, light, and salinity, as well as wave action, ultraviolet radiation, and periods of drought (Ortega-Morales et al. 2010). Intertidal microbial communities preferentially grow as biofilms on natural and artificial surfaces. Within these protective microenvironments, they are subjected to intense biological and chemical interactions, leading to the production of various interesting secondary metabolites (de Nys and Steinberg 2002). For example, in response to intense solar radiation, cyanobacteria and other microorganisms inhabiting intertidal or supratidal zones produce UV-absorbing/screening compounds, which present potential for the development of novel UV blockers for human use (Lam 2006). There are certain phylogenetic groups which constitute interesting targets for bioprospection. Actinomycetes (within the phylum *Actinobacteria*) are widely known for their capabilities of producing metabolites,

which include antibiotics, antitumor and immunosuppressive agents, and enzymes, among others. Novel compounds with biological activities have already been isolated from marine actinomycetes.

Many marine microorganisms are able to produce novel metabolites with biotechnological potential which are not often present in microbes from terrestrial origin. It is important to notice, however, that the recovery of a microorganism from the ocean does not necessarily imply that it is truly "marine," as some organisms may be wash-in components from the terrestrial environment.

Indeed, halotolerant species are frequently isolated from marine sources, especially in coastal environments where terrestrial input is significant. The potential contribution of marine organisms to the discovery of new bioactive molecules is increasingly challenging (Skulberg 2000; Sponga et al. 1999). Now marine microorganisms have become a significant attraction as natural source of bioactive molecules which has a broad range of biological activities, viz., antibiotics, antivirals, antitumorals, antioxidant, and antiinflammatory (Okami 1982; Kamei et al. 1987; Nunez et al. 2006; Uzair et al. 2009; Shankar et al. 2010). The biochemical and physiological properties of various marine microorganisms are mostly revealed now. The physical and chemical properties of sea water have great influence on the organisms and their biological activities. There are various types of bacteria, actinomycetes, fungi, and viruses in marine ecosystem which are capable of producing various enzymes and antibiotics. In marine environments, about 90% bacteria are Gram negative with different characteristics (Zobell 1946). This type of cell wall is better adapted to survive in the marine environment. The three domains of life on earth based on 16S rRNA sequencing are Archaea, Bacteria, and Eukarya (Woese et al. 1990). These domains together play a significant role in the marine environment. The heterotrophic bacterial action releases dissolved organic and inorganic substances by means of organic degradation, decomposition, and mineralization processes in sediments and in the water column (Purushothaman 1998; Sabu 2003) suggests that marine microorganisms which are salt tolerant, can be used for therapeutic applications to humans as they have wide range of enzymatic activities and are capable of producing enzymes that are safer with less harmful effects. So that can be used for therapeutic applications to humans. The study of marine microbial diversity is of greater importance since they are the sources of many biocatalysts, antibiotics, and other useful compounds. The culture methods should include innovative approaches by understanding the cell-to-cell communication and other necessities. Organisms associated with marine environments have greater potential to produce hydrolyzing enzymes such as amylase, lipase, protease, chitinase, etc., and hence the bioprospecting for these compounds is of greater importance.

1.3.1 Enzymes from Marine Habitat

The metabolic functions of marine enzymes are dependent on the ecological features of their habitat. Conventional enzymes are completely denatured under the harsh conditions in which thermophilic enzymes can operate. The synthesis and production of polyhydroxyalkanoates by halophiles is one of the current biotechnological topics related to marine microbes (Quillaguaman et al. 2010). The salt and organic tolerance is very often observed for halophilic enzymes because of reducing water activity (Marhuenda-Edgea and Bonate 2002). Enhancement of thermostability in marine microbes can be observed (Demirjian et al. 2001). Thermostability is higher in marine microbes than in freshwater species. The osmoregulation is based on the synthesis and/or accumulation of compatible solutes without interfering the activity of enzymes. In marine animals D-alanine is involved in the response of osmotic stress in several marine animals (Uo et al. 2001). Enzymes derived from extremophilic archaea have higher stability toward heat, pressure, and solvents and are more resistant to proteolytic attack (Egorova and Antranikian 2005). Cellulose is an odorless hydrophilic compound which is most common on earth. Cellulose is derived from D-glucose units, which is condensed through β-(1→4)-glycosidic bonds (Yakubu et al. 2011). It is the structural component of the primary cell wall of green plants, many forms of algae, and oomycetes. Cellulase has the property of recalcitrance to a smaller extent as there are not so many organisms which can digest cellulose. Hence the organisms which produce cellulase enzyme are of particular importance. The cellulolytic bacteria have so many industrial applications and can be used for the treatment of agricultural wastes in process of bioremediation (Raja Brindha et al. 2011). To release glucose from cellulose, cellulolytic organisms generally secrete a variety of endo-acting β-1, 4-glucanases that preferentially attack the amorphous portion of cellulose and randomly cut the glucan polymers, creating free ends of the cellulose polymers (Lynd et al. 2002). β-Glucosidase converts the potentially inhibitory cellobiose and higher cellodextrins to glucose (Zhang and Lynd 2004); most commercially available cellulases are primarily designed to degrade cellulose. Endoglucanases, cellobiose hydrolases, and β-glucosidases are cellulases which act synergistically to hydrolyze cellulose (Percival Zhang et al. 2006). *Saccharophagus degradans* 2-40 is a simple Gram-negative versatile saprophile seen in marine habitat that can use a variety of polysaccharides that can use a variety of polysaccharides as a major carbon and energy source for growth. The enzymes of this bacterium have the potential to solve several of the major problems in the cellulosic biofuel and green chemical industries (Suvorov et al. 2011). Munoz et al. (2008) reported that this bacterium can grow in monoculture using whole plant material as newsprint and corn stoves as the major carbon source to degrade and mineralize these materials. According to Odisi et al. (2012), organisms associated with deep sediments have greater potential for production of cellulase and lipase than those found in the water column. Some microbes in deep sea sediments have the potential to produce both cellulase and lipase at the same time.

1.3.2 Marine Actinomycetes

Actinomycetes are a separate group of Gram-positive bacteria with well-developed morphological and cultural characteristics. Marine actinomycetes are widely distributed in different marine environments and habitats. Actinomycetes have been detected in unique marine environments, such as in marine organic aggregates and deep sea gas hydrate reservoirs, where they were found to be major components of the microbial communities. They are well known for their capacity to produce bioactive secondary metabolites and enzymes. Several studies have been done to isolate actinomycetes from marine environments, and several genera have been reported (Barcina et al. 1987). From the marine habitat actinomycetes, *Actinopolyspora*, *Micromonospora*, *Micropolyspora*, *Nocardia*, *Rhodococcus*, *Streptomycetes*, *Streptosporangium*, and *Streptoverticillium* have been reported so far. They are capable of producing so many bioactive compounds including different types of antibiotics, and these antibiotics have unique features compared to terrestrial ones (Kokare et al. 2004). Marine actinomycetes have different physiological, biochemical, and molecular characteristics than terrestrial actinomycetes having potential to produce a variety of biologically active enzymes (Jenson and Lauro 2008). Actinomycetes are one of the known cellulase-producing microorganisms. α-Amylases, the starch-degrading amylolytic enzymes, are of great significance in biotechnological applications ranging from food, fermentation, textile, and paper industries (Pandey et al. 2000), as well as in diagnostic settings (Schmid and Verger 1998).

1.3.3 Marine Viruses

Viruses are the dominant component of most of aquatic systems and are more common in marine environment. Virus population biodiversity is totally unexplored (Colwell 1997). Viral growth is abundant in deep sea sediments, and it is controlled by complex interactions with both biotic and abiotic factors including the metabolic state of bacteria and supply of virus from the water column (Danovaro et al. 2002). The viral abundance influences the existence of bacteria and phytoplankton diversity (Giovannoni et al. 1990). Viruses have a key role in the production of dimethyl sulfide which is formed by the hydrolysis of dimethylsulfoniopropionate (DMSP) (Reid and Edwards 2001). Viruses have a great role in carbon budgets also. Viruses influence the composition of marine communities and are a major force behind biogeochemical cycles (Suttle 2007).

1.3.4 Marine Fungi

Hughes classified marine fungi into obligate and facultative forms. Obligate forms grow and sporulate exclusively in the marine habitat (Hughes 1974). The fungi which are native of freshwater or terrestrial habitats and are capable of growing and

sporulating in the marine environment are brought out in the category of facultative. The temperature, availability of substrate or hosts, hydrostatic pressure, and availability of oxygen are important factors controlling the distribution of marine fungi. Marine fungi are important organisms which help in the decomposition and recycling of nutrients by producing suitable enzymes and by meeting the requirements of organic carbon of organisms at higher trophic levels. Marine fungi are found to have antimicrobial properties (Samuel et al. 2011). A diverse array of bioactive compounds with anti-viral, anthelmintic, and anti-tumorigenic activities are found to be produced by marine fungi. Fungal-specific molecular studies in marine environments are so far relatively few, yet many reveal a marine fungal diversity that is significantly higher than other methods suggest but much less diverse than terrestrial environments (Richards Thomas et al. 2012).

These diverse marine environments still remain largely unexplored, understudied, and underexploited in comparison with terrestrial ecosystems and organisms. However, as the success rate in finding previously undescribed active chemicals in marine organisms is 500 times higher than that for terrestrial species, the use of marine biological resources for biotechnological purposes is currently blooming.

1.4 Methods of Bioprospecting in Marine Environments

Both culture-dependent and culture-independent methods have uncovered an incredible diversity of microorganisms whose metabolisms largely have yet to be characterized (Vartoukian et al. 2010; Singh 2010). These methods have been further empowered by genomic-level information, which in turn is supported by sequencing technologies and bioinformatics (Heidelberg et al. 2010; Joint et al. 2010).

1.4.1 Culturing Techniques

Microbial bioprospection and biodiscovery is currently severely limited by the lack of laboratory cultures (Prakash et al. 2013). Although culture-independent approaches have revolutionized environmental microbiology, the development of biotechnological applications from the genetic potential of microbial communities as well as fundamental environmental research must be anchored by the corresponding study of pure cultures. High-throughput dilution-to-extinction culture is one of the most powerful and sensitive approaches for the culture of marine microorganisms such as bacterioplankton.

This technique led to the cultivation of the first member of the widespread but yet uncultured marine SAR11 clade. This method consists in dilution of bacteria up to 1_10 cells per well in microtiter plates, using low-nutrient filtered seawater. High-throughput screening based on fluorescence microscopy clearly improved the technique over conventional methods, allowing rapid and sensitive detection of growing cells. In later studies, this approach was coupled to long-term incubation at low temperatures to allow the recovery of new microbial variants. The diffusion chamber

is a device in which microbial cells are inoculated in an agar matrix separated from the source environment by membranes, isolating the cells but allowing nutrients and growth factors to pass through. The use of this device greatly improved the proportion of culturable bacteria from marine sediments (Kaeberlein et al. 2002).

Another version of this approach is the microbial trap, which selectively enriches for filamentous bacteria (e.g., actinomycetes) by allowing the filament colonization of the sterile agar through membranes with 0:2_m pores (Gavrish et al. 2008). Microdroplet encapsulation in an agarose matrix, combined with growth detection by flow cytometry, led to the recovery of new clades from the marine environment (Zengler et al. 2005). This approach is similar to the diffusion chamber in the sense that the agarose is porous and nutrients and signaling molecules can diffuse into the growing colony and waste metabolites can diffuse out. Another advantage of the approach is that the microdroplets are physically separated and, because they are much larger than bacterial cells, they can be manipulated (Joint et al. 2010).

Currently, second-generation high-throughput automated methods are being developed from these environmental cultivation devices. One example is the development of the isolation chip (Ichip), a culture/isolation device composed of several hundreds of miniature diffusion chambers, each inoculated with a single environmental cell (Nichols et al. 2010).

1.4.2 Culture-Independent Gene-Targeted Methods

In spite of the recent advances in microbial culturing, the majority of environmental microorganisms are still unculturable. Out of the more than 100 bacterial divisions that have been proposed to date, only 30 possess a cultivated representative (Prakash et al. 2013). Moreover, marine microbes are at the top of the list of those unculturable by conventional methods. Culture-independent methods are based on the information provided by biomolecules, mainly deoxyribonucleic acid (DNA), bypassing the need of cultivation by extracting these biomolecules directly from the environmental sample.

Among culture-independent methods, the approach based on the molecular phylogeny of rRNA (ribosomal ribonucleic acid), particularly the small subunit (16S rRNA for archaea and bacteria), continues to be one of the most widely used. This gene has two properties that have positioned it as a building block for a universal molecular phylogenetic framework: its presence in all forms of life and a domain structure with variable evolutionary rates, which enables phylogenetic reconstruction at various levels. Fingerprinting techniques, polymerase chain reaction (PCR) clone libraries, and microscopy-based techniques like fluorescence in situ hybridization (FISH) have been routinely utilized over the last decades to describe and compare the structure and composition of microbial communities (Su et al. 2012). Approaches based on functional genes, which focus on the potential of the community to perform an activity of interest, can give a complementary view to the phylogenetic approach. Genes coding for key enzymes participating in different environmental processes, such as sulfate reduction, denitrification (Braker et al.

2001), nitrogen fixation (Zehr 2011), ammonia oxidation (Marcos et al. 2012), and hydrocarbon biodegradation (Guibert et al. 2012; Hickey et al. 2012) among others, have been studied in the marine environment. Targets include not only bacterial but also archaeal populations and subgroups within these, by means of the use of primers with different specificities. Due to its highly focused nature, this approach is very powerful. However, one of its major drawbacks is the relative lack of database sequence information for functional genes, with respect to the 16S RNA gene. Another shortcoming is the lack of accuracy in taxonomic assignment due to lateral gene transfer (Neufeld et al. 2007).

The most widely used method is DNA-SIP (Stable-Isotope probing), in which DNA is separated in cesium chloride gradients and further purified and analyzed by cloning and sequencing (Dumont and Murrell 2005). The RNA-based SIP approach maintains the sequence-based phylogenetic resolution of DNA-SIP, but focuses directly on the RNA molecule itself rather than its gene, with the advantage of a high copy number and a turnover that is independent of cell replication (Whiteley et al. 2006). Marine environments studied by this method include marine and estuarine sediments (Webster et al. 2006; Freitag et al. 2006; Miyatake et al. 2009) and seawater samples (Neufeld et al. 2008). Biotechnological applications of SIP have mainly addressed issues related to environmental biotechnology (Madsen 2006). SIP depends upon the availability of stable isotopes (13C, 15N, 18O) and of substituted substrate compounds. However, they have the advantage of generating de novo information about the identity of the populations associated with a certain metabolic process.

Microscopy provides information about spatial arrangement and physical interactions of cells, which is applicable to spatially complex environments such as biofilms, consortia, and symbiotic assemblages. The development of fluorescence in situ hybridization (FISH) enabled the detection and identification of single microbial cells in environmental samples by means of rRNA-targeted gene probes (Amann et al. 1995). Microscope-based enumeration of cells makes this method an excellent approach for quantitative estimations, which is more accurate than conventional PCR. Furthermore, the technique is suitable for the use of multiple hierarchical probes in the same sample, which reduces the possibility of false positives. This powerful method has been coupled with the microautoradiography technique (FISH-MAR), which offers the possibility to directly observe the incorporation of substrates labeled with a radioactive isotope into single microbial cells (Wagner et al. 2003). As in SIP, the main limitation of this technique is the availability of radiolabeled substrates, with the additional concern of safety issues. In addition, some environmental samples bearing cells with low ribosome content (e.g., marine oligotrophic environments) can have detection problems with FISH. Horseradish peroxidase (HRP)-labeled oligonucleotide probes and tyramide can be used to enhance the signal intensities of hybridized cells. This approach is sometimes called catalyzed reporter deposition FISH (CARD-FISH (Ishii et al. 2004)), which can also be coupled to microautoradiography, further increasing its potential (Teira et al. 2004). Raman microspectroscopy and nanometer-scale secondary-ion mass spectrometry (nanoSIMS (Behrens et al. 2008; Li et al. 2008)) are other techniques that

are currently under development and may potentially be useful in the future for bioprospecting.

1.5 Omics-Driven Technologies

Omics technologies adopt a holistic view of the molecules that make up a cell, tissue, or organism. The term genome was coined by Hands Winkler (1920). The word "omic" in which the "ome" signifies the "collectivity" of a set of things. They are aimed primarily at the universal detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics), and metabolites (metabolomics) in a specific biological sample in a non-targeted and non-biased manner (Friz 1968). This can also be referred to as high-dimensional biology; the integration of these techniques is called systems biology. Omics technology can be applied not only for the greater understanding of normal physiological processes but also in disease processes where they play a role in screening, diagnosis, and prognosis as well as aiding our understanding of the etiology of diseases (Waaland et al. 2014). Omics strategies lend themselves to biomarker discovery as they investigate multiple molecules simultaneously (Zhiyue et al. 2015).

The aim of this study is to characterize and quantify pools of biological molecules that translate into the structure, function, and dynamics of one or more organisms. This holistic approach to understand complex biological systems in encapsulated "omes" also has important applications in biotechnology.

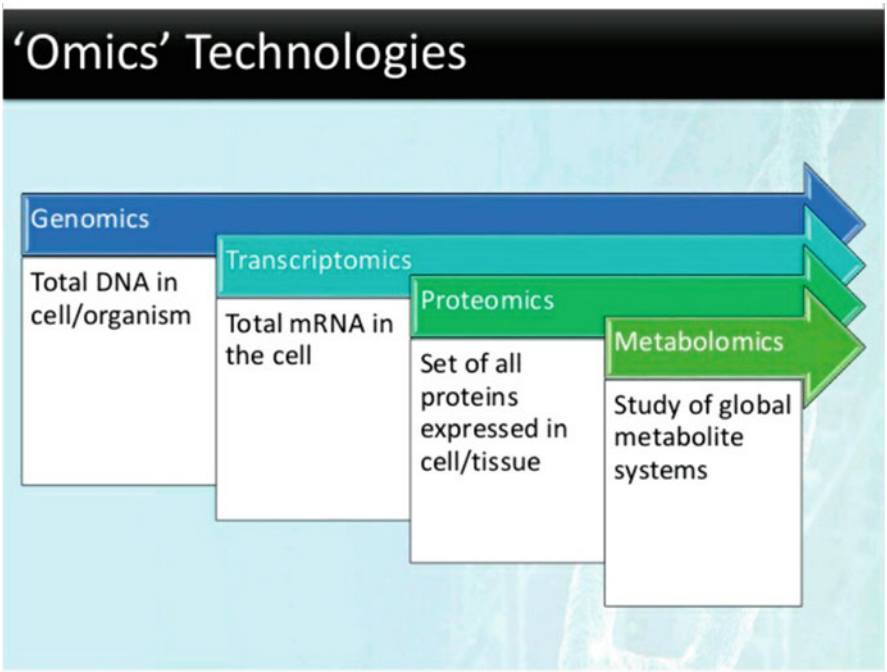
Genomics

Genome is the most fundamental part of many omics. The first complete sequencing of genome was done by Sanger in the 1970s. Genomics is the systematic study of an organism's genome. The genome is the total DNA of a cell or organism. Traditionally, genes have been analyzed individually, but microarray technology has advanced substantially in recent years.

They can reveal abnormalities such as chromosomal insertions and deletions or abnormal chromosomal numbers in a process called comparative genomic hybridization. The most common variations in DNA sequences between people are single nucleotide polymorphisms (SNPs), in which one nucleotide is substituted. Single nucleotide polymorphism profiling also has a role in pharmacogenomics in exploring individual patient responses to drugs.

The transcriptome is the total mRNA in a cell or organism and the template for protein synthesis in a process called translation.

While advances in microarray technology have resulted in progress in genomics and transcriptomics, it is important to highlight some limitations.



1.5.1 Genomics of Marine Organisms

Genomics aims at identifying the functions of as many genes as possible of a complete genome of a given organism. Nowadays about 1000 prokaryotic genomes are completely sequenced and annotated; more than half of them are sequenced in function of medical or industrial relevance. Nowadays over 7500 bacterial species are described.

Viruses are the most common biological entities in the marine environment. Recent metagenomic surveys have shown their unique gene pool and their molecular architecture. Viruses infect a lot of organisms, ranging from archaebacteria to mammals. Until now a huge number of viruses still remain undiscovered or are not sequenced and annotated. Therefore, they are an important untapped resource of potential novel proteins, genetic tools, and unexpected functions.

The study of *eukaryotes* (comprising microalgae, macroalgae, and protozoa) is still in its infancy when it comes to genomics. Compared to prokaryotes, eukaryotes have a much larger genome size and higher cellular complexity, which results in slower progress. Currently only 30 *microalgal* genomes are completed. Many microalgae have chromosomal DNA and mitochondrial DNA just like humans; algae however also possess chloroplast DNA.

Fig. 1.1 *Chaos chaos*, the amoeba with the largest known genome

A few macroalgae genomes have already been sequenced; others are still being completed. For protozoa the case is even more difficult. They have an extremely diverse phylogeny, complex life cycles, and an even larger range of genome sizes than the microalgae.

Chaos chaos, for instance, is a free-living amoeba and has the largest genome ever reported (Wellington et al. 2013). The highly polyploid nature of many protozoans (up to hundreds of small chromosomes) also influences the problems. Yet, most of them of medical relevance (Fig. 1.1).

The study of *metazoan* genomes is mostly focused on the research of mammals because of their medical and economic relevance. Only 11% of the currently planned sequence analyses of metazoan genomes are focused on marine invertebrates. Some mussels and oysters have been sequenced for their importance as aquaculture species. Very few teleost fish genomes have been completed, among them *Takifugu rubripes* and the zebrafish (*Danio rerio*) (Fig. 1.2).

1.5.2 Metagenomics of Marine Communities

A younger science flow is the field of metagenomics concerning the analysis of all the genes of a given community of organisms. In metagenomics studies, all genes in a community are first separated by complete DNA extraction, next they are put in large clone libraries, and afterward, they are available for use in biotechnological applications. Metagenomics allows the discovery of new microscopic life and allows the sampling of a whole community of microorganisms rapidly in order to make an inventory or to understand a certain ecosystem and its diversity.

1.5.2.1 Pharmacogenomics

Pharmacogenomics is the study on how genes affect a person's response to drugs. Pharmacogenomics enables researchers to understand how inherited differences in

Fig. 1.2 *Danio rerio* (Peng et al. 2004)

genes affect the body's response to medications, which can predict whether the drug is effective or ineffective and/or cause side effects for a particular patient.

1.5.2.2 Metagenomics

The term "metagenomics" studies the collection of gene sequences from the environment in a way analogous to the study of a single genome, and a large number of microorganisms exist in the gastrointestinal tract of humans and animals.

The first metagenomics study was conducted on a woolly mammoth (*Mammuthus primigenius*) sample using emulsion polymerase chain reaction and the pyrosequencing technique (Poinar et al. 2006). Metagenomics has been widely applied in the research of obesity, providing an important role of intestinal microbiota for obesity. Metagenomics studies have also demonstrated an imbalanced microbiota composition in various diseases, such as Crohn's disease (Manichanh et al. 2006), necrotizing enterocolitis (Siggers et al. 2008), polyposis or colorectal cancer (Scanlan et al. 2008), and type 2 diabetes (Larsen et al. 2010).

1.5.2.3 Epigenomics

Epigenetics refers to the heritable changes in gene expression without any alteration in DNA sequence. Epigenetic regulation can be complemented by five different mechanisms: DNA methylation (Laird 2010), histone posttranslational modification (Jenuwein and Allis 2001), histone variants (Hake and Allis 2006), RNA interference (Grewal and Elgin 2007), and nuclear organization (Fraser and Bickmore 2007).

1.5.2.4 Transcriptomics

The transcriptome is the set of all RNA molecules, including mRNA, rRNA, tRNA, and other non-coding RNAs. The transcription process of RNA synthesis is the first step of gene expression. Currently, approaches for transcriptome data acquisition and analysis are mainly based on chip technology, including cDNA microarray and oligonucleotide chips (Maskos and Southern 1992), serial analysis of gene expression (SAGE) (Velculescu et al. 1995), and massively parallel signature sequencing

(MPSS) (Brenner et al. 2000). Transcriptomics allows for the discovery of some disease-related gene expression and thus is expected to be applied for clinical diagnosis.

1.5.2.5 Proteomics

Proteome

The proteome is defined as the set of all expressed proteins in a cell, tissue, or organism. Proteomics aims to characterize information flow within the cell and the organism, through protein pathways and networks, with the eventual aim of understanding the functional relevance of proteins. The proteome is a dynamic reflection of both genes and the environment and is thought to hold special promise for biomarker discovery because proteins are most likely to be ubiquitously affected in disease and disease response.

Proteomics is the study of the proteome of a certain type of cell, tissue, or body fluid, particularly their structures and functions, at a large-scale, high-throughput, and systematic level (Anderson and Anderson 1998; Blackstock and Weir 1999). Currently, two-dimensional chromatography with mass spectrometric detection (2DLC-MS) (Bennett et al. 2011), two-dimensional gel electrophoresis-liquid chromatography with mass spectrometric detection (2DE-LC-MS) (Irar et al. 2014), capillary electrophoresis with mass spectrometric detection (CE-MS) (Stalmach et al. 2013), and other chromatographic techniques are increasingly being applied in proteomics. Mass spectrometry (MS) is another essential tool in proteome analysis.

Proteomics provides new ideas for research in the medical and life sciences and has produced remarkable achievements in the past two decades. In the field of cancer research, especially early clinical diagnosis, a series of cancer-related proteins are discovered, such as cathepsin B (Chen et al. 2004), heat shock protein 27 (Ping et al. 2005), mRNA junction protein P62 (Poon et al. 2006), oral squamous cell carcinoma-related protein of HPA/sAa/K-10/GA-HAS (Mu et al. 2014), and pfetin (Kubota et al. 2014). Drug development is the most promising field for the applications of proteomics.

1.5.2.6 Glycoproteomics

Glycoproteomics is a branch of proteomics that identifies, catalogs, and characterizes those proteins containing carbohydrates as a posttranslational modification (Tissot et al. 2009). Glycosylation, which exists in over 50% proteins, is recognized as an important posttranslational modification (Hagglund et al. 2004). Protein glycosylation is involved in a variety of biological processes of cellular immunity, cell adhesion, regulation of protein translation, protein degradation, and so on.

Glycoproteomics is now widely studied for the identification of biomarkers for the diagnosis of cancer and other diseases, such as cancers of the breast (Yen et al. 2014), lung (Ahn et al. 2014), stomach (Bones et al. 2011), and ovary (Wu et al.

2012), liver fibrosis (Ito et al. 2012), and Alzheimer's disease (Butterfield and Owen 2011).

1.5.2.7 Chemoproteomics

Chemoproteomics uses a chemistry-based approach to characterize protein structure and functions. In general, functional small molecules are often used to interfere with certain aspects of the proteome, and target proteins may be detected and isolated due to chemical-protein interactions (Adam et al. 2002). Chemoproteomics is a kind of function-based proteomics and is increasingly applied in several fields of drug target discovery and validation (Terstappen et al. 2007).

1.5.2.8 Metabolomics

The term "metabolome" refers to the complete set of small molecule metabolites to be found within a biological sample, such as a single organism. Metabolomics is defined as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification" (Nicholson 2006). The metabolome represents the physiological or pathological status of organisms. Metabolomics thus can be used in toxicology (Robertson 2005), disease diagnosis (Sabatine et al. 2005; Zhang et al. 2014), molecular pathology (Denkert et al. 2008), and a number of other fields.

1.5.2.9 Pharmacometabolomics

The term "pharmacometabolomics" changes in metabolome for an individual patient caused by drug administration, pharmacometabolomics may describe a detailed mapping of drug effects on certain metabolic pathways implicated in the mechanisms of variation of response to treatment.

Pharmacometabolomics can be used for chemosensitivity prediction in the treatment of cancer (Wang et al. 2010). Besides personalized medicine, pharmacometabolomics is also a powerful tool for research on drug toxicity assessment (Amacher 2010; Klawitter et al. 2010), efficacy evaluation (Ichikawa 2006), and mechanisms of action (Howells et al. 2012).

1.5.2.10 Lipidomics

Lipids are the structural components of cell membranes, serving as energy storage sources and also participating in many important cellular functions. It has been proven clinically that many critical diseases are associated with lipid metabolism disorders, such as Alzheimer's disease, diabetes, and some infectious diseases. Studies have shown that mammalian cells contain 1000 to 2000 kinds of lipids.

Lipidomics is the large-scale study of pathways and networks of cellular lipids in biological systems (Han and Gross 2003; Wenk 2005; Watson 2006). Lipidomics analysis is based on multi-dimensional LC/MS and mainly includes the following steps: lipid extraction, lipid separation, lipid detection, lipid identification, quantification, and data processing.

Lipids are often extracted by acid, alkali, or a neutral solvent with traditional procedures established by Bligh/Dyer and Folch (Bligh and Dyer 1959). The

simplest method of lipid separation is the use of thin layer chromatography (TLC), while it has limited sensitivity; thus solid-phase extraction (SPE) and LC are extensively used. Lipid detection is often by using electrospray ionization or matrix-assisted laser desorption/ionization (MALDI).

Lipidomics has been increasingly studied, especially in the field of discovery of lipid indicators for diagnosis (Wenk 2006), drug targets (Marechal et al. 2011), and pharmacological mechanisms (Adibhatla et al. 2006).

1.5.3 Other Omics Technologies

1.5.3.1 Phenomics

Phenomics is the study concerned with the measurement of phenome as changes occur in response to genetic mutation and environmental influences. Phenomics has come to be used to bridge the genotype and phenotype of the organism. The research on phenomics is mainly performed on a phenotype-microarray platform which enables one to monitor simultaneously the phenotypic reaction of cells to environmental challenges observed on microliter plates.

PhenoChipping is a quantitative method for phenomics analysis. Phenomics has been mainly used to study genotype-phenotype relationships (Bilder et al. 2009), genetic basis of complex traits (Joy and Hegele 2008), and crop improvement (Finkel 2009).

1.5.3.2 Immunomics

Immunomics studies the response and regulation process of the immune system on pathogens, which deals with all immune-related molecules, together with their targets and functions. Immunomics includes the techniques of genomics, proteomics, and bioinformatics. On the basis of genomics and proteomics research, immunomics makes full use of bioinformatics, bio-chip, structural biology, high-throughput screening, and systems biology technologies to study the immune system and immune responses, so as to discover new susceptibility genes and new immune-related molecules.

Immunomics is now mainly applied in vaccine development (Pizza et al. 2000; Bambini and Rappuoli 2009), target identification (He 2012), and disease diagnosis (Bulman et al. 2013).

1.5.3.3 Metallomics

Metal elements play an important role in biology in spite of their low levels. It is estimated that one third of proteins need metal ions (usually a transition metal ion, such as copper, iron, zinc, and molybdenum) as a cofactor to perform their biological functions and are often called “metal proteins.”

Metal elements in metallomics include the biological metals combined with biological macromolecules, such as metal proteins, metal enzymes, metal nucleic acid fragments, metal-containing ligands (organic acids, amino acids, etc.), and metal polysaccharides, and also the free alkali and alkaline earth metal ions.

Metallomics aims to reveal the physiological functions and biological effects of the metallome.

Metallomics is also applied in the field of environmental evaluation (González-Fernández et al. 2009) and in drug discovery (Sun et al. 2009; Yan et al. 2007).

1.5.3.4 Cytomics

Cytomics involves research on the structure and function of cellular systems, subsystems, and their functional components at the single cell level. Cytomics study is often based on genome databases and also uses genomics or proteomics technologies. Sensitive, non-invasive, and fluorescence-based methods are most widely employed in cytomics to conduct the integrated analysis of a single cell.

Currently, the main cytomics technologies include flow cytometry, laser capture microdissection (LCM), confocal laser scanning microscopy (CLSM), laser scanning cytometry (LSC), high-content screening (HCS), and bio-imaging. Cytomics provides strategies and effective approaches to pharmaceutical research, such as target validation (Schooley et al. 2003), drug development (Valet 2006), pharmacological and toxicological evaluation (Jang et al. 2007), and clinical efficacy of predictive and personalized medicine (Shanks et al. 2005).

1.5.3.5 Ionomics

It is generally known that ions play a crucial role in all biological behaviors of an organism, especially in energy metabolism, enzyme activity, intracellular signaling, and transportation. In 2003, Salt and colleagues proposed, for the first time, the concept of ionomics (Danku et al. 2009). Ionomics studies the measurement and biological processes of elementals of an organism to address biological problems.

Ionomics is currently applied in functional genomics (Eide et al. 2005), modern plant nutrition (Ziegler et al. 2013), and other research areas.

1.6 Application of Omics

1.6.1 Pharmaceutical Research

Currently, the production of large omics data sets has become routine, and thus pharmaceutical research has entered into the new era of omics. Now, pharmaceutical research increasingly relies on genomics, transcriptomics, proteomics, and metabolomics and even the combination of multiple omics technologies. In almost every aspect of pharmaceutical research and drug development, including target discovery, efficacy evaluation, safety assessment, mechanism research, personalized medicine, and so on, omics techniques can be used as efficient and powerful tools. Omics research is the most essential part of systems biology and network biology and makes it possible to fully understand the pathological processes of diseases and to reveal the key pathways and possible mechanisms of pharmaceutical research and drug treatment. Moreover, omics studies may highlight the potential targets for drug development, allowing for efficient safety assessment and personal medicine. The

characteristics of the global analysis of omics fulfill the requirements of study on the most complicated research subjects. Now, omics has come to be recognized as a powerful approach for pharmaceutical research, especially in studies of target discovery, personalized medicine, and toxicology.

1.6.1.1 Human Health

Most of our medicines come from natural resources, and scientists are still exploring the organisms of tropical rain forest for potentially valuable medical products. More than 2000 years ago, the extracts of marine organisms had been used as medicine. By the early 1950s, Ross Nigrelli of the Osborn Laboratories of the New York Aquarium (New York Zoological Society) extracted a toxin from cuvierian organs of the Bahamian sea cucumber, *Actinopyga agassizi*. He named this toxin as "holothurin," which showed some antitumor activity in mice (Nigreli et al. 1967). After then, the number of potential compounds isolated from marine realm has virtually soared, and this number now exceeds 10,000 with hundreds of new compounds still being discovered every year (Proksch et al. 2002).

A number of promising identified molecules are already in the market, clinical trials, or preclinical trials (Table 1.1). Interestingly, these precious natural products have been obtained from marine microorganisms as well as invertebrates such as sponges, mollusks, bryozoans, tunicates, etc.

1.6.2 Aquaculture and Fishery

Marine aquaculture is now a very successful example of progress in marine biotechnology. Fish is one of the most important protein supplies of the human nourishment in the world. In the past, aquaculture was traditionally done in fishponds. However, due to recent industrialization of this sector, it now supplies high-quality food in a sustainable way. Biotechnological research to improve aquaculture procedure is focused on species diversification, optimum food and feeding, health of cultured organisms and disease resistance, as well as minimum environmental impact. By using recombinant technology, efforts are underway to develop genetically modified organisms (GMOs) with particularly useful features, such as fast growth, resistance to pathogens, temperature and salinity tolerance, etc. Production of transgenic fish through electroporation has also been successfully carried out since 1980. Furthermore, molecular biological methods have resulted in invention of new feed stocks and vaccines for aquaculture to increase its productivity. Use of marine microorganisms as probiotics in aquaculture is a gift of biotechnological research.

1.6.3 Environmental Biotechnology

Degradation of hazardous material is an important issue worldwide. It has been found that marine microorganisms express novel biodegradation pathways for breaking down a variety of organic pollutants. Several such groups have been

Table 1.1 Examples of marine by-products, which are currently in market or in clinical phases

Product	Source	Application area	Status
Ara-A	Marine sponge	Anti-viral	Market
Ara-C	Marine sponge	Anticancer	Market
Okadaic acid	Dinoflagellate	Molecular probe	Market
Manoalide	Marine sponge	Molecular probe	Market
Vent DNA polymerase	Deep sea hydrothermal vent bacterium	PCR enzyme	Market
Aequorin	Bioluminescent jelly fish, *Aequorea victoria*	Bioluminescent calcium indicator	Market
Green fluorescent protein (GFP)	Bioluminescent jelly fish, *Aequorea victoria*	Reporter gene	Market
Phycoerythrin	Red algae	Conjugated antibodies used in ELISA and flow cytometry	Market
Cephalosporins	Cephalosporins so., marine fungi	Antibiotic	Market
Yondelis	Sea squirt	Cancer	Clinical phase II/III
Ziconotide	Cone snail	Chronic pain	Clinical phase III
Dolastatin	Sea slug	Cancer	Clinical phase II
Bryostatin-1	Bryozone	Cancer	Clinical phase II
Squalamine lactate	Shark	Cancer	Clinical phase III
PL512602 (steroid)	Sponge	Inflammation, asthma	Clinical phase II

described and many others are being explored. Extensive development of such bioremediation processes will be an important area of environmental biotechnology. For example, *Pseudomonas chlororaphis* produces pyoverdin, which catalyzes the degradation of organotin compounds in seawater. Studies were carried out using immobilized cells of the above bacterium in 2% alginate beads, and the results suggested that immobilized cells could be applied to in situ bioremediation of organotin (Inoue et al. 2003). Organic solvent-tolerant bacteria and crude oil-degrading marine cyanobacteria are also reported for their possible implications in environmental bioremediation (Sardessai and Bhosle 2004; Raghukumar et al. 2001).

In addition to this, marine microorganisms frequently produce eco-friendly chemicals, such as biopolymers and biosurfactants that can also be applied in environmental waste management and treatment. Researches are also on track to study the interaction of marine microbes with toxic heavy metals and suggested their use in various biosorption, bioprecipitation, and biocrystallization applications for the treatment of contaminated water systems (Karna et al. 1999; Cohen 2002).

Biosensors are widely used for the assessment of environmental parameters of biological relevance, such as inorganic and organic nutrients, toxic products of marine organisms, and harmful pollutants (Nielsen et al. 2004). Marine microorganisms provide the basis for the development of sophisticated biosensors and diagnostic devices for medicine, aquaculture, and environmental bio-monitoring. Some bioluminescent proteins from marine organisms are currently under study in order to produce gene probes that can be employed to detect human pathogens in food or fish pathogens in aquaculture system (Anonymous, Marine biotechnology 2001).

Biofouling refers to the assemblage of marine organisms on man-made structures and devices submerged in the sea. It causes deterioration and heavy economic penalties to marine industries (Wagh et al. 1997). Several attempts are made to control biofouling with the application of physical, chemical, and biological measures, but results, to the greater extent, are achieved with the use of antifouling paint coatings. Though the life span of these effective coatings is longer, they have toxic proposition, and excessive leaching rate of them causes abnormality in non-target organisms. Environmental concerns about the use of such toxic antifoulants increased the interest in the development of non-toxic alternatives. Efforts in this area have proved that marine natural products could be a good source of eco-friendly antifouling compounds. The sessile marine organisms, which do not allow other organisms to come and settle on their surfaces, may provide key to control biofouling.

Marine organisms synthesize chemicals with bioactive properties, such as metabolites, proteins, enzymes, polysaccharides, and lipids, which have led to new industrial processes. A natural "soap" (biosurfactant), produced by oil-eating marine bacterium, *Acinetobacter*, is a gift of biotechnology (Your world biotechnology and your teacher's guide n.d.). Improved technology, allowing to sample organisms from ocean floor, has explored different group of organisms (extremophiles). These organisms have evolved to live and thrive in extreme conditions. Uniquely adapted enzymes (and other proteins), with extra stable chemical bonds, help these organisms to survive in these conditions. Few such enzymes have led to the breakthrough processes of biotechnology, and some others will surely bring new advances to medicine and industry in the future. For instance, thermostable polymerases, such as "Taq" and "Vent" from aquatic extremophiles, *Thermus aquaticus* and *Pyrococcus furiosus*, are commercially available enzymes used in molecular biology. The best-known commercial success of thermostable enzymes is the Taq DNA polymerase, obtained from *T. aquaticus* (Yellowstone hot spring).

Deep sea hydrothermal vent microorganisms are reported to produce unusual microbial polysaccharides with interesting chemical properties. Among these polymers, poly-β-hydroxyalkanoates (PHAs) are of special interest. In the same range of high molecular weight biopolymers, chitin and chitosan are found to be associated with crustacean shells and fungi. These natural, non-toxic, biodegradable polymers have applications in food and pharma as well as cosmetics. Seaweeds are abundant source of natural polysaccharides, many of which have commercial uses. Algal products, such as agar and agarose, have been used in the laboratory for many

years as nutrient media and gels for electrophoresis. Carrageenan, another algal derivative, is used as a thickener in processed food. Algae are also sources of vitamins, other nutrients, iodine, animal feed additives, fertilizer, and pharmaceuticals. Some other marine natural products include enzymatic hydrolysate, having antioxidant property, from fish, mollusk, or shellfish. Fish oils are sources of polyunsaturated fatty acids (PUFA) and are of interest due to their physiological effects, like prevention of atherosclerosis, and their role in anti-aging and in brain development in premature infants.

1.7 Impact of *Omics* upon Drug Discovery

Pharmacogenomics may benefit many stages of clinical drug development. It will significantly affect trial design, primarily through improved inclusion/exclusion criteria and more effective assessment of patient responses. Genes linked with drug metabolism in preclinical studies could be genotyped in patients recruited for phase I trials. Any genotype that correlates with adverse effects could then be used to screen out relevant patients in subsequent trials. Furthermore, if efficacy data are collected during phase I trials, polymorphisms in the drug target gene could be typed in phase I participants to assess whether they are linked with side effects or with variations in drug response. That analysis could obviously be further refined in phase II trials, enabling companies to undertake phase III trials in a subgroup of patients that responds well and exhibits fewer side effects. The resultant drugs would be expected to have not only better efficacy but also a better safety profile.

While this approach may maximize the medical utility of existing pharmaceuticals, it could also rescue dead drugs. Several products that have failed in recent years in late-stage clinical trials may on retrospective analysis be effective in subsets of patients, although, at the time, there was no clear way of recognizing such subsets clinically. Consequently, traditional approaches that focus on broad groups of patients with a diagnosis (e.g., Alzheimer's disease) may need to be much more precisely divided into subsets of patients who may have a traditionally defined disease amenable to treatment based on a particular molecular target. These pharmacogenomic developments should lead to smaller, more rapid, and cost-effective trials and ultimately to more individually focused and effective therapeutics.

The primary goal of genomics research in the pharmaceutical industry in the 1990s was to identify not only new molecular targets but also more of them and to be the first to gain proprietary rights to use those targets (Ward 2001). Genomics explore new opportunities for the drug discovery, especially through technologies like high-throughput sequencing and characterization of expressed human genes. Knowledge of all the human genes and their functions may allow effective preventive measures.

Genomics may also be used to select out adverse effects before drugs enter the clinic. For example, the gene expression pattern for the liver of an animal administered a drug can indicate whether gene pathways related to toxicity have

been turned on. Variations in gene expression levels may prove just as useful as genetic variation in predicting drug response at any stage in the clinic and as a diagnostic.

Pharmacogenomics benefits many stages of clinical drug development. It will significantly affect trial design, primarily through improved inclusion/exclusion criteria and more effective assessment of patient responses. During preclinical studies, the genes linked with drug metabolism could be genotyped in patients recruited for phase I trials.

Accordingly, "omics" technologies contribute to all stages of drug development, from target identification to target validation. Target identification is based upon molecular information derived from genome sequences and protein structures. Overall, pharmacogenomic approaches offer interesting perspectives for molecular design and development of more specific drugs with significant benefits to patients (Issa 2000). By applying genomics technology, companies can on average realize savings of nearly US$300 million and two years per drug, largely as a result of efficiency gains. Current research activities aim at going beyond the area of human genome sequencing to expand the list of identified proteins and genes. This, ultimately, is expected to help in improved understanding of disease mechanisms and the development of corresponding therapeutics.

1.7.1 Role of Proteomics in Drug Development

Recent advances in applied genomics helped in the target identification process, since it allowed for high-throughput screening of expressed genes. However, studies have shown that there is a poor correlation between the regulation of transcripts and actual protein quantities. The reasons for this are that genome analysis does not account for posttranslational processes such as protein modifications and protein degradation. Therefore, the methods employed in the drug discovery process started to shift from genomics to proteomics.

1.8 Marine Pharmaceutical Biotechnology: Challenges and Opportunities for the Future

Infrastructure Challenge: There is a strong need to continue to build research and innovation capacity in the research and business sectors. This would improve science and technology research infrastructure, providing access to a range of new research support tools and facilities to strengthen marine biotechnology.

Economic Challenge: It is crucial for them to have a clearly defined strategy; otherwise, the risk of failing and running out of cash quickly is high. It is important to know that the cost of technology and manufacturing processes, sometimes with poor yields, increases the cost of the market per kilogram and can make these products economically uncompetitive (Hurst et al. 2016).

Technical Challenge: Access to the ocean and the deepest of its "hot spots" remains very difficult, and new robotic and technical technologies are needed.

Taxonomy: The lack of taxonomic knowledge for marine species, and the still large number of unidentified species and strains, is also a major bottleneck facing marine natural product programs.

Scientific Challenges: Exploration of the potential of marine biodiversity has increased, so it is a rich source of new natural compounds. Some of these compounds are already used in food, cosmetic, agricultural, chemical, and pharmaceutical products, but their diversity has not been fully characterized or used. Other possibilities exist for the use of ocean bio-resources in the markets for industrial enzymes, functional foods, cosmeceuticals, biomaterials, bioprocesses, and medical devices. Since traditional medicinal knowledge associated with marine organisms is almost non-existent, the search for biologically active compounds from marine sources has been done through a random selection of organisms. But initial studies are underway to develop directed selection methods.

Environmental Challenge: The main sources of marine biomass come from species harvested from the wild and those that can be grown. Securing sustainable marine biomass presents challenges, particularly if the only source comes from wild stocks, where over-exploitation can threaten marine biodiversity as well as future supply of target species.

Production Challenge: Consistency, safety, and quality of biomass supply must be balanced to meet environmental challenges and sustainability requirements. The well-managed and well-controlled culture of marine biomass, while facing the challenges of production, provides sustainable sources of biomass.

With the rapid development and application of high-throughput technologies and bioinformatics, omics is increasingly respected by the majority of pharmaceutical researchers. Omics techniques are widely employed in all areas of biological science, agriculture, medicine, and research fields. However, there are still great challenges in omics research, including data acquisition, multi-omics data analysis, and modeling. In the future, in order to fully describe a biological process, the combination of multiple omics techniques will be commonly used and produce a vast and complex data surge on various levels of DNA, RNA, SNP, protein, metabolites, and so on.

Omics data is generally acquired from either experimental results or Internet databases. However, the data is difficult to process due to many factors, such as the diversity of the data type, database redundancy, and lack of uniform data description standards. How to deal with such a mass amount of data, especially multi-omics data from different sources, is the most difficult challenge for omics research. A possible efficient solution for this challenge might be network biology, which may describe biochemical systems as a network based on multi-omics data (Droste et al. 2011). With the multi-omics databases continually expanding, some novel technologies on data analysis and processing are recently developed; for example, grid and cloud computing are applied for database services (Wolstencroft et al. 2013) and biomarker discovery (McIntyre et al. 2014), and Consensus Principal Component Analysis (CPCA) is proposed for multi-omics modeling (Hassani et al. 2010). How to conduct dynamic analysis is another challenge of omics research. Many

researchers have come to realize that the research objects of omics, including the genome, proteome, metabolome, and lipidome, are dynamic and ever-changing, even for the same sample under the same analytical conditions.

However, presently most of the omics data that are generated are "static," ignoring its dynamic nature over time, which may cause bias in research. In the future, some high-quality approaches of "flash analysis" are expected to be developed, which may enable researchers to rely less on the slow separation process of liquid chromatography, with possibly no separation procedure needed. Marine derived pharmaceuticals provide a novel and rich source of chemical diversities that can contributes to design and development of new and potentially useful pharmaceutical agent.

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MarinOmics – Current and Future Perspectives

2

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Abstract

Marine ecosystem is considered vital, coupled with huge variations and disarray among themselves adding to its diverse and sustainable nature. Genomics is a broad discipline with various subdivisions such as metagenomics, proteomics, metabolomics, etc., that primarily encompass studies related to the genetic and protein makeup of a species. By adopting this technology, we will be able to preserve the complete genome and proteome of a species. Also, genomic studies on the marine organisms serve the purpose of understanding the synergy between the eronment and the genome as the marine organism gets accustomed to it, thus acting as an excellent tool to marine biologists. This genomic data assists molecular toxicologists to evaluate their practical uses in drug safety evaluation. In addition to it, advancements in single nucleotide polymorphism (SNP) studies alleviate the understanding in genetic variations between every single organism portraying its uniqueness. The sequencing techniques facilitate to obtain a complete insight about the DNA under study and also the causes for the genetic

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variations in the organism within same species or of different species. Further, new techniques have been evolved that induce sensitive, highly specific, quantitative measurements with high probability for identifying novel sequences and structure. These genome techniques can be done by marker-based genotyping or by using next-gen sequencing (NGS) techniques or also by whole genome sequencing. Performing all these studies provides complete information about the organism. This chapter will enclose the novel and traditional approaches that help in obtaining the complete enlightenment about marine genomics and its related fields of study.

Keywords

Marinomics · Fishes · Coral genomes · Algal genome

2.1 Introduction

Genomics is an emerging part of science that analyses the entire genome of organisms, their structure, function, evolution, as well as mapping of genomes and incorporates elements from genetics. To be more precise, genomics is a branch that aims at the characterization and quantification of genes (Vailati-Riboni et al. 2017). Owing to its rapid gain of popularity among the global scientific community, this new discipline is forcing some of the world's largest companies to reinvent themselves as borders between pharmaceutical, biotech, agricultural, food, chemical, cosmetics, environmental, energy, and computer industries blur and erode (Enríquez 1998).

Marine genomics studies encompass diverse fields, which match the diversity of marine environments and the organisms that inhabit them. Its studies are useful in generating information on population connectivity. Genomic studies provide accurate genetic stock identification and traceability. The data shows that from 1% to 5% of genetic markers distinguish important population structure and many of these informative genetic markers have signatures that indicate adaptive processes that are helpful in finding how these marine species have wide ranges and long pelagic larval dispersal stages. These species often encountered the variable environmental conditions has important implications for their survival, especially in the face of global change (Oleksiak 2016). The ending -ome is employed to handle the objects of study of such fields, such as the genome, proteome, transcriptome, or metabolome, respectively (Vailati-Riboni et al. 2017). Omics stands for collective technologies that help to explore the roles, relationships, and actions of the various types of molecules that make up the cell of marine organisms.

2.1.1 Classification of MarinOmics Studies

Genomics: The classification of omics deals with DNA sequencing, genetic profiling, genetic mapping, recombinant DNA technology, and the structural and functional analysis of genome (Fig. 2.1).

Proteomics: Proteomics is an emerging field in the omics platform that focuses to evaluate the entire set of proteins that are produced, modulated, and/or further modified (e.g., phosphorylation, post-translational modifications, protein–protein interaction) (Bantscheff et al. 2012; Altelaar et al. 2013). Nowadays, the term proteomics covers abundant and sensible analysis of the cistron products or "functional genomics," including large-scale identification or localization studies of proteins and interaction studies using the yeast two-hybrid system (Pandey and Mann 2000). Transcriptomics: deals with analysis of quantitative and qualitative differences in gene expression related to comparing with multiple RNA sequencing, expression profiling, transcriptional regulation (Tan et al. 2009). A transcriptome represents a smaller percentage of the genetic code that is transcribed into RNA molecules – it is estimated be less than 5% of the genome in humans (Frith et al. 2005).

Metabolomics is a study of metabolite profiles, metabolic intermediates, hormones, and other signaling molecules. Metabolomics deals with describing the non-targeted identification as well as quantification of the metabolome, while metabolome is termed as the quantitative measure of low-molecular-weight metabolites present in a cell under certain physiological conditions (Tan et al. 2009).

Phenomics is a major study of phenotypes at a system-wide level. A phenotype is basically the set of observable traits and characteristics of an individual or a population. This depends on both genotype and environmental factors. It is the technologies that qualitatively and quantitatively measure phenomes such as bioimaging, medical imaging, computational neuroinformatics, neurophysiology, or cell cytometry (omicstool.com). It deals with the evaluation of morphological, biochemical, and physical traits, and establishes a link between genetic, epigenetic, and environmental factors. In this chapter, we described the view on how the field of the omics platform is employed in the world of marine organisms.

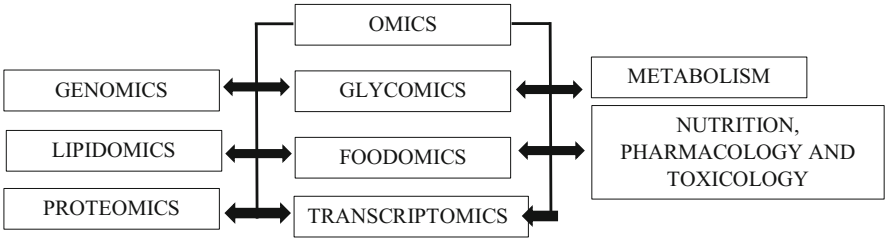

Fig. 2.1 Schematic representation of Marine Omics platform

2.2 Molecular Genomics of Coral

Coral reefs are highly diverse and they serve a great economic purpose to both local and national zones (Reaka-Kudla 1997). Coral reefs generally establish a stable coastline and provide a safe zone of habitat for the flora and fauna of that specific zone (Moberg and Folke 1999). They spread about 28.4 million hectares where nearly 20% has been entirely destroyed showing no traces of recreation (Spalding et al. 2001; Wilkinson 2004); in addition, many are under the threat of short- as well as long-term collapse. Though the recovery rate of coral reefs has been reformed after the coral bleaching crisis, they may be affected further due to the tremendous rise in ocean waters. Furthermore, there are predictions stating that the corals may alter themselves showing a huge level of tolerance to the climatic changes and coral bleaching than decamping themselves completely (Hughes et al. 2003). Numerous factors such as degradation of the quality of water, increased usage of the veteran coral reef species, and drastic change in the climate resulted in mass bleaching and decline in the coral reef population (Hoegh-Guldberg 1999), which is considered one of the important characters of coral organism and is observed as the crucial element for their survival where their response can be studied elaborately through comparative genomics.

2.2.1 Genomics in Evolution

Predicting the changes that can be encountered by the coral organism has been done by many researchers; however, in order to improve the methods of predicting the future of *Cnidarians* requires a proper and clear understanding regarding the adaptability and flexible nature of the species, which includes the basic understanding about the organism can accept and retreat from that event along with its shift from one stabilized state to the other (Nyström et al. 2000). Nevertheless, a proper understanding of the evolutionary aspect is mandatory for successful genome profile prediction of future events. The gaps occurred between the history of development among the corals can be investigated by molecular phylogenetic studies to some extent (Van Oppen and Gates 2006). The construction of molecular phylogenies for *Scleractinian* corals have been executed, which initially encountered with problems related to the development of the relevant methodology and also the genome markers (van Oppen et al. 2002). Here the molecular biology and genomic studies play a crucial role in preserving the organism (Fig. 2.2) (Van Oppen and Gates 2006).

2.2.1.1 Classification Based on Coral Genomics

The molecular data has been studied due to advancement in genomics and the results often contradict to the conventional method of basic grouping (Kitahara et al. 2010; Huang 2012). Generally, the time of origin and the divergences in *Scleractinia* remain ambiguous, the available molecular results and data show that the important corals are grouped into two dominant clades (“superfamilies”) referred as *complexa* (complex corals) and *robusta* (robust corals). The classification depended upon the

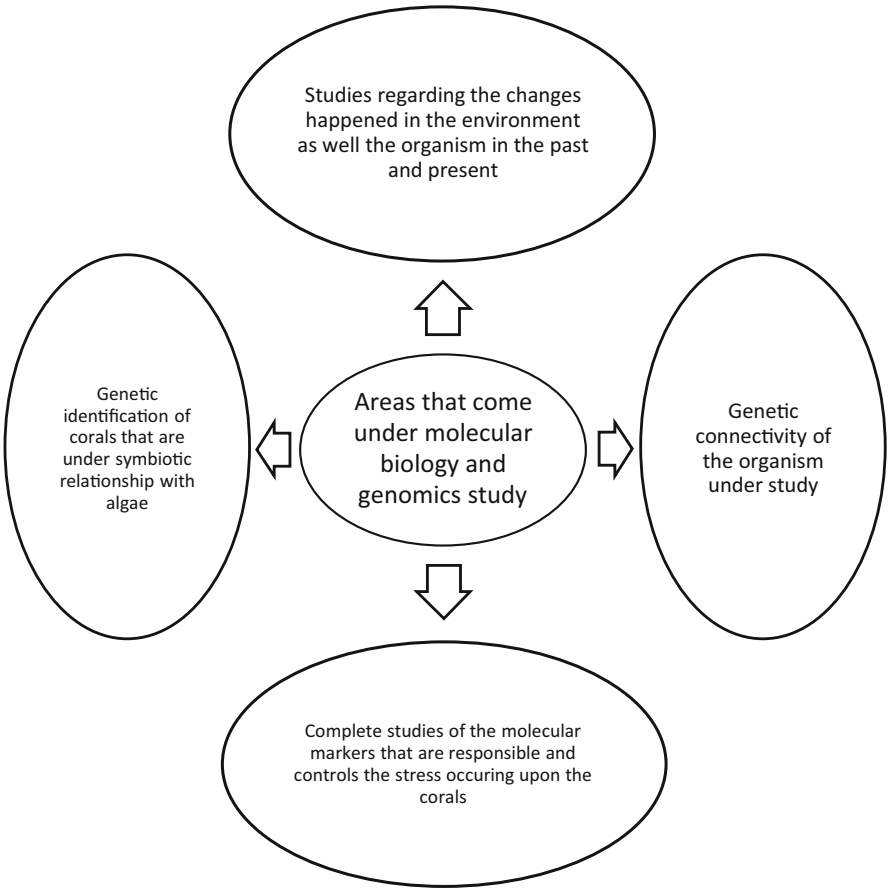

Fig. 2.2 Major fields and areas that come under molecular biology and genomics

16S rDNA data (Romano and Palumbi 1996), which lacked in most of the molecular analysis (Bhattacharya et al. 2016; Lin et al. 2016). Among the characteristic factors that help in distinguishing the complex and robust corals is the mitochondrial genome composition, where the G + C content of the robust corals are comparatively lower than in *complexa* (Kitahara et al. 2014).

2.2.2 Coral Population Genetics

Population genetics also gives an in-depth knowledge about the hybridization process that takes place between different species. Studies related to molecular phylogenetic and population genetic analyses of *Acroporid* corals suggest that the hybridization event occurs naturally but it is occasional (Márquez et al. 2002; Wolstenholme 2004). Many studies have been undergone to prove the above

statement, notably two independent studies, using the nuclear ribosomal DNA (van Oppen et al. 2000) and mitochondrial DNA (Vollmer and Palumbi 2002). The studies concluded that the genus *Acroporais* is a result of hybridization process between the two wild species namely *A. palmata* and *A. cervicornis*, which undergoes the process of backcrossing with only one parent for the confirmation of new hybrid, *A. prolifera* (Vollmer and Palumbi 2004). Further, the three accepted species of *Montastraea* were presumed to express intraspecific variants and further research acknowledged that the differences observed in their colony morphology were matched by their individual differences in growth rate, aggressive behavior, corallite structure, life history, etc. (Fukami et al. 2004). In addition to the above statements, the genetic differences found in these organisms were very limited, comprising changes in an intron in β-tubulin gene (Lopez and Knowlton 1997), amplified fragment length polymorphism (Lopez et al. 1999), mt DNA (Fukami and Knowlton 2005), etc. Though the evidence was clear, the huge debates regarding the above research prolonged over a decade and finally the research has been revised (Levitan 2004), which concluded that the species *M. faveolata* is completely distinct from *M. annularis* and *M. franksi*, where the latter two are similar to each other both genetically and morphologically, thereby acting as a sister species (Fukami et al. 2004). Previous studies revealed the understanding of stress markers present in the corals and the molecular stress markers investigated the heat-shock proteins and the antioxidant enzymes like peroxidase dismutase (Tom et al. 1999). Notably, Snell and co-workers have studied the genes that are more sensitive to the environmental stress and are highly responsive in nature (Morgan et al. 2001; Snell et al. 2003) and ended up with the development of the first cDNA (complementary DNA) array for coral organism (Edge et al. 2005).

2.3 Marine Algal Genomics

2.3.1 Significance of Marine Algae

Marine algae, known more commonly as seaweeds, are further divided into red, brown, and green based on their pigments. They are a major source of new compounds demonstrated to have properties useful in treating cancer, such as cytotoxic and antitumor potential (Alves et al. 2018). They have also been proposed to be of importance in developing novel pharmaceutical agents, due to their ability to produce bioactive secondary metabolites.

In order to obtain better understanding of the marine population, vast amounts of genomic research has been employed. The marine algae genomics originated with the publication of the 551-kilobase remnant nucleomorph genome of the *Cryptomonad Guillardia* theta. Since then, genomes of many more species have been rapidly sequenced, including diatoms, green algae, *prasinophytes*, etc., (Douglas et al. 2001).

2.3.2 Genomic Insights of Algae

Algal genomics have proved to be a valuable tool for understanding algal evolution as well as their non-algal relatives. Whole genome sequences have bolstered the theory of a plastid endosymbiosis event at the base of *Archaeplastida* and undermined the acquisition of red plastid outside the species (Price et al. 2012; Stiller et al. 2014). Phylogenomic studies regarding gene conservation have revealed many instances of mosaicism and the presence of bacterial genes like those of cyanobacteria in chloroplast (Dagan et al. 2012; Deusch et al. 2008; Martin et al. 2002). The whole genome sequencing has also helped to distinguish algae from one another, given that algal supergroups have diverged within 300 million years of the last eukaryotic universal ancestor 1.9 billion years ago (Eme et al. 2014). Phenomena such as horizontal gene transfer (HGT) have been estimated as contributing at least 2000 genes to the diatom *Phaeodactylum tricornutum*. However, around 6000 proteins are unique to *P. tricornutum* and other *stramenopiles* (Rastogi et al. 2018). Similarly, *Emiliania huxleyi*, a marine *Coccolithophorid*, also exhibits amazing diversity where more than 5000 genes in the reference genome were absent in at least one of its isolates (Read et al. 2013).

2.3.2.1 Lateral Gene Transfer in Marine Algae

Lateral gene transfer (LGT) is the movement of genetic material between organisms other than through reproduction – the vertical transfer from parent organism to its offspring. It helps organisms evolve and plays a role in the development of antibiotic resistance in bacteria and maintenance and transmission of virulence for viruses. LGT is detected by looking out for homologous genes in phylogenetically distinct organisms, which is further validated. Solid background knowledge is needed regarding aspects such as potential interaction of the concerned species, GC content, and codon usage. Subsequently, a plausible mechanism by which the LGT occurred is put forward, taking into account the life histories of the recipients and donors (Parker et al. 2008).

The genes, encoding 3-dehydroquinate synthase, AroB, and O-methyltransferase (OMT), present adjacent to each other in cyanobacteria, were transferred via LGT to the dinoflagellates *Heterocapsa triquetra*, *Karlodinium micrum*, and *Oxyrrhis marina*. These genes formed a fusion protein with a plastid targeting peptide (Waller et al. 2006). However, in subsequent analysis of derived dinoflagellates, the genes have been found to revert to being independent, but with a separate copy of the plastid targeting peptide retained with both genes (Parker et al. 2008). For example, whole genome sequencing of *O. Tauri* revealed alien chromosome 19, which represents transferring the entire chromosome from either parent (Derelle et al. 2006). The non-native chromosome differs in many properties from the rest of the chromosomes of the *prasinophytes*, such as GC content, splicing, and codon usage. Phylogenetic studies carried subsequently revealed that most protein coding genes were more similar to those found in bacteria than any other green algae. Since most

of these genes have been found to be involved in cell surface processes, they have been thought to lend pathogen resistance to the organism (Parker et al. 2008).

The earliest discovery of gene transfer through transduction is the presence of marine cyanobacterial photosynthetic genes (psbA and hliP) in cyanophage genomes (Sullivan et al. 2006). The infection of *Ectocarpus* is caused by brown microalgal viruses, EsV and FsV, the viral genomes contain a phytochrome gene that was also detected in the genomes of *T. pseudonana* and *P. tricornutum* (Delaroque et al. 2001). Sequencing has pointed to the presence of unexpected genes in some such viruses (Parker et al. 2008). The EhV virus possesses four genes for the biosynthesis of sphingolipids, which is a precursor of ceramide that is used in apoptotic intracellular signaling and is thought to trigger the death of the host cell to disperse virions (Wilson et al. 2005) (Table 2.1).

Table 2.1 Genome transfer between different organisms

Host marine algae	Gene donor organism	Gene name	Reference
Teleaulax amphioxeia	Firmicute bacterium	*dna*X for DNA polymerase III	Kim et al. (2015)
Gracilaria tenuistipitata	*Buchnera*	*leuC* and *leuD* subunits of leucine biosynthesis	Lee et al. (2016)
Pyropia haitanensis	*Porphyra pulchra*	Some sequences of ptDNA	Lee et al. (2016)
Gracilaria chilensis	*Gracilaria robusta*	mtDNA	Zhang et al. (2012)
Gracilariopsis chorda	*Gracilaria robusta*	mtDNA	Zhang et al. (2012)
Gracilariopsis lemaneiformis	*Gracilaria robusta*	mtDNA	Zhang et al. (2012)
Lotharella oceanica	*Bigelowiella natans*	Mitochondrial rpl16 and rps4 genes	Tanifuji et al. (2016)
Heterocapsa triquetra Karlodinium micrum, Oxyrrhis marina	Cyanobacterium	AroB, OMT (for 3-dehydroquinate synthase and O-methyltransferase, respectively)	Parker et al. (2008) and Waller et al. (2006)
Ostreococcus tauri	Bacteria	Chromosome 19	Parker et al. (2008) and Derelle et al. (2006)
Phaeodactylum tricornutum	Bacteria	784 genes (7.5% of the genome)	Parker et al. (2008)
Prochlorococcus Synechococcus	Cyanophages	psbA, hliP for photosynthesis	Sullivan et al. (2006)
Phaeodactylum tricornutum Thalassiosira pseudonana	EsV, FsV	Phytochrome genes	Parker et al. (2008)

2.3.3 Brown Algal Genomics

Brown algae are another class of algae that contain large amounts of the fucoxanthin pigment. They are highly diverse, ranging from smaller filamentous algae to much larger and more complex seaweeds (Wehr 2015). The sequenced genome of *Ectocarpus* has been studied, providing information about its evolution and adaptation mechanisms. The genome has an abundance of light harvesting complex (LHC) genes, which, along with light stress-related genes, help *Ectocarpus* cope with the variable light conditions in the intertidal and subtidal zones. Metabolically, the flavonoid pathway enzymes and those involved in metabolizing reactive oxygen species help in survival in the presence of abiotic stresses such as ultraviolet radiation (Cock et al. 2010). Phylogenetic studies of the genes involved in the biosynthesis of cell wall in *Ectocarpus* attribute the origin of this pathway to a red alga using secondary endosymbiosis, conferring the brown alga with a plastid. However, the terminal steps of the pathway for alginate biosynthesis were acquired from an actinobacterium via LGT (Michel et al. 2010).

The rise of multicellularity in algae itself seems to have many causative features such as the retention of many ion channel families, transcription factors, *Rad51* family proteins, and GTPases during evolution. Such a feature was a family of membrane-spanning receptor kinases, acquired by brown algae since their divergence from diatoms. The feature evolved only because of the transition to multicellularity, further bolstered by the presence of fucoidans and alginates that improve algal flexibility. Genes of *Ectocarpus* also have been found to be intron-rich – containing an average of seven introns per gene – and are quite long (about 704 bp), like the 3′ untranslated regions (about 848 bp). Genes are also more or less ordered, resulting in alternation of genes as the chromosome is scanned. It has been observed that genes situated close to each other have similar expression patterns, but it is yet to be determined if this is a consequence of the gene orientation (Cock et al. 2010).

2.3.4 Evolution of Diatom Genomes

Diatoms are a class of photosynthetic secondary endosymbionts most prominently known for some of the most beautifully symmetric designs in organisms found in nature. Although microscopic, they are responsible for about 20% of the primary productivity on the earth (Falkowski et al. 1998; Field et al. 1998). Genome sequencing of the pennate diatom *Phaeodactylum tricornutum* has been compared with that of the centric *Thalassiosira pseudonana* to get a clearer idea on the evolution, functional importance, and universality of certain features throughout diatoms. It is surprising that despite these two broad forms of diatoms diverging for only 90 million years, over about 40% of genes differ between these two lineages. Relative to yeasts and metazoans, diatoms are reported to exhibit rapid rates of gene diversification. Factors responsible include differential gains and losses of introns and genes, selective gene family expansions, and differential mobilization

of transposable elements. Even more striking is the presence of hundreds of bacterial genes in these diatom genomes (Purcell et al. 2007; Swartz et al. 2007). Of these, at least 300 genes are found in both the representative diatoms, pointing to ancient origins. These findings will prove fundamental to understanding how diatoms perceive environmental cues and manage metabolites to survive in marine conditions (Bowler et al. 2008).

2.4 Omics of Marine Fishes

The completion of the human genome project paved a way for scientists to explore the marine fish genomics. Comparison of the genomes of different species will guide future approaches to understand genetic evolutions and gene variations. Evaluation of the tiger pufferfish (*Takifugu rubripes*) genome was proposed as a cost-effective way to illuminate the whole human genome sequence to have a comparative analysis within the vertebrates (Khora and Navya 2016).

The Fugu genome is around 350–400 million bases long (Elgar et al. 1999) and is merely one-ninth the size of the human genome (Venter et al. 2001). Fugu was introduced as a "genetic model" organism, specifically because of its compact genome that permits efficient comparison with the human genome (Brenner et al. 1993), and approximately 1000 human genes have been identified (Aparicio et al. 2002). A major motive behind having a comparative study with mammals is to generate a better understanding of the genetics of human diseases including cancer (Amatruda et al. 2002; Pichler et al. 2003). Both *Tetraodon* (freshwater pufferfish) and *F. rubripes* (marine pufferfish) are considered to be important species as they are vertebrates and their genome sizes are eight times smaller than the human genome, having many of same genes as well as regulatory content as in humans but with lesser "junk" DNA. The sequences of the two pufferfish will provide key tools for gaining insights into the human genome, which will in turn translate into practical knowledge toward developing better therapies in the future (Khora and Navya 2016).

2.4.1 Marine Medaka (*Oryzias melastigma*)" "A Genome Model"

Marine medaka also popularly known as the Indian medaka is a native species to coastal waters in India, Sri Lanka, Pakistan, and Thailand (Naruse 1996). It is considered to be an excellent model organism for marine ecotoxicological studies due to its small size, short generation time, distinct sexual dimorphism, and the ability to quickly adapt to a wide range of salinities (Dong et al. 2014; Kim et al. 2016). The omics platform can pave a way for marine researchers to have a better understanding of the molecular mechanisms and responses of the marine flora and fauna in detoxifying marine pollutants (Van Aggelen et al. 2010). Marine medaka's transcriptome and genome are used for marine environmental research (Kong et al. 2008).

2.4.1.1 Potential Applications of Medaka Omics in Marine Environmental and Toxicology Research

Estuarine and coastal regions are often threatened by diverse aquatic contaminants through fresh water run-off from land (Kennish 2002). The regions are highly vulnerable to the environment fluctuations such as UV-B radiation, temperature, and salinity and hence for this very reason ecologically and ecotoxicologically relevant model organisms are required that can express reliable biomarkers in response to environmental changes should be developed for the biomonitoring of estuarine and coastal regions (Monserrat et al. 2007). The marine medaka is a sentinel species that can provide a common platform between laboratory-based molecular toxicology and ecotoxicological investigations on marine environment. The function or purpose of individual molecular biomarkers such as cytochrome P450 activity, antioxidant enzymatic activity, etc., has been very well documented in the marine medaka. However, there is a need for further efforts in developing an omics platform and an integrative omics approach to better understand the molecular pathways underlying these biomarkers (Chen et al. 2009).

2.5 Future Perspectives of MarinOmics

The accessibility to whole genome sequences and the rapid development of genomic tools created a new and valuable research paradigm in the aspects of marine toxicogenomics, evolution, and pharmacogenomics. Using MarinOmics as a tool for detecting the evolutionary aspects, the connectivity within marine organisms and between marine and terrestrial organisms can be mapped. The genome makeup of an organism will provide methods for protecting those species that are at the verge of extinction or already extinct. Gaining an in-depth knowledge in the field of marine genomics will also help in the field of aquaculture for improvising the breed quality, thereby raising the profit for the fish farmers indirectly elevating the economy of the nation. Decoding the genome of marine organism will help in drug development in pharmacogenomics and therapeutics such as gene therapy for numerous diseases. Studying genomes will also help in understanding the environmental changes that occurred and the modifications within the genome of an organism to understand its impact. Transcriptome profiling of marine organisms can be performed to evaluate the effects of environmental pollutants, to simplify the interpretation of molecular and/or biochemical markers for biomonitoring, and to understand the signaling pathways involved in the immunological responses of the marine organism, thereby leading to the utilization of genome information for the further functional genomics studies. Recently, proteomics along with genome information has been successful in analyzing the effects of environmental stressors in marine organism.

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Marine Nutraceuticals

3

Ramakant Joshi, Navneet Garud, and Wasim Akram

Abstract

In modern era nutraceuticals have received considerable interest to cure or prevention of various diseases. Marine nutraceuticals are the products or substances which are obtained from the marine sources such as fish, prawn, sponges, and many other marine organisms. Nutraceuticals comprise of various proteins, peptides, carbohydrates, essential minerals, bioactive peptides, vitamins, enzymes, phenolic phlorotannins, and polyunsaturated fatty acids (PUFA). Many of these are also used as therapeutic agents to cure diseases. Marine sources are well-recognized for biologically active materials by their numerous potentials to be used as nutraceuticals. Moreover, these functional foods are also used to enhance human lifestyles, in that marine-derived nutraceuticals are considered a natural means of achieving healthy lifestyles. Fish and shellfish are considered the main sources of marine-based nutraceuticals. Fish bone is a worthy source of calcium, while the fish membrane and frame are a potent material used for isolation of bioactive peptides. In addition, the global market for nutraceuticals is growing in comparison to conventional therapy, including in countries such as Western Asia, East Asia, South Asia, Southeast Asia, and Oceania and Japan, Europe, China, India, and the United States.

Keywords

Marine nutraceuticals · Bioactive material · Natural supplements · Health benefits

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3.1 Introduction

Nutrition and pharmaceuticals together make "nutraceutical." They are the middle chain of food and medication. In 1994, Food and Nutrition Board stated that nutraceutical is "any substance that is a food or part of food which provides medicinal or health benefits including the prevention and treatment of disease, beyond the traditional nutrients it contains." Nutraceuticals is not equivalent to medications, as it is not outlined particularly for therapeutic utilization, but, it has therapeutic benefits beneath the normal observation. Essentially, nutrient-rich foodstuff is defined as the "products derived from natural sources, whose consumption is likely to benefit human health and enhance performance." Nutraceuticals and functional foods are also widely used as food for the human body after physical effort or as an equally preventive measure against sickness (Haefner 2003; Blunt et al. 2003).

Among the marine resources, microbes play a major role in the arena of pharmacology. During earlier times, one-fourth of the explored dynamic auxiliary metabolites were derived from filamentous fungi (Appleyard et al. 1995; Walton 2000). From a long time, chemists and pharmacologists have been paying attention to marine microbial products and natural derivatives (Pietra 1990; Fenical 1993; Davidson 1995). Marine microbes are the source for bizarre natural particles with novel analogues useful for artificial approaches to treat several diseases (Fsulkner 2000).

Nowadays, normal bioactive compounds or components equivalent to nutraceuticals are widely treasured by customers. Compounds which are a derivative of an oceanic source have worked as a wealthy source of health-promoting components. On earth, about 70% of space is inhabited by marine life, so a wide-ranging variety of marine lives is composed of potential natural resources. Marine nutraceuticals are the products or substances, which are obtained from the marine sources such as fish, prawn, sponges, and many other marine organisms. These are composed of various proteins, peptides, carbohydrates, essential minerals, bioactive peptides, vitamins, enzymes, phenolic phlorotannins, and polyunsaturated fatty acids (PUFA) that are used as therapeutic agents to cure diseases (Fitton et al. 2008; Kim and Wijesekara 2010; Ngo et al. 2011; Wijesekara and Kim 2010; Wijesekara et al. 2010; Wijesekara et al. 2011).

The oceanic environment has a huge variety of living animals in comparison to earthbound biological systems, making various assets available for human sustenance and welfare (Hill and Fenical 2010). These maritime invertebrates are found in complete sea ecosystem from the intertidal zones to the deep oceans, and includes various taxonomic groups, that is, arthropods such as crawfish, crabs, lobsters, prawns, shrimps; cnidarians such as hydrozoans, corals, ocean anemones, jellyfish; bryozoans or green creatures, or ocean mats; annelida such as ocean worms and polychaetes; echinoderms such as ocean urchins, ocean cucumbers, and ocean stars; and mollusks such as shellfish, octopuses, cuttlefish, squid, abalone. etc. (Thorpe et al. 2000). Apart from these, the ocean also incorporates macroalgae, some small fish species, cyanobacteria, microalgae, and microorganisms that yield auxiliary metabolites as per the adjustment required toward their oceanic surroundings.

Research on marine-based molecules has found new bioactive compounds, which are suitable for various nutraceutical-based applications (Voultsiadou 2010). About 36–40% of seafood and fisheries are obtained from the marine invertebrates, which are highly composed of additional bioactive compounds such as taurine and carotenoids; numerous peptides; some minerals like iodine and selenium; and polyunsaturated fatty acids (PUFAs) that have health benefits (Børresen 2009).

3.2 Sources of Marine Nutraceuticals

3.2.1 Marine Algae

In the Protista kingdom, some algae, namely Rhodophyceae, usually known as red algae; Phaeophyceae, also known as brown algae; and Chlorophyceae, also known as green algae are universally found in Asian countries like Korea and Japan; Pacific countries like the Philippines and Maori; and in smaller proportions at Norway, Ireland, as well as the Canadian sea areas, in addition to American Atlantic Ocean and in the waters of France. These edible marine algae, also known as ocean weeds or vegetables of the sea, are the common dietary supplements of the above mentioned countries. In Philippines, *Porphyra* spp. (red algae), a cold-water seaweed; *Caulerpa racemosa* (green algae); and *Sargassum* (brown algae) seaweeds have been conventionally consumed, for instance, new or blenched algae used as a trendy garnishing material for salads and soups (Trono 1999). The key components of traditional Japanese Sushi are *Porphyra tenera* and *Pyropia yezoensis* known as Nori or Gim. Some other species of brown algae like *Undaria pinnatifida* or Wakame, *Hijikia fusiformis* or Hijiki, and *Laminaria* spp. are used as condiments and flavors in Korea and Japan. *Lonicera japonica* or Haidai is used in China for soups. Red algae species such as *Porphyra umbilicalis* (Laver seaweed), *Palmaria palmate* (Dulse seaweed), and *Porphyra columbina* (karengo seaweed) are used in bakery products like laverbread in wales; as components of salads, soups, and snack foods in North America; and as a fermenting agent, chewing gum, steaming process and as fresh or sundried constituents in New Zealand. Also, green algae, namely *Ulva fasciata*, (Limu Palahalaha) is used as components of salad, condiments, and stews in Hawaii (Yuan 2007; Cambie and Ferguson 2003). When we talk about the food industry, several red and brown seaweeds, such as *Gracilaria*, *Gelidium*, *Gelidiella* spp., *Chondrus crispus*, and *Macrocystis pyrifera* play a remarkable role as an emulsifying, gelling, and thickening agent. Recently, restaurants use fresh or dried seaweed in gourmet cooking, such as side dishes, garnishes, and condiments.

Nowadays, a developing zone is interested in investigating possible positive health impacts of oceanic algae and their components as useful functional foods and nutraceuticals; they are used as a source of antioxidants and in decreasing the dangers from numerous diet-related prolonged diseases such as atherosclerosis and hyperlipoproteinemia in circulatory diseases (CVD). Definitely, in Asian and Western countries, people pay a great deal of fascination for marine algae as functional

foods and nutraceuticals, in order to reduce the chance of long-lasting illness by the usual intake of seaweed.

3.2.2 Marine Fish

The role of certain food materials has added more attention as a health promoter above their nutritional significance. Hence, natural resources such as marine animals, plants, organisms, and bacteria are explored extensively for bioactive compounds and functional foods. As a result, the production of nutraceuticals and functional foods is growing on a commercial scale. Nutraceuticals possess the confined bioactive constituents otherwise filtered from foods to be used in the therapeutic form. Functional foods are the compounds that improve health with their efficient constituents to supply medicinal and biological benefits to diminish the chance of dangerous chronic diseases beyond their fundamental dietary capacities (Schmidl 1993). In its entirety, physiological functional food and nutraceuticals equally illustrate numerous benefits toward physiological well-being (Defelice 1995).

Gradually, the willingness to eat marine products for related health benefits are expanding demand for seafood varieties. Especially, in health-conscious ethnic gatherings, fish and shellfish are significant nutritious components. Southeast Asian nations, including Korea, have led the way as an eminent foundation and are engaged in significant fish production. Korea consumed 59% of the entire marine fish population captured for the generation of prepared foods in 2001. The most important concern popular in the oceanic fish-handling trade is the dietetic worth of the foodstuff. As an outcome, in recent years various drug candidates have been founded by this bioresources identification of biological properties and nutraceutical development. However, the majority of these composites are in their formative phases. Fish protein hydrolysates and fish bone-derived functional constituents have been paid attention among functional bioactive ingredients recognized from marine fisheries by-products. A few bioactive peptides from the fish protein hydrolysates are directed toward antioxidants (Park et al. 2001; Saiga et al. 2003) and angiotensin-converting enzyme (ACE) inhibitors (Meisel 1997; Kim et al. 2007a, 2007b) that bring down the blood pressure by inhibition of ACE. The natural surroundings of those biomaterials intended to be used as a source of nutraceuticals or physiological functional foods need to be secured so that their higher scale productions are not impacted. Additionally, derivates of crustacean exoskeleton chitin such as chitosan and their oligomers have gained ample attention due to their wide-ranging variety of uses in various areas of medicine and pharmacy.

3.2.3 Marine Sponges

Marine sponges are present to prove the best foundation of proteins such as silicateins and cathepsins. In this way, separation, characterization, and ultimate encoding of those proteins might lead to commercially interesting components. Most

of the sponges have siliceous spicules that work as their skeletal components. They utilize a protein framework to accelerate biogenic opal in an highly well-ordered manner (Vrieling et al. 1999). Various extracts from distinctive sponges of different areas have been used as bases of bioactive formulations. Extensive studies utilizing quarantined experimental human myocytes revealed the possibility of a couple of sponge abstracts, which can be used as successful agents in the treatment against various cardiac diseases (Christ et al. 2004).

Apart from this, a recently discovered bisindole alkaloid—namely dragmacidins that have anticancer and antiviral activities—has been separated from a diversity of marine sponges. Another species of marine sponge, that is, *Spongosorites* spp., offered a novel alkaloid Dragmacidin E in the Australian southern coastline. Co-metabolite of Dragmacidin E is Dragmacidin D. This when mixed with Dragmacidin D can work as a powerful serine-threonine protein phosphatase inhibitor. Scientists have found interesting biochemical compounds from marine sponge called *Plakinistrella*, found within the Indian seas, that may lead to modern management of fungal infections that appear among survivors of cancer and acquired immunodeficiency syndrome (AIDS) patients (Capon et al. 1998; Venugopal 2008).

3.2.4 Marine Cucumber

Marine cucumbers have been found to have anticoagulant, antifungal, antibacterial, and antioxidant activities. These cucumbers originate in low aquatic ranges of the oceans toward sea bottoms. Antibacterial action is identified in their distinctive body fragments and also in the eggs of the *Cucumaria frondosa* ocean cucumber. High antioxidant activity is seen in the animal's respiratory organs, alimentary canal, muscles, and gonads. They contain flavonoids and 2.8–59.7 mg of rutin, equaling 100 g; phenol substance like 22.4–235 mg; and gallic acid equaling 100 g of desiccated mass that are responsible for antioxidant action. The oxygen radical–activating capacity (ORAC) standards extend beginning at 141 micromoles and move toward 798 micromoles of Trolex/g. The action remained most noteworthy within the acetone excerpts of the alimentary canal, taken later by the muscle, gonads, and respiratory system. The outcomes proposed that *Cucumaria frondosa* tissues may remain valuable bases of antioxidants intended for human usages (Seymour et al. 1996).

Leucospilotaside A is a Triterpenes glycoside produced from *Holothuria leucospilota* or the ocean cucumber (Mamelona et al. 2007). *Asterias rubens*, a starfish, is responsible for the potent antibacterial action displayed within the eggs and gastrointestinal organs; some of the tissues also possess lysozyme-like action. Some species are recognized for hemolytic activity, especially within the extracts from the body wall. These happenings also existed and was recognized within the green ocean urchin *Strongylocentrotus droebachiensis*, recommending that maritime echinoderms may remain latent bases of unique antimicrobials (Han et al. 2007; Haug et al. 2002). Patagonicoside A, a modern Triterpenes glycoside is produced from *Psolus patagonicus* ocean cucumber. This compound have impressive

antifungal action against pathogenic fungi *Cladosporium cucumerinum* because it turns into disulfate tetrasaccharide with a replacement of a glycan moiety (Murray et al. 2001). In addition, *Actinopyga lecanora* is an ocean cucumber known for their antifungal action (Kumar et al. 2007).

Other ingredient known as sulfated glycans, who contrast the common arrangement of fucose sulfated polysaccharides, separate the oceanic algae from animal tissues glycosaminoglycans and also from the ocean echinoderms eggs jelly coat. These compounds are responsible for the maintenance of the integrity of the body wall of ocean cucumber, in a relationship by the part of extra macromolecules within the vertebrate animal tissue (Paulo et al. 1987). The polysaccharide found in ocean cucumber cell wall was analogous to the spine structure of mammals with the chondroitin sulfate of mammalian, but several glucuronic acid remainders showed sulfated fucose divisions. The definite spatial array of the sulfated fucose divisions within the fucosylated chondroitin sulfate has conversed into great anticoagulant action and similarly decides contrasts within the approach that one prevents thrombin (Mourão et al. 2001). Some extensive ranges of biological actions like recombinant human immunodeficiency virus (HIV) reverse transcriptase action, anticoagulant action, and venous antithrombic action are shown by the sulfated glycans. Fucoidans have been well recognized for their various activities like immunomodulation, antiviral action, anti-inflammatory action, hypolipidemic activity, , anti-angiogenic action, anti-adhesive properties, antitumor action, hypoglycemic action, anti-mutagenic action, and anticoagulant activity (Shanmugam et al. 2001).

3.2.5 Mollusks

An additional significant class that possesses properties to be good for extended use in pharmacology is the Mollusks. In literature, more than 400 compounds from molluscan sources have been designated for their pharmacological effects. A few of these can work as antiviral, muscle relaxants, antitumor agents, hypotensive agents, and cardioactive substances (Proksch et al. 2002; Alam and Thomson 1998). The marine environment covers numerous cases of remarkable robust bounding approaches which will arouse the planning of innovative synthetic adhesives. Marine beings such as reef-building worms, mussels, and barnacles employ particular protein to glue together—mussel adhesive proteins (MAPs)—which help stick to fine shells in spite of their nearness to liquid consistency. The composition of MAPs is catecholic amino acid and l-3,4-dihydroxyphenylalanine (DOPA) that play a key role in building up chemical initiative among MAPs and many metallic, metallic oxide, and polymer exteriors. The presence of oxidizing metal ions like $Fe3^{+}$ and MnO_4^{-} play an ideal role in efficacy of the MAPs. these polymers are utilized in mucoadhesive drug delivery systems and injectable fluids intended for operating tissue hold. The polymer imitates are being outlined for medicinal and nonmedical applications (Messersmith 2007; Monahan and Wilker 2004; Venugopal 2008).

3.3 Types of Marine Nutraceuticals

The choice of bioactive moiety, by using widespread presentations such as nutraceuticals within the foodstuff as well as supplement manufacturing, is delivered through oceanic environments. This comprises vitamins, polysaccharides, mineral deposits, proteins, probiotics, peptides, enzymes, fatty acids, and polyphenols. The residual portion of the current study discusses the physicochemical possessions of those diverse moieties that confer bioactivity within the framework of nutraceutical uses.

3.3.1 Proteins

Human beings need protein for nutrition, which is significantly fulfilled by the outstanding derivation of functionally dynamic as well as nutritious proteins originating from the marine ecosystem (Clarkson and Rawson 1999). Often, different kinds of seafood, especially fish, have been accepted, for instance, inexpensive origin of animal protein. The population in a nations with low per capita gross domestic product (GDP) will, in general, prefer a better percentage of fish protein in their protein utilization. The proportion of fish protein in entire animal protein expenses is higher for lower-income groups, and the poor public eats generally low rate fish. So this displays the significance of the low rate fish by way of a principal basis of protein among the poor households in rising nations. For example, the proportion of animal protein derived from marine products in the diet of the population in West Africa is extraordinary, that is, in Senegal (46%), Ghana (62%), and Gambia (61%) (Béné and Neiland 2003). In several countries, oceanic fish is frequently used to improve the palatability of diets, which in turn upsurge the overall food ingestion and thus improve the nutritional status of the consumer. Fish is utilized regularly to increase the total protein content of cereal-centered intakes, and this usually reduces the necessity of aminoalkanoic acid, lysine. On the other hand, throughout the past 40 years, the portion of fish proteins toward animal protein intakes revealed a small undesirable tendency, owing to more rapidly growing options of additional animal products.

About 30% of the total quantity of protein is found in cooked tuna fish, although 16–21% (mean 18%) of protein quantity is found in most of the raw finfish flesh (Venugopal and Shahidi 1996). Apart from that, greater protein content is found in various crustacean-like oysters, crab, and shrimp flesh. Some of the cephalopods, as well as crustaceans such as oysters, shrimps, crabs, scallops, krill, mussels, squids, and lobsters, contain 9–12%, 16.9–22%, 14.9–18.3%, 14.7–17.6%, 11–12%, 9–12%, 13–19.5%, and 18–19% content of proteins, respectively. The aminoalkanoic acid arrangement of fish exists similar to that of meat. Gelatin, collagen, and elastin are found in the stroma protein of muscle. Fish meat comprises merely 3% of stroma protein, excepting skates, sharks, and rays in comparison to meat. The nonprotein nitrogen composites that are present usually exceed that of the land animals by 1–40%, making the fish muscle more delicious. Nucleotides,

trimethylamine oxide, creatine, amino acids, creatinine, trimethylamine, and small peptides are found in nonprotein nitrogen composites. Squids, other shellfish, shrimps, crabs, and lobsters generally comprise higher quantities of amino acids, which cover alanine, arginine, glycine, and glutamic acid when compared to finfish. The higher contents of these amino acids throughout the wintertime season make squids more pleasant as linked with those harvested in summer. Demersal fish generally comprise superior measures of trimethylamine oxide than pelagic fish, and its contents differ from 20 mg% to 190 mg% (Martin 1982).

3.3.2 Fatty Acids

For the maritime ecology, fats are vital within the composition and generative procedures of marine creatures and reveal the distinct biochemical and environmental circumstances of the oceanic atmosphere. The interface in a maritime fatty acid is basically because they comprise considerable quantities of long-chain polyunsaturated fatty acids, like ω-3 PUFA, which are acknowledged to be significant in human health maintenance and nourishment. Preliminary readings on oceanic fatty acid included representation of their constituents, encouraged by the introduction of strategies like gas-liquid chromatography (GLC) for easy investigation, followed by the radioisotope tracer methods, based on the molecular biodiversity and distinguishing of main sources of ω-3 PUFA (Bergé and Barnathan 2005). During the previous couple of decades, research on the dietary perspectives of oceanic fatty acids, especially ω-3 PUFA, unlocked a remarkable outlook for those composites in health safety. The work began in the 1970s with recognition of the role of diet in the health of native Greenland Eskimos. The study detected that the life span and coronary well-being of Eskimos were associated with their calorie counts, which enclosed a mean 450-g greasy fish each day (Dyerberg et al. 1975). Very high ingestion of fish is assumed to add a positive healthy outlook to the people of Japan, who eat almost 80 g of fish and shellfish each day, consuming around 1000–2000 mg per day of ω-3 PUFA. Due to the known positive health outlook, greasy fish types, which comprise noteworthy quantities of ω-3 PUFA (Table 3.1), were considered as a functional food (Garg et al. 2006; Nettleton and Katz 2005).

However, the modern decline in certain fisheries, together with the preference of certain sections of the population for foods of vegetable origin, has led to a search for alternative sources of these fatty acids, such as transgenic plants and microalgae. It is supposed that the food processors are locked in a "fish oil arms race"; several businesspersons have an interest in the improvement of genetically modified crops that could challenge the supremacy of fish as the best source of ω-3 fatty acids (Graham et al. 2004). However, it is difficult to challenge the supremacy of marine products as a source of PUFA, at least in the near future.

The relative percentage of lipids and greasy acids in oceanic creatures is specific to their genus and species and additionally on their atmospheric behavior. The chief builders of oceanic lipids within the maritime surroundings are microalgae, which sustain both pelagic and benthic food networks. Sea lipids are the composition of

Table 3.1 ω-3 Fatty acids contents in marine foodstuff (1g/100 g flesh) (Venugopal and Shahidi 1996)

≤0.5	0.6–1.0	≥1.0
Haddock	Red snapper	Bluefin tuna
Atlantic Pollock	Channel catfish	Atlantic herring
Rockfish	Swordfish	Pink Salmon
Atlantic cod	Atlantic mackerel	Anchovy
Catfish	Indian mackerel	Atlantic salmon
Pacific halibut	Spiny dogfish	Pacific herring
Oil sardine	Silver hake	Pacific mackerel
Skipjack tuna	Torbot	Rainbow trout
Sole	Trout	

few uncommon fats, including hydrocarbons, esters, sulfolipids, glycolipids, and neutral lipids such as wax esters, phospholipids, triacylglycerols, and sterols. The maximum the distinctions in lipid originates within the triacylglycerol portion, and the phospholipids seem to possess less variations. The phospholipids of fish muscle limit by and large more phosphatidylcholine than phosphatidylethanolamine. The phospholipids of tropical fish are highly saturated than temperate water fish. The neutral lipids ensure lesser specific gravity than ocean water; consequently, the parts in regulating flexibility have frequently been suggested, particularly intended for wax esters (Bergé and Barnathan 2005).

Oceanic fish is normally categorized according to the fat content of fillets. Fishes are divided into three categories, that is, high-fat fishes contain over 8% fat, intermediate-fat fishes contain 3–8% fat, and lean-fat fishes contain less than 3% fat. In lean fish, lipids are set down within mature gonads, liver, and muscle tissue, while it is generally deposited in subcutaneous tissue in fatty fishes. High-fat fishes like Pollock and cod look off-white to white; lean fat fishes such as sole have whitish appearance. Salmon's (high-fat fish) flesh is typically pigmented such as grey fish, yellow fish, and pink fish. Depending on the diet, anatomical position, season, sex, the site within the body, and age, the quantity of fat may vary in fishes expansively from 0.3–24%. This lipid content decreases toward the tail in every fish, through an improved level of fat deposit within the abdomen flap and dark muscle. The lipid content found in pelagic fish is 3–5% throughout the summertime, whereas 11–20% during wintertime. Some fishes associate the reproducing cycle to fat levels such as before going to freshwater for the reproducing cycle, anadromous fish store fat. About 2.5 g of total fat is approximately 85 g of cooked portion of various seafood such as whiting, sole, mahi-mahi, clams, perch, tuna (skipjack), haddock, red snapper, northern lobster, tuna (yellowfin), grouper, shrimp, halibut, monkfish, squid, pike (Northern eye), orange roughy, scallop, cod, flounder, and Pollock. Although about 5–10 g of total fat is found by an equal quantity of cooked portion of bluefin tuna, herring, whitefish, butterfish, salmon (Atlantic, coho, or sockeye), Spanish mackerel, and lake trout fish (Ackman 1995; Silva and Chamul 2000; Venugopal 2005).

Overall, 50–90 mg per 100 g of fish's flesh contain oceanic steroids, which contain cholesterol. In a few pelagic fish species such as diverse mackerels, bluefin

fish, anchovy, and pilchards, saturated fat might be equal to 150 mg each of 100-g meat, and it is ought to be as high as 50–650 mg in liver and roe. Shellfish tend to comprise somewhat greater quantities of cholesterol. In this way, shellfish such as shrimp, crab, and lobster comprise 69–100 mg each of 100 g, while squid and octopus could comprise 120–250 mg of the steroid each of 100 g. Shellfish and a few mollusks require a nutritive origin of sterol for development and existence since they lack de novo sterol-synthesizing capacity (Kanazawa 2001).

3.3.3 Polysaccharides

The ability of gel formation in various oceanic polysaccharides like carrageenan, alginate, fucoidan, and chitosan are principal raw resources for biodegradable or eatable films. Arrangement of the gel assemblies includes intermolecular affiliation or cross-linking of polymer to create a semi-rigid three-dimensional (3-D) network; this captures as well as restrains the solvents. The improvement of film's properties is performed via the conversion of a gel by surplus technology like the incorporation of additives and copolymerization with multicomponent structures, including added hydrocolloids. The propensity to biodegradation of these films by various microbial and other enzyme sources like chitinase and carrageenase makes them eco-friendly. In addition, as they are nonhazardous, the films can also be utilized for wrapping foodstuff products meant for ecological safeguard in contrast to microbial contamination, moistness forfeiture, and regulator of oxidation. They can similarly play a role as transporters of bioactive composites (Prashanth and Tharanathan 2007; Lopez-Rubio et al. 2006; Srinivasa and Tharanathan 2007).

Chitin and chitosan are economical, besides being harmless, decomposable, and biocompatible. Chitosan is much handy as associated with its precursor (Prashanth and Tharanathan 2007). Chitosan can create semipermeable coverings, utilized in diets and extend their shelf life by performing obstructions to air and moistness. The utilization of chitosan in the diet is especially favorable since it is biocompatible and nontoxic. The method utilized and the kind of solvent system can impact the features of the chitosan film, such as chitosan commencing crawfish excess softened in formic acid or acetic acid produced elastic and apparent films that are useful in wrappings. Chitosan acetate films preserve lesser humidity levels associated with chitosan formate films. The sorption isotherm is considerably subjective of chitosan formate films by its molecular weight but not its acetate films (Figs. 3.1 and 3.2). The apparent tackiness of the coverings is reliant on the degree of de-acetylation of chitin, the precursor of chitosan (Nadarajah et al. 2006; Huei and Hwa 1996; Caner et al. 1998).

There are various kinds of carrageenan's such as ι-carrageenan, λ carrageenan, and κ carrageenan, which could be utilized to produce biodegradable films. In this direction, the humidity loss for mackerel mince film equipped by mixing 2% κ-carrageenan, 0.75% glycerol, 0.75% polyethylene glycol, and 0.1% potassium chloride was studied to be used as a possible packing material. The fish mincemeat patties were vacuity packed by the film and kept at temperature starting from +20 to

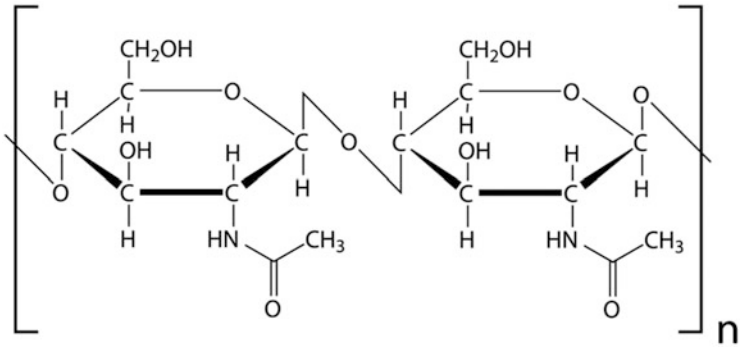

Fig. 3.1 Chitin chemical structure

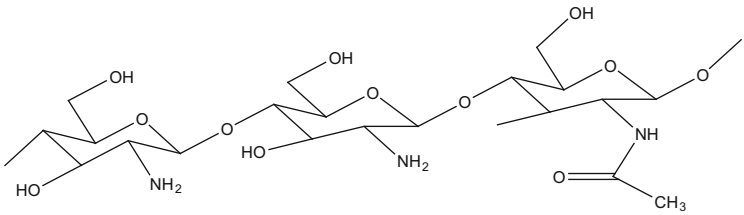

Fig. 3.2 Chitosan chemical structure

−15 degrees C. Mass damage and fat oxidation were dignified throughout storing. Packed or non-packaged samples kept at 20 degrees C, 10 degrees C, and 0 degrees C exposed a 60% weight loss among 2–15 days of storing, while the resultant weight loss once kept at −15 degrees C was ∼3% after 25 days. Non-packaged sample kept at 0 degrees C exhibited a gentle rise in lipid oxidation, while carrageenan enfolded produce showed a lesser degree of lipid oxidation (Hwang et al. 1997). Moistness loss throughout storing at 4 degrees C of beef patties, apple slices browning reduction (Lee et al. 2003), and oxidation of lipids is also reduced by films of carrageenan. Its film was, in effect, polyvinylchloride films in decreasing moisture loss (Wu et al. 2000). Carrageenan can substitute polyethene, which is engaged in covering the paper utilized for packing oleaginous or greasy foodstuff. Both carrageenan covered papers and films were extremely impervious to lipid, as a κ-carrageenan-coated paper displayed maximum impermeability than by λ- carrageenan and ι-carrageenan films. κ-carrageenan layer increased, thereby the lipid impermeability improved. Carrageenan-coated papers that were between 4 kg/ream and 5 kg/ream or 278 m^2 displayed lipid impermeability similar to that of polyethene-coated papers. The lipid impermeability of carrageenan films was just about ten times above that of carrageenan-coated paper. κ-carrageenan-coated papers consuming more than 4 kg/ream were revealed to possess acceptable lipid blockade possessions to be utilized for packing fatty foodstuff (Rhim and Ng 2007; Rhim et al. 1998).

In addition, another polysaccharide algin has multipurpose uses in the separation of whey, as microsphere vectors for drug delivery system, food products, dressing absorbents, dental impression creation, dye manufacturing, anti-reflux therapies, and many more. Alginate gel films appeared to stop moisture loss and lipid oxidation to a particular degree in fishery foods. The arrangements of solvent alginates are colorless, translucent, non-coagulable on warming, and ensure a good sort of viscosity. Alginate features a solid empathy for liquid and might be utilized to regulator moistness in foodstuff (Brownlee et al. 2005).

3.3.4 Peptides

Current studies revealed that various oceanic animals such as seaweeds, mollusks, fishes, sea anemones, ascidians, and sponges are amazing sources of bioactive peptides. Those are designed, moreover, by the activity of endogenous proteases or amid the activity of exogenous proteolytic enzymes. Within fresh fish, the quantity of peptides could also be little, while their substance may increase during storage, thanks to proteolytic degradation of their muscles. During research on the development of peptides, acid-soluble portions in fresh fish kept up to 2 weeks in frost were separated by high-performance liquid chromatography (HPLC) and analyzed by mass spectrometry (MS). A minimum of 25 polypeptides of molecular weights of 2–33 kDa were recognized; those with molecular weights of 3.9, 11.4, and 32.8 kilodaltons, respectively, reduced throughout ice-cold storing, while those with molecular weights of 12.5 kDa and 16.5 kDa improved. The rise in peptide shapes throughout storing connected by fluctuations in entire aerobic and anaerobic amounts within the fish is suggestive that the peptide investigation might suggest a lead of decay. Biochemical alterations within the muscle proteins in the seasoning originated to communicate to the deprivation of the myofibrillar arrangement of the muscle. Hydrolysis of muscle proteins was momentous for the period of the first 6weeks, by proteins of relative molecular mass greater than 35 kDa being further probable to be hydrolyzed. Myosin heavyweight chains were the foremost complex myofibrillar protein, although actin, a-actinin, and tropomyosin were further opposing to enzymatic deprivation (Hernández-Herrero et al. 2000).

3.4 Health Profits of Marine Nutraceuticals

Marine nutraceuticals might ensure a positive impact on human health, as they will secure the physical body, in contrast to destruction by receptive oxygen species, which assault macromolecules like deoxyribonucleic acid (DNA), proteins, and deposit lipids, ultimately, causing numerous disorders like inflammatory disorder by severe tissue damages, diabetes mellitus, a neurodegenerative disorder, and cancer. (Ngo et al. 2011). Recently, chitooligosaccharides (COS) are the topic of increased attention in terms of their pharmaceutical and medicinal uses (Kim and Mendis 2006), owing to their omitted toxicity and elevated solubility, also their

progressive physiological actions such as adipogenesis inhibition, antimicrobial action, anticoagulant possessions, antioxidants, hypocholesterolemia, ACE enzyme inhibition, anti-Alzheimer's, anticancer, and hypoglycemic and antidiabetic activity (Kim 2012).

In the prevention of various human diseases such as some of the chronic diseases, cardiovascular diseases, and cancer, carotenoids are believed to be liable for valuable possessions. Moreover, marine-derived sterols have considerable responsiveness due to their cholesterol-lowering properties. Further, marine algal-derived sulfated polysaccharides exhibit a variety of health-beneficial biological activities like anti-cancer activities, anticoagulant action, anti-HIV-1, and immunomodulating action (Wijesekara et al. 2011).

Besides, some bioactive peptides from marine organisms are recognized to possess nutraceutical abilities for human health elevation and disease threat reduction (Shahidi and Zhong 2008), and currently the probable roles of food-derived bioactive peptides in reducing the danger of cardiovascular diseases have been demonstrated (Erdmann et al. 2008; Zhang et al. 2013). Additionally, saringosterol, a derivative of fucosterol, discovered in numerous algae (Phaeophyceae), like *Lessonia nigrescens* and *Sargassum ringgoldianum*, have been revealed to prevent the expansion of tubercle bacillus.

Although shellfish like abalones contain diverse nutrient content, which often can be changed, depending on temperature (season), extraction time (growth stage), and reproductive stage, the consumption of abalone provides several benefits, including rich nutrition diet and defense against various diseases (Yoo and Chung 2007; Zhou et al. 2012). Abalone contains lower amounts of calories in comparison to other animal foods. Current dietary recommendations signify that reducing total calories within the diet leads to maintaining weight and decreasing the danger of varied disorders, especially cardiovascular diseases (Larsen et al. 2011). Numerous studies have shown that abalone-like mollusks carry balanced nutritional characteristics that improve human health (Benkendorff 2010). Abalone may be a rich source of protein that helps to take care of all types of health benefits. Analysis of nutritional composition shows that the edible segment of 100 g of abalone consists of 20 g of protein. Collagen is the supreme copious structural protein within the physical body. Two categories of fibril-forming collagens are identified from *Haliotis discus* foot muscle (Yoneda et al. 1999). Also, a considerably higher quantity of taurine is available in abalone that aids in shielding the intestine, reducing the oxidative damage via antioxidant function, maintaining a healthy liver, a quick improvement from fatigue, preventing myocardial infarction, and minimizing allergies (Kim et al. 2006; Kim et al. 2007a, 2007b). Seven and eight sorts of non-volatile organic acids are identified from dried–boiled and fresh abalone, respectively. The most copious organic acids are reported as succinic, lactic, and pyroglutamic acids (Jo and Park 1985).

3.5 Conclusion

With numerous novel types of maritime resources still to be discovered, the forthcoming for new marine-derived bioactive nutraceuticals is immense with useful effects on human health, and therefore the food manufacturing is poised for enhanced expansion soon. Marine resources are accepted for their naturally dynamic constituents with immense perspective to be used as nutraceuticals. Furthermore, ample consideration has been given in recent times by the consumers in the direction of a healthy way of life by natural bioactive components. Modern research has delivered proof that marine-derived bioactive nutraceuticals play an important role in human health.

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Part I

Marine Microbes: Potential Candidates for Medical Applications

Small in Size, Big in Impact: Marine Microbes, a Boon for Biotherapeutics

4

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Abstract

Due to re-emerging infections and multiple drug-resistant pathogens, scientists initiated the investigation of marine microbes for their anti-infective characteristics. About 70% of the Earth's surface gets cover by the ocean, which is a vast habitat for marine microbes, and out of those few microbial classes endure only in the sea. The benefit with the marine microbes is that it fits in the traditional pharmaceutical "model," so there is no need for the extra effort for drug extraction from them. The marine microbes secrete the secondary metabolites, having a variety of bioactivities. In the 1950s, two drugs (Ara-C as anticancer and Ara-A as antiviral) isolated from a shallow-water sponge of the Florida coast launched in the market for the first time opened the gate for the marine microbes as the promising source of new drugs. The marine microbes also produce nutritional supplements, for example, marine alga secretes the docosahexaenoic acid (DHA), which is an essential unsaturated fatty acid of breast milk, and, nowadays, this is used in the formula milk of infants. The ocean should be explored more in search of novel marine drugs because the preclinical marine pharmacology pipeline is found to be very productive.

Keywords

Marine microbes · Secondary metabolites · Bioactive components · Biopharmaceuticals · Nutraceuticals · Marine natural products (MNPs)

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4.1 Introduction

The marine environment has become a hub for research on natural drugs because of its unexplored biodiversity in comparison to the terrestrial environment. The ocean covers about 71% of the Earth, and the sea has still not been explored thoroughly in search of natural products. The ocean represents a virtually untouched resource for the discovery of new pharmaceutical compounds. The ocean has representatives of every phylum, out of which 12 are found exclusively in the marine domain.

It is not known when the exploration of the marine environment for medicinal purposes began., Still, few pieces of evidence prove that both the Chinese and the Japanese were using marine products in their herbal medicines (Newman and Cragg 2016). During the Roman times, "Tyrian Purple," a dyestuff isolated from the Mediterranean mollusk, was utilized for treating various cancers, and the dye was used in traditional medication for the treatment of leukemia (Newman and Cragg 2016). Since the 1950s, over 20,000 marine natural products (MNPs) have been determined, and their chemical diversity and bioactivity have led them mentioned as "blue gold" in the search for novel drugs.

In this chapter, we have tried to summarize the journey of marine bioactive products as drug and food supplement for human welfare.

4.2 Ocean: A Source of Chemical Diversity

The ocean is known for its biodiversity, as organisms from almost every phylum reside there, and, among them, 12 phyla are reported to be exclusive marine dwellers. In the ocean, the existence of more than 200,000 species of invertebrates and algae are found (Winston 1992); however, it is predicted that this number is a small fraction of the number of species discovered and described. Marine plants, animals, and microbes produce plenty of secondary metabolites that are not required for their regular metabolism., Still, these metabolites provide protection and, at times, are involved in their defense process. These also play a role in their evolutionary changes. The highly distributed marine invertebrate is the sponge, which occurs in every marine environment from the intertidal to the abyssal region and possesses a vastly distributed range of secondary metabolites. The variation in the chemical structure of secondary metabolites shows the deviation in their biotherapeutic properties. Like marine sponges, the other marine organisms containing bioactive molecules with therapeutic potential are bryozoans, ascidians, mollusks, cnidarians, and algae. Several chemical compounds have been isolated from marine organisms, but only a few of these compounds have been tested clinically for their therapeutic competency.

4.3 Marine Microbes as a Novel Resource for Drugs

The finding of penicillin in the late 1920s from soil-derived microorganisms opened the scope for identifying novel drugs from the microbes. Currently, around 120 microbially produced drugs are in clinical use to treat infectious diseases and facilitate organ transplantation by suppressing the immune response. These highly used drugs include the penicillin, cephalosporins, streptomycin, and vancomycin as the antibiotics; actinomycin and mitomycin as the anticancer drugs; and cyclosporin as the immunosuppressant. These useful microorganisms are also cultured industrially for the large-scale production of drugs.

For the past 50 years, microbial-derived drugs were studied mainly from terrestrial microorganisms. Later, it was observed that marine microbial resources possess different potential profiles; therefore, researchers began to focus on marine microbes. Furthermore, it has been discovered that many classes of microorganisms live only in the ocean in addition to the common organisms found on land. Due to difficulties in cultivating marine microbes, the pharmaceutical industry did not benefit from this enormous resource. Now, times have changed, and marine microorganisms can be successfully grown, opening up the entry of marine environments to the scientific world (Davidson 1995).

4.3.1 Metabolites from Marine *Actinobacteria*

Actinobacteria cover two-thirds of the antibiotic-producing bacterial population, but most of the antibiotic-producing actinobacteria are land dwellers. The first marine mycelium having the characteristic similar to terrestrial actinobacteria was studied around 1969 (Weyland 1969). After that few more studies revealed marine *Actinobacteria* as a new therapeutic agent (Manivasagan et al. 2013, 2014). These inhabit marine environments such as mangroves, seaweeds, beside seawater, sediments, and even marine hosts such as the mollusks, fishes, sponges, etc. These organisms possess unique bioactive compounds such as anticancer and immunosuppressive agents, antibiotics, antioxidants, enzyme inhibitors, and pigments.

Although marine *Actinobacteria* show broad pharmacological effects, it has been extensively studied for its antibacterial property. A novel polycyclic polyketide antibiotic, abyssomicin C, isolated from marine *Verrucosispora* sp., was found to be effective against Gram-positive bacteria as it inhibits the para-aminobenzoic acid biosynthesis and, hence, inhibits the folic acid biosynthesis more effectively in comparison to synthetic sulfa drugs (Bister et al. 2004). Another antibiotic essramycin, produced by *Streptomyces* sp., was active against Gram-positive and Gram-negative bacteria (El-Gendy et al. 2008). Micromonospora produces a farnesylated dibenzodiazepinone alkaloid, diazepinomicin, which, along with anti-microbial activity showed anti-inflammatory and antitumor properties (Charan et al. 2004). A novel macrolide, curvularin-7-o-α-d-glucopyranoside, isolated from marine actinomycete *Pseudonocardia* sp., showed the suppression of cell proliferation in six tested cancer cell lines along with antibacterial activity against

Escherichia coli (Ye et al. 2016). Two compounds isolated from *Streptomyces* sp., bisanthraquinone, and tirandamycin C, act as antibacterial against vancomycin-resistant *Enterococcus faecalis* (Socha et al. 2006; Carlson et al. 2009). Bisanthraquinone also shows bioactivity against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-susceptible *S. aureus* (MSSA), and tetracycline-resistant *S. aureus* (TRSA) (Socha et al. 2006). Salinisporamycin, a new polyketide antibiotic isolated from *Salinispora arenicora,* displayed cytotoxic activity by inhibiting the growth of human lung adenocarcinoma cell lines along with antibacterial activity against *S. aureus* (Matsuda et al. 2009).

In the last few years, cases of fungal infection accidentally increased in the immunocompromised individuals, and due to several side effects of synthetic antifungal compounds, the researchers moved to the natural products. Chandrananimycin A, a novel Phenoxazin-3-one antibiotic isolated from *Actinomadura* sp., showed antifungal activity against *Mucor miehei*. Alternatively, this compound also unveiled as anticancer, antibacterial, and antialgal compound (Maskey et al. 2003). Saadamycin, an antifungal obtained from *Streptomyces* sp. showed potent antifungal activity against *Candida albicans*, *Aspergillus*, and *Cryptococcus.* An antibiotic isolated from *Nocardia dassonvillei*, N-(2-hydroxyphenyl)-2-phenazinamine (NHP), shows the antifungal activity against *C. albicans* and a high level of cytotoxicity (Gao et al. 2012).

Several compounds isolated from marine actinomycetes also show antiviral activities. An alkaloid isolated from *Streptomyces nitrosporeus*, benzastatin C, showed antiviral activity against vesicular stomatitis virus (VSV), and herpes simplex virus (HSV) type-1 and type-2 (Lee et al. 2007). In 2014, Strand et al. reported anti-adenoviral characteristics of butanolides 1a, 1b, 2, 3, and 4, extracted from *Streptomyces* sp. Out of all butanolides, compound 3 was reported as the most effective anti-adenoviral agent (Strand et al. 2014). Antimycin A1a, the compound extracted from *Streptomyces kaviengensis*, displayed the antiviral activity against the Western equine encephalitis virus by inhibiting the cellular mitochondrial electron transport chain (mETC) complex III (Raveh et al. 2013).

Cancer is considered as the malignant tumor, and presently, few compounds of marine actinobacteria show the bioactivity against cancer. The compound streptoanthraquinone A, isolated from *Streptomyces* sp., inhibited the proliferation and induced apoptosis in four different glioma cell lines (Liang et al. 2016). Another bioactive compound obtained from marine *Streptomyces* sp., strepsesquitriol, presented moderate inhibitory activity against lipopolysaccharide-induced tumor necrosis factor- α (TNF-α) production in macrophages (Yang et al. 2013). Salinosporamide A, a novel rare bicyclic beta-lactone gamma-lactam isolated from *Salinispora tropica*, acts as a proteasome inhibitor, which further leads to apoptosis in multiple myeloma cells (Prudhomme et al. 2008).

The multiple roles of natural products derived from marine actinobacteria provide anticipation that natural marine compounds will formulate a new wave of drug discovery in the coming years.

4.3.2 Metabolites from Marine Cyanobacteria

Cyanobacteria (blue-green algae) live in terrestrial and marine environments under the extreme conditions where normal survival is impossible. In the marine environment, cyanobacteria show dual habitat: free-living plankton in the open ocean and near shores, and as symbionts with invertebrates such as sponges. Cyanobacteria were the first studied photosynthetic bacteria and played an essential role in the global cycles. The extraordinary characteristic of cyanobacteria has attracted researchers for the biomedical properties of secondary metabolites. Although the cyanobacterial strains belonging to freshwater and brackish water are known to produce toxic metabolites (Wiegand and Pflugmacher 2005), secondary metabolites of the marine cyanobacteria appear to have potential pharmacological properties, especially in case of cancer therapy. The diversity in the metabolites of freshwater and marine cyanobacteria reflects their evolutionary fluctuations in the metabolic enzymes. The chemical compounds secreted by cyanobacteria comprise peptides, glicomacrolides, macrolactones, fatty acid amides, and swinholides, which show a wide range of pharmacological activities, such as antifungal, antiviral, antibacterial, and antitumor (do Rosário Martins and Costa 2015). Considering cancer as the primary cause of the death of the human population, the researchers focused on the marine cyanobacteria-derived compounds having potential antitumor bioactivities.

Among the marine cyanobacteria, most of the chemical compounds that possess potential bioactivity have been isolated either from the filamentous *Lyngbya* or from some strains of *Moorea* (Engene et al. 2011, 2012). Further, the investigation progressed toward other cyanobacterial species. A new anticancer compound named hierridin B was isolated from a *Cyanobium* strain, which proved to be effective against the colon cancer cell line (Leao et al. 2013). After this, the anticancer potential of the compounds, extracted from 28 different cyanobacterial strains of the genera *Nodosilinea*, *Leptolyngbya*, *Pseudanabaena*, *Romeria*, *Cyanobium*, *Synechococcus and Synechocystis*, and *Synechococcus* were tested and the results obtained from these studies proved these strains as the source of novel compounds (Costa et al. 2013).

Apoptosis is considered as an effective process for the cytotoxicity by drugs due to the lack of inflammatory response in it. So, the researchers focus on such compounds in cancer therapy, which act as apoptotic inducers. Few compounds (cryptophycin 1, calothrixin A, dolastatin 10, lagunamide A, and somocystinamide A) belonging to the cyanobacteria are found to induce the apoptotic morphological changes in the cancer cell lines, which comprises cell shrinkage, blebbing of plasma membrane, chromatin condensation, nucleolus segregation, nuclear fragmentation, and formation of apoptotic bodies (do Rosário Martins and Costa 2015). Some of the cyanobacterial strains also induce the caspases and so finally help in the promotion of cytotoxicity by apoptosis. Caspases are the proteases, which are initiators and effectors, and these are involved in the execution phase of apoptosis. The compounds dolastatin 10, coibamide A, cryptophycin 1, biselyngbyaside, and curacin A stimulate caspase 3, while lagunamide A induce caspase 9 activation at

the nM range. Another cyanobacterial compound somocystinamide A is found to act as the potent inductor of apoptosis by activating caspase 8 in numerous malignant tumors (Barnhart et al. 2004). The Bcl-2 protein family also regulates apoptosis, and this family has both pro- and anti-apoptotic proteins. The pro-apoptotic proteins cause the cytosolic release of cytochrome c. In the cytosol, cytochrome c promotes apoptosome formation, which then activates the caspase 3, while the anti-apoptotic proteins constrain the activity of pro-apoptotic proteins. In this apoptotic process, also, two cyanobacterial compounds—dolastatin 10 and symplostatin 1—modulate the apoptotic process in the cell by enhancing the pro-apoptotic proteins (Martins and Costa 2015).

Few of the extracted compounds from cyanobacteria displayed the anticancer potential by disrupting the microtubular structure and, lastly, affecting cellular integrity. The marine cyanobacterial compounds that cause the depolymerization of microtubule in G2/M phase include dolastatin 10, symplostatin 1, and symplostatin 3, and the linear peptide belamide A isolated from *Symploca* (Luesch et al. 2001; Simmons et al. 2006).

Hence, undoubtedly, the compounds isolated from marine cyanobacteria proved to be the rich source of potential anticancer drugs with their capability to target distinctive metabolic pathways involved in cancer.

4.3.3 Metabolites from Marine Fungi

The natural products (matrix metalloproteinases [MMP] inhibitors, topoisomerase inhibitors, protein kinase C [PKC] inhibitors, cytotoxic-inducing, apoptosis-inducing, and anti-inflammatory metabolites) derived from the marine fungi are found to be useful for the cancer therapy because these products hinder the various signaling pathways that lead to tumor generation.

Matrix metalloproteinases (MMPs) are the zinc-dependent protein-degrading endopeptidases, which destroy the extracellular matrix (ECM). Generally, these peptidases express at a low level, but the high expression of MMPs causes unwanted tissue damage, inflammation, tumor progression, and metastasis. The marine fungal species of *Microsporum* produces few secondary metabolites: chrysophanol, physcion, and emodin, which act as MMP inhibitors and hence show antitumor properties (Karuppiah et al. 2015). These antitumor compounds mainly act on two matrix metalloproteinases, MMP-2 and MMP-9, capable of degrading type IV collagen. This collagen provides structural support to cells and, hence, maintains the tissue organization, and the damage to the basement membrane leads to the metastatic progression in most cancers.

Topoisomerases are the enzymes that maintain the deoxyribonucleic acid (DNA) helix integrity during cell division. During the tumor progression, the topoisomerase level suddenly increases because of its importance in cell multiplication. So, for cancer treatment, anticancer drugs have been developed against the DNA topoisomerases I and II. Two drugs, leptosins (Leps) F and C, against topoisomerase, were isolated from a marine fungus, *Leptoshaeria*. Lep F hinders the activity of

topoisomerase I and II, while Lep C inhibits topoisomerase I only. Later, it is found that Lep C prevents the G1 to S phase transition in the cell cycle. The marine lichen-derived *Gliocladium* sp. T31 fungus secretes secalonic acid D (SAD) that inhibits topoisomerase I's binding to DNA (Hong 2011).

The protein kinase Cs (PKCs), several serine-threonine kinases, are a well-known player in tumor progression and maintenance of the malignant phenotype. Protein kinase inhibitors block the downstream signaling and so act as an effective anticancer drug. Many protein kinase inhibitors have been isolated from marine fungi. The marine fungus *Chaetomium* sp. produces chaetominedione, a novel benzonaphthyridinedione derivative, which inhibits the activity of the p56lck tyrosine kinase (Abdel-Lateff 2008). Another tyrosine kinase inhibitor was isolated from *Ulocladium botrytis*, *Microsphaeropsis*, *Aplysina aerophoba*, and *Halorosellinia* (Karuppiah et al. 2015).

The fungal-derived products also act in regulating apoptosis. The fungal compound anthracenedione and its derivatives isolated from mangrove endophytic fungi *Halorosellinia* sp. and *Guignardia* sp. inhibits the mitochondrial function in cancer cell KB and thus lead to apoptosis (Zhang et al. 2010). A new metabolite, namely protuboxepin A isolated from marine *Aspergillus* sp. SF-5044 showed antiproliferative activity in several cancer cell lines by stabilizing the tubulin polymerization and so affected the microtubule dynamics, and finally arrest the cell cycle at metaphase (Asami et al. 2012). Phomopsidin, a microtubule assembly inhibitor, has been obtained from the *Phomopsis* sp., which shows potency similar to colchicine and rhizoxin. Wentilactone B (WB), a tetranorditerpenoid, isolated from the endophytic fungus *Aspergillus wentii* EN-48, induces cell division arrest at G2/M phase and induces apoptosis in hepatoma cells (Zhang et al. 2013). Mycoepoxydiene (MED), a polyketide isolated from the marine fungus of the mangrove forests, induces cell cycle arrest by affecting the tubulin polymerization and activates apoptosis by activating caspase-3 and triggering the release of cytochrome c (Wang et al. 2010).

The epidemiologic studies support the idea that inflammation and cancer are co-related because the production of reactive oxygen and nitrogen radicals in the inflammatory cells cause mutagenic alterations and result in tumor progression (Okada 2002). A variety of mediators, including cytokines, chemokines, and enzymes, promote cancer growth from inflammation. A cytochalasan-based alkaloid chaetoglobosin Fex (Cha Fex), isolated from the marine endophytic fungus *Chaetomium globosum* QEN-14, inhibits the production of tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and monocyte chemotactic protein-1 (MCP-1) in macrophages (Dou et al. 2011). Two more alkaloids, neoechinulins A and B, isolated from the marine fungus *Eurotium* sp. SF-5989, show the inhibitory effect on nitric oxide (NO) production. Additionally, neoechinulin A reduces the secretion of pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) (Song et al. 2013). Many potent new classes of anti-inflammatory compounds have been identified from marine fungi.

Some cytotoxic compounds have been isolated from the marine fungi, which are found to be effective in leukemia and other cancer cell lines. The compounds efficient in leukemia include the gymnastatins A, B, and C of *Gymnascella dankaliensis* (Amagata et al. 1998) and asperazine isolated from *Aspergillus niger* (Varoglu and Crews 2000). Leptosin, one of the largest classes of cytotoxic fungal metabolites produced by *Leptosphaeria* sp., and pyrenocine E, isolated from the *Penicillium waksmanii*, show cytotoxicity against the leukemia cells (Karuppiah et al. 2015). The polyphenols, expansols A and B, isolated from marine-derived fungus *Penicillium expansum*, show cytotoxicity against the HL-60 cell line (Lu et al. 2010). Coriolin B, along with three new chlorinated cyclic sesquiterpenes, has been isolated from the marine fungi, and findings revealed that these metabolites are effective against breast cancer and neuroblastoma cell lines (Efferth 2010). The cytotoxic lipopeptide isolated from marine *Aspergillus versicolor* is effective against neuroblastoma and colon cancer cell lines (Lee et al. 2010). The prenylated polyketides, epoxyphomalin A and epoxyphomalin B, isolated from *Phoma* sp., showed superior cytotoxicity toward human tumor cell lines (Mohamed et al. 2009).

4.3.4 Metabolites from Marine Algae

Currently, the polyphenols (phlorotannins) isolated from the marine algae are found to be pharmacologically active against human disorders. More than 8000 phenolic compounds have been found in the present scenario, and most of them belong to the marine algal origin. The marine algae survive in the harsh environment, so they produce potent polyphenolic compounds that are generally not manufactured in the plants. These phenols differ structurally in nature from simple phenolic acids to the complex phlorotannins. The two most studied marine brown algae for their bioactive compounds include *Ecklonia cava* and *Eisenia bicyclis* (Thomas and Kim 2011). The compounds extracted from them found to act as anti-inflammatory and antidiabetic, in addition they also affect the hyaluronidase activity.

Polyphenolic compounds are effective anti-diabetic compounds. At present, diabetes mellitus (DM) has emerged as a common and challenging disease due to the deficiency of insulin secretion. Out of type I and II DMs, type II therapy is a challenge because of its insulin-independent nature. In the human body, carbohydrate digestion occurs by two enzymes α-glucosidase and α-amylase, and it is found that for the regulation of DM, these two enzymes should be inhibited. The phloroglucinol derivatives isolated from *E. bicyclia* have shown the inhibitory effects on glycation and α-amylase (Okada et al. 2004). The compound isolated from *Ecklonia stolonifera* proved as the antidiabetic compound due to its inhibitory effect against the advanced glycation end products, angiotensin-converting enzymes, rat lens aldose reductase, peroxynitrile, and reactive oxygen species in the diabetic complicated case (Jung et al. 2008), and the methanolic extract from *E. stolonifera* acts as an inhibitor for α-glucosidase. Diphloroethohydroxycarmalol (DPHC), a phlorotannin isolated from the brown algae *Ishige okamurae*, has shown

the inhibitory effect against α-glucosidase and α-amylase, and this DPHC also lowered the dietary carbohydrate absorption in the intestine (Heo et al. 2009a).

Phlorotannins also possess anticancer properties, which proved by examining its effect on the cancer cell lines. The phlorotannin extract (PE), derived from brown algae *Laminaria japonica*, was found to inhibit the cell division in the hepatocellular carcinoma cell lines (BEL-7402) and the murine leukemic cell line (P388) in a dose-dependent manner (Yang et al. 2010). Another polyphenol, dioxinodehydroeckol, isolated from *Ecklonia cava*, has shown a remarkable antiproliferative effect on human breast cancer cell line (MCF-7), by inducing the apoptosis in these cells by enhancing the NF-κB-dependent pathway (Kong et al. 2009). The extracts from red algae, *Palmaria palmate,* and three kelp *Laminaria setchellii*, *Macrocystis integrifolia*, and *Nereocystis leutkeana*, showed an antiproliferative effect in the human cervical adenocarcinoma cell line (HeLa cells) (Yuan and Walsh 2006).

Blood pressure has also emerged as one of the major health problems, nowadays, that further could lead to the progress of cardiovascular and renal diseases. Angiotensin I-converting enzyme (ACE) plays a major role in blood pressure regulation, by converting the inactive form of decapeptide angiotensin I to potent vasopressor octapeptide angiotensin II. Due to the emergence of side effects against synthetic drugs, scientists progressed their research in search of natural compounds for controlling blood pressure. Three phlorotannins—eckol, phlorofucofuroeckol A, and dieckol—isolated from *E. stolonifera* are found to be ACE inhibitors (Jung et al. 2006). ACE belongs to the family of zinc protease, so it requires Zn^{2+} for its activity. The three phlorotannins mentioned above (eckol, phlorofucofuroeckol A, and dieckol) act as the inhibitor by blocking Zn^{2+},. These might form a complex with proteins or glycoproteins that are involved in the inhibition of ACE activity. The flavourzyme, isolated from *E. cava*, also possesses the ACE inhibitory effect (Athukorala and Jeon 2005). The aqueous extract from the two red algae—*Lomentaria catenata* and *Lithophyllum okamurae*—is also found to have the ACE inhibitor property (Cha et al. 2006).

Extracts from the brown algae are also reported as the defender of skin damage. The human skin provides primary protection, but continuous exposure to ultraviolet (UV) radiation causes skin damage. The UV rays have damaging oxidative components, which lead to photooxidative stress. In that case, the intake of antioxidants is preferred, and the brown algae's phlorotannins were found to be the primary source of the antioxidants, and they also absorb UV light (Henry and Van Alstyne 2004). Phlorotannin and dieckol, isolated from *E. cava,* have been investigated for their ability to inhibit melanogenesis as well as against photooxidative stress (Heo et al. 2009b). Two phlorotannins—eckol and dieckol—isolated from another brown alga *E. stolonifera* deteriorate the expression of matrix metalloproteinase-1 in dermal fibroblast (MMP-1) (Joe et al. 2006). MMP-1 is responsible for the ageing process, so the abovementioned two phlorotannins are used to treat skin ageing. The polyphenol isolated from the brown algae inhibits cyclooxygenase-2 (COX-2) expression and cell proliferation in SKH-1 hairless mouse skin model (Hwang et al. 2006). These results also recommend brown algae

as a potential cancer chemopreventive agent against photo-carcinogenesis and other adverse effects of UV-B exposure.

The bioactive compound, 6,6'-bieckol, a phloroglucinol derivative isolated from *E. cava,* has been reported to inhibit HIV-1-induced syncytia formation, lytic effects, and viral p24 antigen production in vitro and in cellular experiments (Artan et al. 2008). Another group reported the inhibitory effect of 8,8′-bieckol and 8,4″-dieckol on HIV-1 reverse transcriptase and protease (Ahn et al. 2007). Their study proved the phlorotannin to be a more potent inhibitor of reverse transcriptase in comparison to protease. These outcomes intimate phlorotannins as a potent inhibitor of HIV-1 infections.

4.4 Novel Nutraceuticals Derived from Marine Microbes

Along with the pharmaceutical bioactive compounds, the marine microorganisms also contain compounds having nutritional values. Few marine algae are known to possess vitamins in more concentration compared to conventional food. Ingestion of these algae can cope up with the world requirement of nutritional supplements. The microalgae, which are known for having maximum concentrations of the different vitamins, are *Dunaliella tertiolecta* for β-carotene, folic acid, riboflavin, and cobalamin; *Tetraselmis suecica* for ascorbic acid, pantothenic acid, thiamin, nicotinic acid, and pyridoxin; and *Chlorella stigrnatophora* for biotin and tocopherol (vitamin E) (Fabregas and Herrero 1990).

Docosahexenoic acid (DHA) and arachidonic acid (ARA) are the widely distributed polyunsaturated fatty acids (PUFAs) of breast milk and are also the predominant structural fatty acids in brain gray matter (Agostoni 2008). So, it is believed that these PUFAs can lead to brain development, so they have been recommended as nutritional supplements for infants. A marine microalgal species has been discovered to produce large quantities of the fatty acid docosahexaenoic acid (DHA). It is used in an infant formula supplement Formulaid® (Martek Biosciences, Columbia, MD) (Kanase and Singh 2018).

Marine-derived nutritional supplements, or "nutraceuticals," present a new opportunity for research in the application of marine natural products to human health issues.

4.5 Potential Evolution of Marine Natural Products as the Biotherapeutics

Few of the marine natural products have been introduced as pharmaceutical compounds in the 1950s (Bergmann and Feeney 1951; Bergmann and Burke 1955), which opened the gate for novel compounds and those studies led to introduction of two marine-derived medicines that are clinically available today. These two market-available drugs are the antileukemic drug, Ara-C (cytarabine), used to treat acute myelocytic leukemia and non-Hodgkin's lymphoma, and the antiviral

drug, Ara-A (vidarabine), used for the treatment of herpes infections (Mayer and Gustafson 2008). Both are derived from nucleosides isolated from a shallow-water marine sponge collected off the coast of Florida. After the success of Ara-C and Ara-A, the US Food and Drug Administration also approved other MNPs as biotherapeutics (Table 4.1).

The bioactive compounds derived from soil-dwelling and marine microorganisms are equally effective for diseases. So, the systematic search of bioactive compounds from marine organisms has opened the gateway for the pharmaceutical companies.

The ocean covers 70% of the earth's surface, but the researchers are still unable to exploit the resources because of several pitfalls. For the proper cultivation of marine microorganisms and isolation of drugs from them, precise taxonomic determination of the microbial species is necessary. The suitable metagenomic approaches are needed for the screening of microorganisms. Further study of microorganisms at the transcriptomic and proteomics levels is required for determining the secondary metabolites within them. For isolation of bioactive compounds from marine microorganisms living in deep sea regions and extreme conditions, the advancement in the sampling techniques is needed. like terrestrial drugs, the market access for marine-derived drugs also requires a long time and a lot of money. In such a case, many marine-derived products get damaged, and so the drugs become unproductive.

In the future, through interdisciplinary collaboration, researchers could overcome the hindrances mentioned earlier, and pharma companies can approach the market with novel drugs for the treatment of incurable diseases like cancer.

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Conflict of Interest The authors declare no competing interests.

Table 4.1 Marine organism–derived drugs and their clinical status

Marine organism	Compound	Molecular target	Disease	Clinical status	References
Cryptotheca crypta (sponge)	Cytarabine (cytosar-U®)	DNA polymerase	Leukemia	FDA approved	Kim (2014)
Tethya crypta (sponge)	Vidarabine (Vira-A)	Viral DNA polymerase	Herpes simplex virus infection	FDA approved (current status: Discontinued)	Field and Clercq (2004)
Conus magus (marine snail)	Ziconotide (PRIALT®)	DNA polymerase	Severe and chronic pain	FDA approved	Williams et al. (2008)
Fish (fish oil)	Omega-3-acid ethyl esters (Omacor®)	Triglyceride-producing enzymes	Hypertriglyceridemia	FDA approved	Rupp (2009)
Dorabella auricularia Lightfoot (sponge)	Eribulin mesylate (Halaven®)	Microtubule inhibition	Metastatic breast cancer	FDA approved	Gerwick and Moore (2012)
Mollusk/ cyanobacteria	Brentuximab vedotin (Adcetris®)	CD30, microtubules	Anaplastic large T-cell systemic malignant lymphoma and Hodgkin's disease	FDA approved	Chen et al. (2015)
Ecteinascidia turbinate (sea squirt)	Trabectedin (Yondelis®)	DNA (minor groove)	Soft tissue sarcoma and ovarian cancer	EU approved	Kim (2014)

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Marine Microbial Pharmacognosy: Prospects and Perspectives

5

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Abstract

Modern scientific advancements and research on marine microbes has revealed their significance as producers of therapeutic products useful in treating various human diseases. Microbes in marine habitat have evolved to adapt to the harsh condition that prevails in the ocean. Their struggle to compete for space and nutrients has paved way for the synthesis of different novel enzymes possessing distinctive characteristics. Thus, marine habitat hosts many remarkable microorganisms that offer unique biologically active compounds, enzymes endowed with astonishing properties, and mechanism to survive in extreme environmental conditions. The utilization of marine biotic resources grows at an extraordinary growth rate of 12% per annum and is evident from about 4900 patents filed connected with marine genetic resources and 18,000 natural compounds. This concern has boosted research all over the world to explore the untapped potential hidden in marine microbes, which has lot of biotechnological

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applications that includes bioactive compounds (metabolites) for therapeutics, novel enzymes, cosmetics, and nutraceuticals. This book chapter will meticulously deliberate the utilization of marine resources by biotechnological applications for therapeutics like antibiotics, chemical compounds, biopolymer, enzymes, and various microbial biomedical purposes such as drug delivery and tissue engineering from marine biota (bacteria, fungi, and algae).

Keywords

Microbial Pharmacognosy · Bioactive compounds · Biomedical application

5.1 Introduction

The ocean engulfs about 70% of the area on planet earth whereas the aquatic ecosystem houses nearly 80% of living organisms on the whole biosphere. The marine environment hosts 178,000 different species of microorganisms (34 phyla) as reported by the United Nations Environment Programme on Global Biodiversity Assessment (UEPA 2006). The marine unicellular organisms play a crucial role in the conservation and sustainability of the marine ecosystem. The marine microbes are competent in enduring from volcanic eruptions to Antarctic glacier, and they possess numerous distinctive adaptations compared to the terrestrial microbes. Marine microbes adapt to environmental variations like high salt concentration, extreme temperature, low or higher concentrations of organic matter, high hydrostatic pressure, and other external physiochemical factors. Due to their continued exposure to various environmental changes, they have developed unique defense and survival mechanisms employing secondary metabolites that can sense, adopt, and protect them from such harsh conditions.

Microbes are the modern day marvel, whose potential has not been fully explored, yet they offer extensive application in diverse fields like heavy metal bioremediation (Rainbow 1995), antibiotics and enzyme production (Okami 1986), biodegradation and bioremediation of hydrocarbons (Mohanrasu et al. 2018), biosurfactant production (Maneerat and Phetrong 2007), degradation of plastic debris (Mohanrasu et al. 2018), anti-biofilm activity (Jiang et al. 2011), and polyhydroxybutyrate (bioplastics) synthesis (Arun et al. 2006). Recently, researchers have discovered a number of novel metabolites from marine bioresources including macro or micro algae, bacteria, and fungi that are used as antimicrobial, anti-obesity, antitumorous, antidiabetic, immunological, and therapeutic potential biomolecules. For example, the *Pseudomonas* genus serves as a wellspring of bioactive compounds such as andrimid, bushrin, moiramides, phthalate, pseudopeptide, phloroglucinol, phenazine, pyrroles, pyrrolidinedione, phenanthrene, quinolone, and zafrin for the treatment of many diseases (Romanenko et al. 2008).

The marine microbial biosphere delivers a variety of biomolecules that cater diverse novel biologically active compounds for pharmaceutical applications. This

Fig. 5.1 Pharmaceutical importance of marine microbes

chapter exclusively focuses on biologically active compounds synthesized by marine microbes for pharmaceutical applications; we highlight the varieties of biological compounds from marine-based algae, bacteria, and fungi (Fig. 5.1 and Table 5.1).

5.2 Past, Present of Marine Microbial Pharmacognosy

In today's modern world, with increasing population and demanding food industry, marine habitat acts as a crucial food source that caters around 90 million tons of food per year. Due to the huge biodiversity, marine environment offers a variety of biologically active compounds that could be efficiently employed to treat new diseases. Emerging infectious diseases with newly emerging drug-resistant microbial strains demand pristine compounds that would be tailored by marine microbes as researchers have shifted their interest toward the quest for bioactives from marine

Table 5.1 Novel bioactive compounds produced by marine microbes

Source	Compound	Activity	Literature
Micromonospora sp.	Thiocoraline	Antitumor, antimicrobial	Romero et al. (1997)
Streptomyces sp.	Salinamides	Anti-inflammatory	Moore et al. (1999)
Streptomyces sp.	Himalomycins	Antibacterial	Maskey et al. (2003)
Streptomyces sp. KS3	Komodoquinone A	Neuritogenic	Itoh et al. (2003)
Streptomyces sp. BD21-2	Bonactin	Antimicrobial	Schumacher et al. (2003)
Salinispora tropica	Salinosporamide A	Anticancer	Feling et al. (2003)
Streptomyces sp. B8652	Complex compounds	Anticancer, antimalarial	Maskey et al. (2004)
Verrucosispora	Abyssomicin C	Antibacterial	Riedlinger et al. (2004)
Verrucosispora sp.	Abyssomicins	Antibacterial	Riedlinger et al. (2004)
Streptomyces aureoverticillatus NPS001583	Aureoverticillactam	Antitumor	Mitchell et al. (2004)
Verrucosispora maris	Abyssomycin C	Antibacterial	Bister et al. (2004)
Streptomyces sp. M045	Chinikomycins	Antitumor	Li et al. (2005)
Streptomyces sp.	Glyciapyrroles	Antibacterial	Macherla et al. (2005)
Streptomyces sp.	10α,11-dihydroxyamorph-4-ene, 10α,15-dihydroxyamorph-4-en-3-one, and 5α,10α,11-trihydroxyamorphan-3-one	–	Macherla et al. (2005)
Thermoactinomyces sp.	Mechercharmycins	Antitumor	Kanoh et al. (2005)
Streptomyces sp. CNQ-085	Daryamides	Antitumor, antifungal	Asolkar et al. (2008)
Streptomyces CNQ766	Actinofuranones	Cytotoxic	Cho et al. (2006)
Streptomyces sp. KORDI-3238	Streptokordin	Anticancer	Jeong et al. (2006)
Streptomyces corchorusii AUBN1/7	Tetracenomycin D	Cytotoxic	Adinaryan et al. (2006)
Streptomyces sp. QD518	Selina-4(14),7(11)-diene-8,9-dio	Anticancer	Wu et al. (2006)
Streptoverticillium	Butenolides	Cytotoxic	Li et al. (2006)
Streptomyces sp. NTK 937	Caboxamycin	Anticancer	Hohmann et al. (2009c)
Streptomyces sp.	Piericidins	Anticancer	Hayakawa et al. (2007)

(continued)

Table 5.1 (continued)

Source	Compound	Activity	Literature
Nocardiopsis lucentensis	Lucentamycins	Cytotoxic	Cho et al. (2007)
Streptomyces sp.	Essramycin	Antibacterial	El-Gendy et al. (2008a, b)
Marinispora sp.	Lynamicins	Antibacterial	McArthur et al. (2008)
Salinispora arenicola	Saliniketals	Anticancer	
Salinispora arenicola	Arenicolides	Antitumor	Williams et al. (2007)
Brevibac illus laterosporus	Tauramamide tauramamide ethyl ester	Antimicrobial	Desjardine et al. (2007)
Marinispora sp. (NPS008920)	2-alkylidene-5-alkyl-4-oxazolidinones, lipoxazolidinone A, lipoxazolidinone B	Antimicrobial	Macherla et al. (2007)
Streptomyces sp.	Piperazimycins	Cytotoxic	Miller et al. (2007)
Marinispora sp. (NPS12745)	Lynamicins B	Antimicrobial	McArthur et al. (2008)
	Lynamicins C		
Salinispora pacifica CNS-237	Salinipyrones	Cytotoxic	Oh et al. (2008)
Salinispora pacifica CNS-237	Pacificanones	Cytotoxic	Oh et al. (2008)
Salinipora arenicola	Arenamides	Cytotoxic	Asolkar et al. (2008)
Streptomyces sp.,	Cyclomarines	Anti-inflammatory	Schultz et al. (2008)
Pseudomonas stutzeri	Zafrin	antimicrobial	Uzair et al. (2008)
Nocardia sp.	Ayamycin	Antifungal	El-Gendy et al. (2008a, b)
Streptomyces sannurensis	Marinopyrroles A	Cytotoxic & MRSA	Hughes et al. (2008)
	Marinopyrroles B		
Verrucosispora sp.	Proximicins	Cytostatic	Fiedler et al. (2008)
Streptomyces sp. CNQ-418	Marinopyrroles	Antibacterial	Hughes et al. (2008)
Streptomyces sp. Merv8102	Essramycin	Antibacterial	El-Gendy et al. (2008a, b)
Streptomyces sp.	Mansouramycins	Cytotoxic	Hawas et al. (2009)
Streptomyces sp.	Albidopyrone	Cytotoxic	Hohmann et al. (2009a)
Streptomyces sp.	Carboxamycin	Antibacterial, cytotoxic	Hohmann et al. (2009b)
Streptomyces sp.	2-Allyloxyphenol	Antimicrobial, antioxidant	Arumugam et al. (2010)
Dermacoccus sp.	Dermacozines	Cytotoxic, radical scavenging	Abdel-Mageed et al. (2010)
Streptomyces sp.	ML-449	Cytotoxic	Jørgensen et al. (2010)

(continued)

Table 5.1 (continued)

Source	Compound	Activity	Literature
Nocardiopsis sp.	TP-1161	Antibacterial	Engelhardt et al. (2010)
Actinomadura sp.	Halomadurones A–D	Potent Nrf2-ARE activation	Wyche et al. (2014)
Nocardiopsis sp.	Nocapyrones H–J	Pro-inflammatory factor, stronger inhibitory effect on nitric oxide	Kim et al. (2014)
Micrococcus sp.	Microluside A	Antibacterial activity	Eltamany et al. (2014)
Micromonospora sp.	MBJ-0003	Moderate cytotoxicity	Kawahara et al. (2014)
Actinomycetospora chlora	Thiasporines A–C	Cytotoxicity	Fu and MacMillan (2015)
Nocardiopsis sp.	Diketopiperazine 1	Sterol *O*-acyltransferase inhibitor	Kobayashi et al. (2015)
Verrucosispora sp.	Glycerol 1-hydroxy-2,5-dimethyl benzoate	Anti-MRSA activity	Huang et al. (2016)
Micromonospora sp.	Quinoline alkaloid	Antibacterial activity	Thi et al. (2016)
Pseudonocardia carboxydivorans	Branimycins B and C	Antibacterial activities	Braña et al. (2017)
Nocardiopsis sp.	Nocazines F and G	Excellent cytotoxicity	Sun et al. (2017)

sources. Though terrestrial plants and microbes have severed as an important source in last couple of decades for biomedical drug discovery and health, the untapped potentials of marine microbes have emerged as widespread resources. 1940s penicillin was discovered by Alexander Fleming whereas the in same decade penicillinase (resistant to penicillin antibiotic) produced by *Staphylococcus aureus* was reported similarly in 1950 *S. aureus* was also found to have developed multi resistant strain to various antibiotics such as tetracycline, aminoglycoside, macrolides, and minoglycoside (López et al. 2018). To counter this drug resistance, modern pharmaceutical industry has ventured the use of marine environments to foster the next generation of antibiotics. Scientists have isolated in the 1950s the first marine bioactive compounds (spongouridine and spongothymidine) from *Cryptotheca crypta* (Caribbean sponge) and demonstrated its significant anti-cancer and antiviral properties (Leary et al. 2009). Since, the discovery of marine bioactive metabolites, several interesting molecules were isolated from marine environment, which was evident from accelerated research that resulted in a diverse array of applications like pharmacology, biology, biochemistry, organic chemistry, and ecology (Leary et al. 2009).

The marine diversity has immense untapped potential, awaits for researchers to unravels it, recently more than 1277 new compounds has been published in 432 papers during 2016 alone and a peak 17% rise in research output during 2018 compared to latter 1490 novel compounds from 477 papers (Blunt et al. 2018). The enormous evolution of technologies in the field of marine biotechnology leads to tremendous therapeutic potent compound breakthroughs from the marine ecosystem. Scientists have found numerous deleterious components possess astonishing therapeutic novel value compounds that are castoff as predator defense mechanisms by marine microbes. Many marine microorganisms have been rigorously investigated over the past 50 years from which 270,000 natural products and 30,000 compounds have been isolated among which 9 compounds have been approved as medical drugs (Blunt et al. 2011; Gerwick and Moore 2012; Rangel and Falkenberg 2015).

5.3 Pharmacological Potential Biomaterials from Marine Algae

Humans had utilized algae mainly for nutrients (protein) produced by *Spirulina* (Chlorophyta). Marine microalgae are mainly classified into three types based on the pigmentation as red (Khan et al. 2015). With the development of improved technologies, a diverse array of application for algae has been recognized from health care, cosmetics, and pharmaceutical. Polyunsaturated fatty acids (PUFAs) from microalgae have started gaining commercial value (Roy and Pal 2015). There are several compounds isolated from algae that are promising bio alternatives to the existing drug, which exhibits higher efficacy, with nearly less side-effects, and some of them are briefly discussed. The primary producers of marine algae n-3 PUFA have several health benefits such as in treating cardiovascular diseases, brain development, function and as healing for inflammatory conditions. Awad (2000) segregated 3–0-β-D-glucopyranosyl stigmasta- 5,25-diene compound from green alga *Ulva lactuca*, which have potential anti-inflammatory activity. The bioactive compound, Isorawsonol have been isolated by Chen et al. (1994) from tropical green alga *Arrainvill arawsonii* that exhibited potential anticancer and immunosuppressive effects (Chen et al. 1994).

Cycloeudesmol isolated from marine alga *Chondria oppositiclada* exhibited potent antibiotic activity against *Staphylococcus aureus* (Fenical and Sims 1974). Ascosalipyrrolidinones A and B isolated from green alga *Ulva* spp. presented potential antiplasmodial activity against *Plasmodium falciparum* strains NF-54 and Kl (Osterhage et al. 2000). Halitunal compound isolated from *Halimeda tuna* displays antiviral toward *murine coronavirus* A59 in in vitro condition (Koehn et al. 1991). Two new compounds, Capisterones A and B, are triterpene sulfate esters isolated from green alga *Penicillus capitatus*, which shows antifungal activity against marine algal pathogen *Lindra thallasiae* (Puglisi et al. 2004).

The brown algae color is mainly due to the presence of xanthophyll and fucoxanthin pigments, which mask the presence of other pigments (chlorophyll *a* and *c*, *β* carotenes). Currently there are 1200 compounds reported from brown algae (Phaeophyceae). Leptosins K, K1, and K2 compounds from *Sargassum tortile*

exhibited antitumor activity against sarcoma 180 as cites and cytotoxicity against cultured P388 cells (Takahashi et al. 1995). The compound Stypoldione from *Stypopodium zonale* brown alga is found to possess ichthyotoxic effect (Gerwicket al. 1979). Cis-dihydroxy tetra hydrofuran isolated from brown alga *Notheia anomala* found in southern coast of Australia showed nematocidal activity against parasitic nematodes such as *Trichostrongylu scolubriformis* and *Haemonchus contortus* (Capon et al. 1998). Lobophorolide isolated from brown alga *Lobophora variegate* possesses potent anti-fungal activity against *C. albicans* and is highly specific against *Dendroyphiella salina* and *Lindra thalassiae* (Kubanek et al. 2003).

Lopophorins A and B compounds from brown alga *Lobophora variegate* compound illustrated good anti-inflammatory activity (Jiang et al. 1999). *Dictyota pfaffi* isolated compound displayed strong anti-human syncytial virus (HSV)-1 activity and moderate activity against human immunodeficiency virus (HIV)-1 reverse transcriptase (Pereira et al. 2004); *Dictyota dichotoma* obtained compounds such as diterpenes, dictyolactone, and sanadaol that showed algicidal activity against dinoflagellate *Alexandrium catanella* (Finer et al. 1979); Ecklonia cava derived 8,8″- bieckol (Fukuyama et al. 1989); and 8,4″- bieckol showed activity against HIV-I and fucosterol from *Pelvetia siliquosa* that displayed antidiabetic activity (Lee et al. 2004).

In red algae, the presence of pigments phycoerythrin and phycocyanin are responsible for red coloration, whereas those compounds suppress other pigments xanthophylls, β- carotene, and chlorophyll a and thus are termed as red algae (Bold and Wynne 1985). The red algae *Portieria hornemanii* produced halmon (polyhalogenated monoterpene) and showed antitumor activity in in vitro condition (Fuller et al. 1992). The red algae *Gigartina tenella* compounds Sulquinovosyl diacylglycerol, sulfolipid KM043, and KM043 are a class of 6-sulf-α-D-quinovopyranosyl-(l → 3_)-1,2 diacylglycerol (SQDG) compounds and have potential antiviral activity against HIV-l reverse transcriptase (Ohata et al. 1998). Chondriamide C and 3- indol acrylamide were isolated from red algae *Chondria atropurpurea* and displayed anthelmintic activity toward *Nippostrongylus brasiliensis* (Davyt et al. 1998). Vidalols A and B were isolated from red alga *Vidalia obtusaloba* shown DPPH radical scavenging activity (Choi et al. 2000).

The red alga *Symphyocladia latiussula* produce cyclohexanone shown free radical scavenging activity (Choi et al. 2000). *Digenea simplex* derived amino acid (α-alkokainic acid) shown potent neurophysiological activity in mammals (Biscoe et al. 1975; Ferkany and Coyle 1985). *Laurancia pinnata* synthesized compound Laurepinacine and isolaurepinnacin showed insecticidal activity (Fukuzawa and Masamune 1981). *Laurencia brongniarti* derived four polybrominated indoles has a potential antimicrobial activity against *Saccharomyces cerevisiae* and *Bacillus subtilis* (Carter et al. 1978). *Tichocarpus crinitus* red algae obtained tichocarpols A and B showed antifeedant activity against *Strongylocentrotus intermedius* (Ishii et al. 2004).

Fucoxanthin is a member of carotenoid present in various species of brown algae exhibited different pharmaceutical applications such as antioxidant activity,

anticancer, anti-inflammatory, antiobesity, neuroprotective effect, antiangiogenic, and skin protective effect (Kim and Pangestuti 2011). The marine algae–derived sulfated polysaccharides are the source for numerous health beneficial activities such as antioxidant, anticoagulant, anti-allergic, anti-human immunodeficiency virus, immunomodulating activities, and anticancer activities (Ngo and Kim 2013).

5.4 Marine Bacteria: A Promising Resource for Biomedical Application

Ever since the inception of mankind, nature has been nourishing us with valuable resources for the sustainability of humans by providing necessity for survival like food, shelter, and protection. Extensive screening of marine actinobacteria was started from early 1969 to formulate antagonistic compounds (Weyland 1969). Early evidence shows members of actinomycetes like *Aeromicrobium marinum*, *Dietzia*, *Marinophilus*, *Rhodococcus*, *Salinibacterium*, *Salinispora*, *Solwaraspora*, *Streptomyces*, *Verrucosispora*, *Arthrobacter*, *Streptomyces*, *Corynebacterium*, *Frankia*, *Micrococcus*, and *Micromonospora* synthesize numerous important compounds that have a huge variety of pharmaceutical applications (Solanki et al. 2008).

Marine actinobacteria are the main source for novel secondary metabolites, around the 1970s there were only 11 genera of actinomycetes reported and then in 2005 the number rose to 100 whereas in 2010 the numbers doubled to around 220. The reason behind such a sharp increase in genera is the advancements in sequencing techniques that revealed novel actinomycetes (Subramani and Aalbersberg 2013). Actinobacteria are filamentous, Gram-positive bacteria belonging to the Actinomycetaceae family. *Streptomyces* are known for their unsurpassed amount of secondary metabolite productions that account for 80% actinobacterial natural products (Manivasagan et al. 2014a, b). The marine actinobacteria are found in diverse biological sources (seawater and sediment, sponges, seaweeds, fish, mollusks, and mangroves) and several reports indicated that marine actinobacteria have several biotechnological applications such as antitumor agents, antibiotics, enzyme, immunosuppressive agents, and pigments (Fenical and Jensen 2006; Bull and Stach 2007; Dharmaraj 2010; Mayer et al. 2011).

The extensive search of bioactive compounds from microorganisms has led to the discovery of 23,000 antibiotics, and several reports have been published related to marine actinobacteria that are biologically active molecules (Lam 2006; Solanki et al. 2008; Zotchev 2012; Manivasagan et al. 2014a, b; Subramani and Sipkema 2019) Apparently, only minor fraction of marine actinomycetes natural products were discovered, but with recent sophisticated techniques made accessibility for isolation and identification of numerous bioactive compounds, which are in pipelines for antimicrobial, anticancer, anti-inflammatory and neuromodulating drugs.

5.4.1 Antibacterial Activity

Typically, antibacterial activity implies any element that kills the bacteria or inhibits bacterial growth or it will help to inhibit or kill the infectious diseases causing antibiotic-resistant microorganisms. Riedlinger et al. (2004) isolated novel polycyclic polyketide (Abyssomicin C) antibiotic form *Verrucosispora* sp., a potent inhibitor of p-aminobenzoic acid biosynthesis that will lead to inhibition of folic acid biosynthesis, an earlier stage inhibition than the well-known synthetic sulfa drugs. Abyssomicin C has potential antibacterial activity against vancomycin-resistant, against Gram-positive bacteria and multi-drug resistant *Staphylococcus aureus*. A novel compound, bonactin displays antimicrobial activity against both Gram-positive and Gram-negative bacteria that are obtained from the liquid culture of *Streptomyces* sp. BD21–2, the culture was accumulated from Kailua Beach, Oahu, Hawaii (Schumacher et al. 2003).

El-Gendy et al. (2008a, b) isolated *Streptomyces* sp. Merv8102 from sediments of the Mediterranean Sea at the Egyptian Coast and extracted Essramycin (triazolopyrimidine) antibiotic. The compounds shown antibacterial activities against Gram-positive, Gram-negative bacteria like as *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 10145), and *Micrococcus luteus* (ATCC 9341). Hughes et al. (2008) isolated *Streptomyces* sp. CNQ-418 from La Jolla, California, and extracted densely halogenated and axially chiral metabolites of marinopyrroles A that contain uncommon bispyrrole structure. The marinopyrroles have potential antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Caboxamycin is a new antibiotic (benzoxazole) produced by *Streptomyces* sp., are isolated from deep-sea sediments of Canary Basin has inhibitory activity against Gram-positive bacteria (Hohmann et al. 2009a, b, c).

5.4.2 Antifungal Activity

Several unique structural features of bioactive compounds were obtained from a variety of marine actinomycetes, yet research is conducted to find the novel antibiotics against pathogenic fungi (Subramani and Sipkema 2019). The common saprophytic nature *Streptomyces* species are significant to produce complex antibiotics and biopolymers (Wanner 2009). In south China, the sponge (*Craniella australiensis*) associated Marine *Streptomyces* sp. DA11 was found to produce chitinase enzyme that exhibited antifungal activities against *Candida albicans* and *Aspergillus niger* (Han et al. 2009).

Daryamides are a novel antifungal compound isolated from marine sediment *Streptomyces* strain, CNQ-085, that shows antifungal activities against *Candida albicans* with moderate or weak cytotoxicity against human colon carcinoma cell line HCT-116 (Asolkar et al. 2008). The antibiotic N-(2-hydroxyphenyl)-2-phenazinamine (NHP) was obtained from *Nocardia dassonvillei*, which has antifungal activity against *C. albicans* (Gao et al. 2012). Numerous compounds revealed

antifungal activity such as Trioxacarcins, Bonactin, Aureoverticillactam, Rapamycin, FK520 Ascomycin, and Jinggangmycin against some clinically important pathogens like *Aspergillus flavus, Trichoderma ressei*, C. albicans, *Aspergillus niger*, and *Alternaria alternate*.

5.4.3 Anticancer Activity

In recent years, cancer has been the second leading disease with high fatality of about 9.6 million death in 2018. Thus a huge urge for anticancer compounds have raised, result in diverse avenue of researchers extending further pursuit in finding novel anticancer compounds from actinobacteria. Cancer is one of the leading human health problems, breast cancer is responsible for second most causes of deaths in women (Ravikumar et al. 2010). Several therapeutic treatments are available to counter cancer, which includes immunotherapy, radiotherapy, and chemotherapy even though cancer could not be defeated till date as a major issue for mankind (Gillet et al. 2007). Salinosporamide A has shown inhibitory effects against various malignant cell types that were isolated from *Salinispora tropica* in oceanic sediments (Prudhomme et al. 2008). Salinosporamide A is a proteasome inhibitor which leads to apoptosis in multiple myeloma cells, subsequently entered to phase I of human trials for solid tumors, multiple myeloma and lymphoma (Jensen et al. 2007; Feling et al. 2003). Stritzke et al. (2004) isolated *Streptomyces* sp. B6007 from mangrove sediment in Papua New Guinea, acquired caprolactones, which showed moderate cytotoxicity and low cytotoxicity against cancer cells. Miller et al. (2007) isolated *Streptomyces* sp. CNQ-593 from marine sediments in Guam, and piperazimycins A-C (cyclic hexadepsipeptides) were extracted from the fermentation broth of a *Streptomyces* sp. with cytotoxic activities against the human colon carcinoma HCT-116 cell line with cytotoxicity of GI50 of 76 ng/mL for each. Piperazimycin A also exhibits potent vitro biological activity against multiple (60) cancer cell lines. *Nocardiopsis lucentensis* strain CNR-712 produced Lucentamycins 3-methyl-4-ethylideneproline-containing peptides and Lucentamycins showed cytotoxicity against HCT-116 cell line (IC50 values of 0.20 and 11 μM) (Cho et al. 2007).

5.4.4 Cytotoxic and Cytostatic Activity

Salinosporamide A has shown potential cytotoxicity against HCT-116 human colon carcinoma, MDA-MB-435 breast cancer, SF-539 CNS cancer, NCI-H226 non-small cell lung cancer, and SK-MEL-28 melanoma cells (Feling et al. 2003). Two new polyketides furanones A and B have been isolated from fermentation broth of *Streptomyces* CNQ766, found in the marine sediments displayed weak in vitro cytotoxicity against macrophages and splenocyte T-cells (Cho et al. 2006). *Nocardiopsis lucentensis* strain CNR-712 isolated from the sediment of saline pond in Bahamas exhibits Lucentamycins compound (3-methyl-4-

ethylideneproline-containing peptides) that showed cytotoxicity against colon carcinoma HCT-116 cell line (IC50 values of 0.20 and 11 μM) (Cho et al. 2007). Arenamides A is a cyclohexa depsipeptide, isolated from the fermented broth of actinobacterial *S. arenicola* CNT-088 strain obtained from a depth of 20 m marine sediments in Kandavu Island chain, Fiji. Arenamides A possess weak in vitro cytotoxicity against human colon carcinoma HCT-116 (IC50 values of 13.2 and 19.2 g/mL) (Asolkar et al. 2008). The cyclic hexadepsipeptides, Piperazimycins were obtained from *Streptomyces* sp. CNQ-593 fermentation broth, exhibited in vitro cytotoxic against human colon carcinoma HCT-116 cell line melanoma (average LC50 of 0.3 μM), leukemia cell line (average LC50 of 31.4 μM), prostate cell lines (average LC50 of 0.6 μM), and central nervous system (average LC50 of 0.4 μM), respectively. Proximicins A, B, and C produced by *Verrucosispora* strain MG-37, *Verrucosispora maris* AB-18-032, displayed strong cytostatic effect against various human tumor cell lines such as hepatocellular carcinoma Hep G2 (GI50 of 0.82, 9.5, and 0.78 μM), adenocarcinoma AGS (GI50 of 0.6, 1.5 and 0.25 μM), and hepatocellular carcinoma Hep G2 (GI50 of 0.82, 9.5, and 0.78 μM, respectively) (Schneider et al. 2008).

5.4.5 Anti-Inflammatory and Anti-Parasitic Activity

One of the major challenges faced by developing tropical countries are infectious diseases that is one of the foremost causes of death. About 335 infectious diseases were reported between 1940 to 2004 (Jones et al. 2008). The prominent new discovery of effective bioactive compounds from marine environment has started countering the burden of infectious disease. Abdelmohsen et al. (2010) reported 90 actinomycetes from 11 different species with anti-infective activities against clinically potential organisms such as Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*), Gram-positive (*E. faecalis*, *S. aureus*) bacteria, human parasites (*Leishmania major*, *Trypanosoma brucei*), and fungi (*C. albicans*). Globally, the parasitic disease is one of the major health problems to humans, and it is responsible for one million deaths every year and it is almost close to the number of deaths caused by AIDS (Antoszczak et al. 2019; Bhatti et al. 2016). The tropical disease caused by parasitic protozoa *Leishmania*, the species are *Leishmania major*, *L. amazonensis*, *Leishmania aethiopica*, *L. tropica*, *Leishmania mexicana*, *Leishmania braziliensis*, *Leishmania donovani*, and *Leishmania Mexicana*. Pimentel-Elardo et al. (2010) obtained secondary metabolites form marine sponge associated *Streptomyces* sp. that showed antiparasitic activities against *T. brucei* (staurosporine IC500.022 μM; valinomycin IC500.0032 μM; butenolide IC5031.77 μM) and *L. major* (staurosporine IC505.30 μM; valinomycin IC50 $<$ 0.11 μM;).

5.4.6 Antimalarial and Antiviral Activity

Malaria remains one of the devastating infectious diseases globally caused by protozoan parasites of the *Plasmodium* genus, and its species include *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi* that are together responsible for two million deaths with 300 million clinical cases annually. The global prevalence evidently showed that *P. falciparum* causes higher mortality rates compared to other species of *Plasmodium* (World Health Organisation 2014). The potential peptide from *Streptomyces* sp. LK3 (JF710608) was isolated from a Nicobar marine sediment sample that exhibited antiplasmodial activity with IC50: 25.78 mg/ml (Karthik et al. 2014). Marinacarbolines (A – D) compounds are produced by *Marinactinospora thermotolerans* SCSIO 00652 which belongs to *Nocardiopsacea* family, exhibited antiplasmodial activity against *Plasmodium falciparum* with IC50 values ranging from 1.92 to 36.03 μM (Huang et al. 2011). Marine actinobacteria *Streptomyces nitrosporeus* derived compound benzastatin exhibits antiviral activity against simplex virus type 1 (HSV-1), *Vesicular stomatitis virus* (VSV), *Herpes simplex* virus type 2 (HSV-2) with EC50 values of 1.92, 1.99, and 0.53 μg/mL (Lee et al. 2007).

5.4.7 Antioxidant and Anti-Angiogenesis

Antioxidant compounds retard or prevent the oxidation of lipid. The marine isolate *Nocardiopsis alba* produced (Z)-1-((1-hydroxypenta-2,4-dien1-yl)oxy) anthracene-9,10-dione compound showed significant in vitro antioxidant capacity (Janardhan et al. 2014). The *Streptomyces* VITSVK5 spp. was isolated by marine sediment at the Marakkanam coast in the Bay of Bengal, India, with a compound 5-(2,4-dimethylbenzyl)pyrrolidin-2-one (DMBPO), which exhibited significant antioxidant activity (50.10% at 5 μg/ml DMBPO) (Saurav and Kannabiran 2012). Secondary metabolites, Dermacozines A-G (phenazine compounds) were obtained from *Dermacoccus*, which shows significant antioxidant properties (Pathom-Aree et al. 2006). Angiogenesis is an essential step for the formation of new blood vessels from pre-existing vessels and it is a vital step for tumor cell proliferation (Risau 1997). *Streptomyces* sp. isolated from the deep-sea sediment at Ayu Trough exhibit Streptopyrrolidine compounds with significant anti-angiogenesis activity (Shin et al. 2008). The compound Cyclo-(L-Pro-L-Met) was isolated from fermentation broth of a marine-derived actinomycete *Nocardiopsis* sp. 03 N67 showed anti-angiogenesis activity against human umbilical vein endothelial cells (HUVECs) (Shin et al. 2010).

5.4.8 Exopolysaccharides (EPSs)

Polysaccharides are high molecular weight polymers that are vital material for synthesizing microbial and plant cell walls, and they can be produced as both intracellular or extracellular polysaccharides (EPSs) during extreme environmental

conditions. These natural polysaccharides have an exceptional physical characteristic that has extensive applications in the pharmaceutical field. Okutani (1984, 1992) reported polysaccharides from *Vibrio* and *Pseudomonas* with antitumor, antiviral, and immunostimulant activities. *A. infernus* produced exopolysaccharide displaying anticoagulant property (Senni et al. 2011).

5.4.9 Biosurfactants

Biosurfactants or biological surfactants are microbial compounds with a wide range of structural variety (fatty acids, glycolipids, lipopeptides, phospholipids and neutral lipids, polysaccharide-protein complexes) produced by bacteria, yeast, and fungi (Mnif and Ghribi 2015). Initially, biosurfactants are used in pollution remediation and some surface-active compounds are used as anti-adhesive agents against several pathogens, anti-biofilm against human multi-drug resistant pathogens, antibacterial, antifungal, antiviral, and anti-cancer activities (Singh and Cameotra 2004).

5.4.10 Microbial Biopolymers

The microbial origin naturally occurring biopolymers are produced by variety of microorganisms, most of them are of bacterial sourced biopolymers. Bacterial poly hydroxyalkanoates (PHAs) are polyesters synthesized by a wide variety of 300 Gram-positive and Gram-negative species as a carbon/energy storage material (Rehm 2003). Due to its microbial origin, PHB is gaining more interest in medical applications. The unique properties of these polymers are utilized as drug carriers, biocontrol agents, antibacterials, tissue engineering, biodegradable implants, anti-cancer agents, and also as memory enhancers (Ray and Kalia 2017).

5.5 Pharmacological Effects of Marine Fungi-Derived Biomaterials

Marine fungi are rich in diversity of species, phylogenetic distribution and natural products (NPs) whereas in recent years extensive research has provided thorough data about marine resources (Richards et al. 2012; Imhoff 2016; Rämä et al. 2016; Taylor and Cunliffe 2016). The diverse physical and chemical growth conditions of fungi are the prime reason for the production of novel drugs whereas certain marine fungal metabolic pathways are entirely distinct from terrestrial fungi (Kijjoa and Sawangwong 2004; Abdel-Lateff 2008). Marine Fungai are a potential producer of secondary metabolites like peptides, alkaloids, terpenes, and mixed biosynthesis compounds. Two new indole alkaloids, (2–3, 3- dimethylprop-1- ene)-epicostaclavine and (2–3, 3-dimethylprop-1-ene)-costaclavine, are known compounds of costaclavine, fumgaclavine with antibacterial activity obtained from *Aspergillus fumigates* (Kossuga et al. 2012).

Several marine fungi such as *Trimmatostroma salinum*, *Phaeotheca triangularis*, *Aureobasidium pullulans*, *Hortaea werneckii*, and *Cryptococcus liquefaciens* produce photo-protective compounds (mycosporines). These compounds absorb UV in the range of 310–320 nm (Kogej et al. 2006). Zopfiellamide A is a pyrrolidinone derivative; it was obtained from marine fungi *Zopfiella latipes*, which inhibits the growth of Gram-negative *Acinetobacter calcoaceticus* and Gram-positive *Bacillus subtilis*, *Bacillus brevis*, *Corynebacterium insidiosum*, *B. licheniformis*, *Micrococcus luteus*, *Corynebacterium insidiosum*, *Arthrobacter citreus*, *Mycobacterium phlei*, and *Streptomyces* sp. (Daferner et al. 2002). Marine fungal antiviral compounds such as phomasetin, equisetin, and integric acid showed significant anti-HIV activities based on bioassay experiments, and Sansalvamide A compound obtained from *Fusarium* sp. was found against pathogenic poxvirus *Molluscum contagiosum* (MCV) (IC50 = 124 lM) (Hwang et al. 1999).

5.6 Conclusion

This chapter provides firsthand information of marine microbial products and its marine genetic resources of commercial interest. The marine microbes possess potentially untapped resources, and if utilized properly they will lead to the discovery of novel compounds that can revolutionize the pharmaceutical industry. In recent years, a number of patents and scientific publications have demonstrated the importance of marine genetic resources to the scientific community. The remarkable new methodologies of underwater exploration, bioassays, recent technology in cultivation of marine microorganisms combined with proteomics, genomics, DNA shuffling, combinatorial chemistry, bioinformatics, and DNA shuffling are used to rapidly screen the bioactive compounds from marine microbes. Marine microbes can produce chemically unique secondary metabolites, will have greatest impact on marine natural products (MNP), and will eventually lead to revealing unexplored pharmaceutical significant bioactive compounds. As a result of improved methodologies in marine microbes and bioactive metabolites isolation has led to successful pipelines in pharmaceutical fields, Carrol et al. (2019) clearly elucidated that in last 10 years there is about 41% jump in discoveries of MNP was observed.

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Molecular Diversity and Pharmaceutical Applications of Free-Living and Rhizospheric Marine Actinobacteria

6

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Abstract

The actinobacteria are well studied from the terrestrial ecosystems for the secondary metabolites production. However, the marine actinobacteria that thrive under various stress conditions are less explored for the novel drugs and therapeutic agents. Among various marine habitats, mangrove sediments, mangrove plants, and natural wetlands are still less explored. Both conventional techniques and modern molecular techniques are the most significant approaches for mining the diversity of actinobacteria that persist in the marine ecosystem. The omics techniques, for instance, marine metagenomics, metatranscriptomics, and metaproteomics, make the basis for the identification of new organisms and the development of new drugs. Further, the sequence and function-based analysis of the genes that encode specific pharmaceutically active compounds allows the identification of novel strains of actinobacteria with several pharmaceutical applications. Moreover, the marine actinobacteria are studied for the hydrolytic enzymes like proteases, amylases, cellulases, etc., that can withstand various extreme conditions of salt, pH, and temperature. They also play an ecological role in nutrient cycling. The bioactive compounds produced by marine actinobacteria are also applicable in phytopathogen control. Thus, the present book chapter focuses on various newly isolated actinobacteria from different marine habitats, their diversity, and pharmaceutical applications.

Keywords

Rhizospheric · Actinobacteria · Marine habitats · Molecular diversity · Novel drugs · Biocatalysts

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6.1 Introduction

The actinobacteria are Gram-positive bacteria that exhibit filamentous growth with high G + C content in their DNA. The large ranges of actinobacteria are explored for antibiotics and enzymes production and among them 80% antibiotics are produced by *Streptomyces* sp. The actinobacteria play an important role in nutrient cycling by degrading organic matter and producing enzymes such as chitinase and lignocellulose (Khanna et al. 2011). The actinobacteria producing secondary metabolites are explored from terrestrial habitats; however, the mangrove, marine sediments, wetlands as well as coastal regions are least explored which would be a source of novel actinobacteria that might produce unique bioactive compounds useful in drug discovery. The marine actinobacteria are more potent producers of novel biological compounds as compared to the terrestrial relatives due to their molecular diversity (Maldonado et al. 2005). The marine actinobacteria are known biofactory that efficiently produces the bioactive compounds that show a range of activities such as antibacterial and antifungal activity against human and plant pathogens, anticancer, antiviral, and antioxidant activities as well as produces various enzymes and their inhibitors (Solanki et al. 2008). The enzymes from the actinobacteria can sustain in the presence of harsh conditions such as high pH, temperature, and salt. The marine actinobacteria are studied as a source of fuel to generate the bioelectricity (Rajagopal and Kannan 2017).

The metagenomic studies revealed the complexity and diversity of microbiome associated with marine habitats. The metagenomics of the rhizosphere of terrestrial plants is studied well but the rhizobiome of the marine plants such as seagrasses is less explored (Cúcio et al. 2016). The novel biological compounds can be explored using metagenomics and biotechnological approaches (Huete-Pérez and Quezada 2013). The metabolic diversity of actinobacteria is because of their large genome size and due to their ability to regulate the gene coding for various metabolites in the stress conditions (Trujillo 2008). More recently, Xu et al. (2018) discovered the antibiotic tunicamycins that have anti-complement properties based on the genome analysis of marine actinobacteria *Streptomyces* sp. DUT11 (Xu et al. 2018). The metagenomic studies also revealed marine actinobacteria that have low G + C (33%) content in their DNA and cell volume was 0.013 μm^3 known as ultra-small actinobacteria. Moreover, the new sub-class, "*Candidatus Actinomarinidae*" was proposed by Ghai et al. (2013).

The novel actinobacteria are also explored from the mangrove environments. The endophytic actinobacteria from mangrove plants have drawn more attention for diverse biological compounds (Qu et al. 2017). As work done previously, the first actinobacterium isolated from the sediment of the ocean was not considered as a marine organism and believed that it was a dormant spore of terrestrial organisms. However, further studies proved that they are marine forms of organisms (Mincer et al. 2002; Lam 2006). Despite this, the marine actinobacteria producing bioactive metabolites are not classified on the basis of various bioactivities instead of structural similarities. The marine actinobacteria also show probiotic potential against pathogens and exhibit multidrug-resistant (Norouzi et al. 2018). They also play a

vital role in nanotechnology as they synthesize nanoparticles such as silver, gold, and other metal ions. It enhances the production of bioactive compounds (Puttaswamygowda et al. 2019).

Therefore, this chapter highlights the role of actinobacteria found in various marine habitats, their diversity based on culture-dependent and independent approaches, and their potential to produce novel bioactive compounds.

6.2 Distribution of Actinobacteria in Various Marine Habitats

6.2.1 Wetlands

The wetland habitats are the most productive ecosystem on the earth and significant biologically. The scientist of China studied the coastal wetlands and wetland soils to explore the novel actinobacteria and pharmaceutically important compounds (Djokic et al. 2011). They studied the Yalujiang coastal wetland of North China and isolated new actinobacteria. The characterization of various sites of this habitat is performed from the sediment samples. The habitat is unique due to saline and alkaline nature of soil, i.e., salinity (% w/v) around 0–30.4% and 5–14 pH. The rhizospheric sites of straw and seepweed of the sediment sample were recently studied (Yu et al. 2015). According to the study, a total of 172 actinomycetes were reported and among them the majority of isolates belonged to *Streptomyces* strains inhabiting the root rhizosphere. The isolates showed antibacterial and antifungal activities that significantly showed that the wetlands are the source of biologically important compounds. The meta-analysis of wetland soils showed that 66% of the isolates belonged to order *Actinomycetales* while the dominant genus was *Mycobacterium* (Lv et al. 2014). The saline wetlands (218.62 g/L NaCl) are studied for the antimicrobial actinomycetes as they are an unexplored environment and important for the screening of novel secondary metabolites (Trabelsi et al. 2016). Thus, it is very significant to explore the wetland habitats for microbial diversity as well as to retrieve novel biologically active compounds.

6.2.2 Mangrove Sediments

Mangrove ecosystems occupy 181,000km^2 area worldwide and mainly found in the tropical and subtropical regions. They are largely unexplored for secondary metabolite producing actinomycetes (Jusoff 2013). The *Streptomyces* produces a total of 80% of the known antibiotics. The *Streptomyces* strains showed antifungal activity against *Candida albicans* isolated from mangrove habitat (Palla et al. 2018). The group of Chinese people worked on the discovery of drugs from mangrove environmental samples such as plant tissue and rhizospheric soil (Hong et al. 2009). The bioactive actinomycetes showed antifungal and antibacterial activities and also showed activity against the tumor cell line. They found that the actinomycetes isolated from the mangrove habitats are a rich source of drugs that can be used for

various diseases. Also, the halophilic actinomycete is isolated from the mangrove ecosystem able to grow on the ISP-2 International Streptomyces Project (ISP) medium containing 5–7% (w/v) NaCl and pH 9 (Priya et al. 2014). The tropical mangrove sediments were explored for isolation of novel actinomycetes sp. such as *Streptomyces pluripotens sp.nov.* MUSC135^T showed antimicrobial activity against pathogens such as *Bacillus subtilis*, *Staphylococcus epidermidis, Klebsiella oxytoca, Enterococcus faecalis, Salmonella typhi*, and *methicillin-resistant Staphylococcus aureus* (MRSA). The actinobacteria isolated from proximal, middle, and distal zones of mangroves located in Andaman and Nicobar Islands, India showed activity against pathogens (GopalakriShnan et al. 2016). As far as the environmental problems are concerned, the marine actinobacteria are studied for the production of antifouling compounds to prevent the marine biofouling from mangrove and estuarine sediments (Gopikrishnan et al. 2013). The rare actinobacteria such as *Actinomadura, Rhodococcus, Nonomuraea, Rhodococcus*, and *Nocardia* were isolated from mangrove habitats in China (Hong et al. 2009). Similarly, the novel compounds such as antibiotic butremycin, 5′-methylthioinosine, and a novel alkaloid 2-(furan-2-yl)-6-(2*S*, 3*S*, 4-trihydroxybutyl) pyrazine from the rare actinobacteria are also reported (Azman et al. 2015; Wang et al. 2014).

6.2.3 Associated with Marine Plants/Sea Grasses

The actinobacteria isolated from the rhizospheric ecosystem of the plant showed plant growth-promoting activities and make nutrients available to the plants. The marine endophytic actinomycetes were isolated on actinomycete isolation agar (AIA) from the various plant parts such as leaves, twings, and buds of the mangrove plant *Avicennia marina*, collected from Muthupet Mangrove region, South East Coast of Tamil Nadu, India (Rajivgandhi et al. 2018). Recently, Jiang et al. (2018) reported endophytic actinobacteria as an important pharmaceutical resource and the genus *Streptomyces* was most abundant. The actinobacteria isolated from the rhizosphere of the mangrove plant were also studied for the L-asparaginase enzyme (Pitamber et al. 2014). The novel actinobacterium *Kocuria pelophila* sp. was isolated from the rhizosphere of a mangrove (Hamada et al. 2016). Since the last few years, the endophytes thrive in mangroves regions have gained attention due to newly isolated novel actinobacteria (Sun et al. 2017; Jiang et al. 2017). The *Streptomyces sp.* BCy was isolated from seagrass *Cymodocearotundata* (Damayanti et al. 2018). Recently, the genera belonging to actinobacteria such as *Sachharomonospora* and *Kocuria* endophytes isolated from roots of seagrass *Cymodocea serrulata* were screened for their in vitro plant growth-promoting attributes such as inorganic phosphate solubilization, IAA (Indole acetic acid) production, and ammonia production (Jose et al. 2014).

6.3 Novel Actinobacteria Isolated from Various Marine Habitats

The novel bioactive compounds from the actinobacteria are most important in drug discovery to combat against pathogens. The novel biological compounds from actinobacteria display antibacterial, antioxidant, antifungal, antitumor, antiviral and cytotoxic activity and are potentially applicable in pharmaceutical industries and agriculture. The *Streptomyces sp*. is a major source of secondary metabolite production. Previously, a novel marine actinobacteria *Streptomyces variabilis* RD-5 with antibacterial and antioxidant activities were isolated from sea sediments of Gulf of Khambhat, Gujarat (Dholakiya et al. 2017). Similarly, a novel marine actinomycete *Streptomyces xiaopingdaonensis sp.* nov. was isolated from Xiaopingdao in Dalian, China (Chen et al. 2015). The physiological characteristics and chemotaxonomic characteristics of various novel actinobacteria from diverse marine habitats are illustrated in Tables 6.1 and 6.2.

6.4 Molecular Diversity of Actinobacteria

The cultural and molecular characterizations are the most significant aspects to study novel actinobacteria from various habitats. It includes both culture-dependent and culture-independent approaches.

6.4.1 Culture-Dependent Approaches

The culture-dependent approach is most important for the isolation of novel actinobacteria. However, the isolation samples are generally pre-treated specially to exclude the commonly found microbes and this enhances the chances to isolate the novel and unique microbes. For the cultural characterization of actinobacteria, standard serial dilution and spread plate techniques are generally used. In addition, the cultural media such as 1 to 7 ISP (*International Streptomyces Project*) medium and starch agar medium are generally used supplemented with the NaCl (w/v). The alkaline pH is maintained as marine bacteria sustain in saline and alkaline environment (Gohel and Singh 2015). For the physiological characterization, the isolates are generally screened for the salt, pH, and temperature tolerance as described in Bergey's manual (Holt et al. 1994). The marine actinobacteria can also be characterized on the basis of morphology such as filamentous structure, aerial mass color, aerial and substrate mycelium color, melanin pigments, diffusible pigments as well as based on spore morphology. The pigment producing marine actinobacteria was used as bio-pigments in textile dyeing and bio lip balm preparations in China (Chakraborty et al. 2015). In addition, the biochemical and enzymatic characterization such as screening of extracellular protease, amylase, catalase, and urease is also most important for the isolation of unique and novel actinobacteria (Singh et al. 2016). Moreover, the BIOLOG assay describes the

Table 6.1 Physiological parameters of various marine actinobacteria

Sr. No.	Name of organism	Study site	Salt (% w/v)	pH	T (°C)	References
1	*Nocardiopsis xinjiangensis strain* OM-6	Okha Madhi, coastal region of Gujarat, India	0–20%	7.0–11.0	30 °C	Gohel and Singh (2018)
2	*Blastococcus litoris* sp. nov.	Sea-tidal flat sediment sample from Gopado, Republic of Korea	0–3%	7.0–9.0	28–37 °C	Lee et al. (2018a)
3	*Glycomyces xiaoerkulensis* sp. nov.	Silt sample from Xiaoerkule lake in Xinjiang province, China	5%	–	35–37 °C	Wang et al. (2018)
4	*Euzebya rosea* sp. nov.	Surface seawater of the East China Sea	0.5–5.0%	6.0–9.0	15–45 °C	Yin et al. (2018)
5	*Glycomyces sediminimaris* sp. nov.	Marine sediment, 12 m depth in Rostami seaport, Bushehr Province in Iran	2.5–5%	6.0–8.0	25–35 °C	Mohammadipanah et al. (2018)
6	*Streptomyces salilacus* sp. nov.	Sediments of a salt Lake, Xiaoerkule Lake, Xinjiang, China	1%	12	28 °C	Luo et al. (2018)
7	*Tessaracoccus aquimaris*	Intestine of a Korean rockfish, *Sebastes schlegelii*	0–4%	7–9	15–37 °C	Tak et al. (2018)
8	*Streptomyces kalpinensis* sp. nov.	Salt water beach at Kalpin, Xinjiang, north-west China	1%	–	–	Ma et al. (2017)
9	*Salinifilum proteinilyticum sp.* nov.	Meighan wetland in Iran	12–25%	5.5–10.5	30–50 °C	Nikou et al. (2017)
10	*Saccharopolyspora aidingensis* sp. nov.	Aiding salt lake in Turpan Basin, north-west China	12%	–	–	Xia et al. (2017)

(continued)

Table 6.1 (continued)

Sr. No.	Name of organism	Study site	Salt (% w/v)	pH	T (°C)	References
11	*Nocardioides flavus* sp. nov.	Sediment sample collected from the Western Pacific	0–10%	6.0–9.0	4–40 °C	Wang et al. (2016b)
11	*Kineococcus magrovi* sp. nov.	Mangrove sediment in Thailand	0–10%	–	17–32 °C	Duangmal et al. (2016)

metabolic potential (aerobic or anaerobic) and phenotypic fingerprints of the actinobacteria (Dholakiya et al. 2017).

6.4.1.1 Chemotaxonomy Analysis

The identification of novel actinobacteria involves chemotaxonomical analysis including the analysis of the cellular fatty acid content quantitatively using gas chromatography [FAME (fatty acid methyl ester)] and identification using microbial identification software (MIS). The cell wall composition of the actinobacteria such as polar lipids, diaminopimelic acid, whole-cell sugar, and menaquinones is generally studied using thin-layer and high-performance liquid chromatography techniques (Table 6.2).

The phylogeny analysis involves the extraction of genomic DNA, amplification of 16S rRNA gene followed by sequencing, and bioinformatics analysis of 16S rRNA gene sequence using Mega 6.0 (Thompson et al. 1997).

6.4.2 Culture-Independent Approaches

The modern molecular techniques are predominantly useful for the identification of actinobacteria. The G + C content and DNA-DNA hybridization techniques are molecular techniques used to determine phylogeny and taxonomy of novel actinobacteria. The RFLP (restriction fragment length polymorphism) technique distinguishes the species on the basis of a unique pattern of DNA fragments generated using restriction enzymes that consequently used for the analysis of diversity among microorganisms. The diversity of marine actinomycete genus *Salinispora* was studied using RFLP analysis (Mincer et al. 2005). Similarly, the diversity of actinomycetes from deep-sea sediments was also studied using T-RFLP (terminal restriction fragment length polymorphism) technique which is also used to determine single nucleotide polymorphism (SNP) (Prieto-Davó et al. 2013). The ARDRA (amplified ribosomal DNA restriction analysis) technique involves the digestion of PCR products with restriction enzymes followed by the analysis of the band pattern profiles. It distinguishes the bacterial species within a genus. It has been reported that the ARDRA technique is convenient for the diversity analysis of actinobacteria associated with sponges and marine sediments (Menezes et al. 2010).

Table 6.2 The cell wall composition and characterization studies of various novel marine actinobacteria

Sr. No.	Actinobacteria	Study site	Cell wall peptidoglycan	Whole cell Sugar	Polar lipids	Predominant menaquinone	Major fatty acids	DNA G + C (%)	Reference
1	*Actinomadura craniellae* sp.	Marine sponge (South China Sea)	*meso*-diaminopimelic acid	Glucose, galactose, mannose and madurose	Phosphatidylinositol and diphosphatidylglycerol.	MK-9(H_6) and MK-9(H_8)	Iso-$C_{16:0}$, iso-$C_{18:0}$, 10-methyl $C_{17:0}$ and $C_{18:1}$ ω9*c*	72.0	Li et al. (2019)
2	*Corynebacterium alimapuense* sp.	Marine sediment (Valparaíso bay, Chile)	Mycolic acid	–	Diphosphatidylglycerol, glycolipids, phosphatidylglycerol, phosphoglycolipid, and phosphatidylinositol	MK-8(II-H2)	$C_{18:1}$ω9*c* and $C_{16:$	57.0	Claverias et al. (2019)
3	*Streptomyces reniochalinae* sp.	Marine sponge (China)	ll-diaminopimelic acid	–	Diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol	MK-9(H_8)	Iso-$C_{16:0}$, anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$	–	Li et al. (2018)
4	*Nonomuraea mangrovi* sp.	Mangroove soil sample (Sanya, China)	*meso*-diaminopimelic acid	–	–	–	Iso-$C_{16:0}$, 10-methyl-$C_{17:0}$, $C_{17:1}$ω8*c* and $C_{16:0}$	73.2	Huang et al. (2018)
5	*Saccharopolyspora maritima* sp	Mangroove sediment (Ranong Province)	*meso*-diaminopimelic acid	Arabinose, galactose and ribose	Diphosphatidylglycerol, hydroxy-phosphatidylethanolamine, hydroxy-phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, unidentified phospholipids and unidentified lipids	MK-9(H_4)	Iso $C_{16:0}$ and anteiso-$C_{17:0}$	69.4	Suksaard et al. (2018)
6	*Jatrophihabitans telluris* sp.	Sediment of wetlands soil (Republic of Korea)	*meso*-DAP	–	Diphosphatidylglycerol, phosphatidylinositol polymannosides, an unidentified phospholipid, an unidentified aminophospholipid, an unidentified aminolipid, two unidentified glycophospholipids, three unidentified glycolipids and two unidentified lipids as polar lipids	MK-9(H_4)	Iso-$C_{16:0}$ and $C_{17:1}$ ω8*c*	68.1	Lee et al. (2018a, b)

7	*Actinoplanes sediminis* sp.	Marine sediments (Syros, Greece)	*meso*-diaminopimelic acid	xylose, arabinose and glucose	Phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides and an unknown phospholipid	MK-9(H_4), MK-9(H_6) and MK-9(H_2	–	71.5%	Qu et al. (2017)
8	*Pseudonocardia profundimaris* sp.	Deep-sea sediment	*meso*-diaminopimelic acid	xylose, galactose and arabinose	Phosphatidylcholine, phosphatidylinositol, one unknown glycolipid, one unknown phospholipid	MK-8(H_4)	Iso-$C_{16:0}$ and iso H-$C_{16:1}$	76.9%	Zhang et al. (2017)
9	*Nocardia xestospongiae* sp.	Marine sponge (Andaman Sea)	*meso*-diaminopimelic acid	Arabinose, galactose, glucose, mannose and ribose	Diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol	MK-8($H_{4\ \omega\text{-cycl}}$)	$C_{17:1}\omega 8c$, $C_{16:0}$ and $C_{17:0}$		Thawai et al. (2017)
10	*Glutamicibacter halophytocola* sp.	Root of a coastal halophyte, *Limonium sinense,* Jiangsu Province, eastern China.	Lysine, glutamic acid, and alanine		Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, one unknown phospholipid, one unidentified glycolipid, and two unidentified lipids	MK-9	Anteiso-$C_{15:0}$ and iso-$C_{16:0}$	60.0	Feng et al. (2017)
11	*Saccharopolyspora aidingensis* sp	Aiding salt lake in Turpan Basin, north-West China.	*meso*-diaminopimelic acid	Galactose, arabinose and ribose	Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol and phosphatidylinositol	MK-9(H_4), MK-10(H_8) and MK-10 (H_4)	Anteiso-$C_{17:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{17:0}$	70.9	Xia et al. (2017)
12	*Nonomuraea purpurea* sp.	Mangrove sediment collected from Ranong Province, Thailand	*meso*-diaminopimelic acid	Madurose, mannose and ribose	Diphosphatidylglycerol, hydroxy-phosphatidylethanolamine, hydroxy-phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, aminophospholipids	MK-9(H_4)	$C_{17:1}\ \omega 8c$ and iso-$C_{16:0}$	70.4	Suksaard et al. (2016)

(continued)

Table 6.2 (continued)

Sr. No.	Actinobacteria	Study site	Cell wall peptidoglycan	Whole cell Sugar	Polar lipids	Predominant menaquinone	Major fatty acids	DNA G + C (%)	Reference
13	*Nocardioides flavus* sp.	Sediment sample collected from the Western Pacific.	Ll-diaminopimelic acid	–	Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, one unknown phospholipid, two unknown glycolipids, and one unknown lipid.	MK-8(H_4) and MK-7(H_4)	$C_{17:\ 1}\omega8c$, iso-$C_{16:\ 0}$ and $C_{17:\ 0}$	70.4	Wang et al. (2016a, b)
14	*Streptomyces ovatisporus* sp.	Marine sediment collected from the southern Black Sea coast, Turkey	l,l-diaminopimelic acid	Glucose and ribose	Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannoside	MK-9(H_8)	Anteiso-$C_{15:\ 0}$, iso-$C_{16:\ 0}$, anteiso-$C_{17:\ 0}$ and iso-$C_{15:\ 0}$	72.5	Veyisoglu et al. (2016)
15	*Nocardiopsis sediminis* sp.	Mangrove sediment collected from Ranong province, Thailand.-	*meso*-diaminopimelic acid	Absent	Phosphatidylcholine, phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, two unidentified phospholipids, and four unidentified lipids.-	MK-11(H_4), MK-11(H_6) and MK-11 (H_8)	Iso-$C_{16:\ 0}$, $C_{18:\ 1}$ $\omega9c$, 10-methyl $C_{18:\ 0}$ and anteiso-$C_{17:\ 0}$.	73.5	Muangham et al. (2016)
16	*Streptomyces verrucosisporus* sp.	Marine sediments collected from Chumphon Province, Thailand	ll-Diaminopimelic acid	Glucose and ribose	Diphosphatidylglycerol, phosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannoside	MK-9(H_6), MK-9(H_8), MK-10(H_6) and MK-10 (H_8)	Anteiso-$C_{15:\ 0}$, anteiso-$C_{17:\ 0}$ and iso-$C_{16:\ 0}$	–	Phongsopitanun et al. (2016a)
17	*Micromonospora sediminis* sp	Mangrove sediment collected from Chonburi Province, Thailand	*meso*-diaminopimelic acid	Glucose, mannose, xylose, ribose and rhamnose	–	MK-10(H_4), MK-10(H_6) and MK-10 (H_8)	Iso-$C_{15:\ 0}$, iso-$C_{16:\ 0}$ and iso-$C_{17:\ 0}$	–	Phongsopitanun et al. (2016b)

18	*Nocardioides rotundus sp*	Deep seawater of the western Pacific	LL-2,6-diaminopimelic acid.		Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, one unidentified lipid, and six unidentified phospholipids	MK-8(H4)	isoC16: 0 and C18: 1v9c	71.2	Wang et al. (2016a, b)
19	*Kineococcus magrovi* sp.	Mangrove sediment in Thailand	*meso*-diaminopimelic acid	Arabinose, galactose, glucose, mannose, and ribose	Diphosphatidylglycerol, phosphatidylglycerol and an unidentified phosphoglycolipid	MK-9(H_2)	Anteiso-$C_{15:0}$ and iso-$C_{14:0}$	74.7	Duangmal et al. (2016)
20	*Micromonospora zhanjiangensis* sp.	Mangrove forest in Zhanjiang, Guangdong province, China	Alanine, asparagine, glycine, and *meso*-diaminopimelic acid	–	Phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside, and diphosphatidylglycerol	MK-10(H_6) and MK-10 (H_8)	Iso-$C_{15:0}$, anteiso-$C_{15:0}$ and iso-$C_{16:0}$.	70.2	Zhang et al. (2015)
21	*Nocardiopsis oceani* sp. nov	Marine sediment samples of the South China Sea	*meso*-diaminopimelic	Absent	Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, and unknown phosphoglycolipids and phospholipids	MK-9(H_4), MK-9(H_6) and MK-10(H_6) for strain 10A08A^T and MK-9(H_4), MK-9(H_6), MK-10(H_4) and MK-10 (H_6)	Iso-$C_{16:0}$ and summed feature 4 (iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B)	70.9	Pan et al. (2015)
	Nocardiopsis nanhaiensis sp. nov.							71.6	
22	*Actinokineospora spheciospongiae sp. nov*	Red Sea sponge Spheciospongia vagabunda	*Mesodiaminopimelic acid*		Polar lipid profile, diphosphatidylglycerol, phosphatidylethanolamine and hydroxyphosphatidylethanolamine	MK-9 (H4) (47%), MK-9(H6) (27%) and MK-9(H2) (15%)	Iso-C16:0, iso-C14:0, iso-C15:0 and iso-C16:1H		Kampfer et al. (2015)

Moreover, the RAPD (rapid amplified polymorphic DNA) is a PCR-based technique that discriminates between the actinobacterial strains. It uses only one pair of primer with arbitrary nucleotide sequences for the determination of interspecific relationship among the actinobacteria (Lee et al. 2012).

6.4.2.1 Metagenomic Approach

The metagenomics is a powerful technique to study the diversity of bacteria. It involves the direct extraction of DNA from nature (Handelsman 2004). The sequence-based metagenomic studies revealed the genome of rare and unstudied marine actinobacteria which are the source of unique secondary metabolites (Schorn et al. 2016). The marine microbes are valuable sources of the novel bioactive compounds. The development of 454 pyrosequencing technology identifies marine microbes based on the study of variable regions. Besides the next-generation sequencing, the study of RNA extracted from environmental sample termed as metatranscriptomics is also important for the analysis of changes in gene regulation as the environmental changes and also useful to study the regulation of metabolic pathways of marine bacterial communities (Martínez et al. 2013; Wu et al. 2013). The metaproteomics study involves the analysis of the protein sequence and it identifies the novel functional genes that encode for bioactive compounds, enzymes, or proteins. In functional metagenomics analyses, the metagenomic library is screened for the clones encoding the genes of enzymes or metabolites. Thus, the marine metagenomics study is largely significant for the discovery of novel species and novel biocatalysts from the unexplored sites and has promising importance in drug discovery research. Recently, metagenomic studies and the phylogenetic analysis of 16S rRNA gene sequencing showed the novel strain that contained the hallmark genes for secondary metabolites such as polyketide synthetase (PKS) and non-ribosomal polyketide synthetase (NRPS) (Lee et al. 2014; Rocha-Martin et al. 2014). The metagenomic analysis of wetland soils and sediments for the bacterial and archaeal diversity revealed that the actinobacteria represented 783 sequences and was clustered into 418 OTUs (operational taxonomic units) (Lv et al. 2014). The microbiome of rhizosphere (rhizobiome) of marine flowering plant, seagrass species such as *Zostera marina, Zostera noltii*, and *Cymodocea nodosa* were studied for the diversity of actinobacteria from the North-eastern Atlantic Ocean (Cúcio et al. 2016). Recently, a review on the diversity of the endophytic actinobacteria associated with plant isolated from various habitats such as mangrove and aquatic ecosystem was published that signifies the use of molecular techniques for the identification of endophytic actinobacteria (Singh and Dubey 2018).

6.5 Pharmaceutical Importance of Marine Actinobacteria

6.5.1 Enzymatic Potential

6.5.1.1 Protease

The protease accounts for 65% of total industrial enzyme and has the applications in detergent, leather, silk, and food industries. Due to these applications, the proteases with novel properties from marine habitats are the focus of recent researchers. The marine actinobacteria *Streptomyces pectum* RA71 isolated from Chennai was studied for screening and characterization of protease enzyme (Fernandez et al. 2018). Recently in the year 2019, novel *Streptomyces radiopugnans* VITSD8 isolated from marine sponge *Agelas conifer* is studied for the production of fibrinolytic protease in the sea coast of Tamil Nadu (Dhamodharan and Naine 2019).

6.5.1.2 Amylase

The amylase enzyme has many significant biotechnological applications in textile, food, paper, and fermentation industries. According to the study conducted by Basha and Rao (2017), mangrove microbes from marine habitats are rich sources of industrial important enzymes particularly actinomycetes from marine habitats are the dominant source of enzymes. The aquatic actinobacteria producing amylase activity that belonged to genus *Streptomyces* were isolated from mangrove sediments in South Iran (Kafilzadeh and Dehdari 2015). Similarly, the α-amylase from *Streptomyces pluripotens* and *Streptomyces chilikensis* was isolated from mangroves and was studied for the geographical effect on the amylase production (Saavedra and Marambio-Alfaro 2019).

6.5.1.3 Cellulase

Celllulose is a renewable resource and cellulase is an important enzyme produced by the microbes that use cellulosic substances for their growth. The marine actinobacteria *Actinoalloteichus* sp. MHA15 was screened for their cellulolytic activity (Rajagopal and Kannan 2017). The actinomycetes especially *Streptomyces* sp. was studied for cellulolytic activities such as hemicellulolytic and lignocellulolytic activities. The lignocellulolytic enzymes are most important for the environment cleaning by the process of biodegradation (Saini et al. 2015). The cellulase-producing actinobacteria were also explored from mangrove sediments (Mohanta 2014) and coastal areas (Kulkarni and Maurya 2017). The cellulase enzyme is applicable in biomass treatment, waste treatment, and detergent industry.

6.5.1.4 Chitinase

The chitin is the main constituent of fungal cell wall, shrimp cells, and marine prawn waste. The chitinase-producing actinobacteria play an essential role in carbon nutrient cycle (Lacombe-Harvey et al. 2018). Recently, the chitinase-producing *Streptomyces* sp. ACT7 was isolated from the coastal region of South India (Thirumurugan et al. 2015). More recently, marine strains *Stenotrophomona*

maltophilia were isolated from the ocean sediments with high chitinase activity (Salas-Ovilla et al. 2019).

Other Enzymes

The actinobacteria also produce lipases, urease, and gelatinase enzymes which are most important for the biodegradation of complex substances in nature. The marine actinobacteria are studied for industrial important enzymes such as L-asparaginase and DNase (Gobalakrishnan et al. 2016). The xylanase enzyme which is important in paper and pulp industry was produced by marine actinobacteria *Streptomyces viridochromogenes* (Liu et al. 2013).

6.5.2 Antimicrobial Potential

The marine actinobacteria are well known for the secondary metabolite production and showed biological activities against pathogens. These metabolites displayed antibacterial activities in the presence of Gram-positive and Gram-negative pathogens such as *Pseudomonas aeruginosa, Escherichia coli, Salmonella enteric, Staphylococcus aureus*, and *Listeria monocytogenes* (Undabarrena et al. 2016). In addition, it displayed antifungal, antiprotozoan, and antiviral activities. The actinobacteria are also pharmaceutically important as they have antitumor activities, and display resistance against model antibiotics such as tetracycline, ciprofloxacin, oxacillin, and enzyme inhibitors. Moreover, in agriculture, secondary metabolites derived from actinomycetes act as herbicides, insecticides, and pesticides against plant pathogens (Zotchev 2012). In Egypt, a marine *Streptomyces sp*. was studied for their antimicrobial and nematicidal activities and it increased plant growth by the production of phytohormones (Rashad et al. 2015). The marine actinobacteria isolated from the Red Sea coast showed cytotoxic activity against breast cancer cell lines MDA-MB-231 and antifungal activity against fungal yeast *Candida tropicalis* (Abdelfattah et al. 2016). A novel marine actinobacteria *Streptomyces* LK-3 isolated from marine sediments was studied for the antioxidant activities such as metal chelating activity, nitric oxide scavenging activity, ferric reducing antioxidant activity, etc. (Karthik et al. 2013). The diversity analysis of marine grass *Halodule uninervis* revealed that the strains belonged to the phylum *Firmicutes* was dominant and showed antagonistic activity against plant pathogenic fungi such as *Rhizoctonia solani, Pythium ultimum, Phytophthora capsici*, and *Pyricularia oryzae* (Bibi et al. 2018). The marine endophytic actinobacteria were studied for their antimicrobial activity against multidrug-resistant bacteria isolated from mangrove plants (Jiang et al. 2018) and antifungal metabolites from mangrove soil (Palla et al. 2018). The antimicrobial activity of actinobacteria from saline wetlands was also studied (Trabelsi et al. 2016).

6.5.3 Novel Bioactive Compounds

The actinobacteria are a major source of the secondary metabolites as well as a source of novel bioactive compounds with various pharmaceutical applications. In 2018, the novel bioactive compounds from the marine actinomycetes were isolated from South China Sea sediments such as a novel α-pyrone compound from marine *Nocardiopsis* sp. NHF48, paulomenol from marine *Streptomyces* sp. NHF86, and a new source of rifamycin B from *Salinispora* sp. NHF45 (Yang and Song 2018). A novel bioactive compound as "(Z)-1-((1-hydroxypenta-2,4-dien-1-yl)oxy) anthracene-9,10-dione" having antiviral and larvicidal activities was extracted from marine actinobacteria *Nocardia alba* KC710971 (Janardhan et al. 2018). As discussed before, the metagenomic approach is very useful for mining the novel bioactive compounds. The metabolomic analysis of isocoumarin metabolite produced by the marine actinobacteria *Streptomyces* species MBT76 revealed two novel secondary metabolites 5, 6, 7, 8-tetramethoxyl-3-methyl-isocoumarin and acetyltryptamine (Wu et al. 2016). In addition to this, a novel bioactive compound aporphine alkaloid SSV has anticancer activity. It was produced by the marine actinobacteria *Streptomyces s*p. KS1908 (Kadiri et al. 2013). A novel antibiotic anthracycline and keyicin were produced from co-culturing of the *Rhodococcus* sp. and a *Micromonospora* sp. isolated from marine habitat (Adnani et al. 2017). The mangroves also explored for the mining of new bioactive compounds such as novel ansamycin and their analogs were isolated from the *Streptomyces* sp. KFD18 (Zhou et al. 2019). Recently, a novel biosurfactant, Dokdolipids A − C which is a hydroxylated rhamnolipid was obtained from the *Actinoalloteichus hymeniacidonis* sediment of coasts of Dokdo island, Korea (Choi et al. 2019) and actinomycin D was derived from marine actinobacteria *Streptomyces costaricanus* SCSIO ZS0073 (Liu et al. 2019). Thus, the rare actinobacteria from various marine habitats are a potential source of unique and novel bioactive compounds (Subramani and Sipkema 2019).

6.6 Conclusion

The marine actinobacteria from various habitats are a potential source of novel bioactive compounds. They are important in drug discovery as well as have many pharmaceutical applications. The enzymatic potential of marine actinobacteria highlights its applications in various biotechnological industries. The actinobacteria associated with marine plants also play a significant role in nutrient cycling and biodegradation of organic matter. Moreover, the new molecular approaches would explore the diversity of marine actinobacteria from unexplored habitats. In nutshell, the marine actinobacteria would be the best source of novel and unique biological compounds.

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Biodiversity of Marine Actinobacteria in Indonesia and Their Potential to Produce Bioactive Compounds

7

Ifah Munifah and Hari Eko Irianto

Abstract

Increased microbial resistance due to the use of antibiotics and the emergence of new pathogenic microbes has inspired the search for new antibiotics from microbes. Some literature states that marine actinobacteria have a lot of potential to produce bioactive compounds, thus increasing the interest of researchers.

It was reported that there are more than 25,000 known microbial bioactive compounds, 75% of which are produced by actinobacteria (most of which are the genus *Streptomyces*), and a further 25% by the fungus, *Bacillus* spp. and other bacteria. More than 10,000 antibiotics are known to have been produced from the genus *Streptomyces*. This makes the genus *Streptomyces* very important than others. The discovery of rare and new actinobacteria is very important and interesting for the discovery of bioactive compounds because of the increasing need for the development of new and potential antimicrobial agents. Provision of access to new sources of bioactive compounds in the form of modern technology is needed for the detection and isolation of marine actinobacteria and their bioactive compounds. This process is then continued through the development of improved cultivation methods with molecular technologies that needs to be done intensively so as to produce antibiotic compounds with novel actinobacteria including new species as previously reported.

Keywords

Actinobacteria · Antimicrobial · Bioactive compounds · Marine · *Streptomyces*

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7.1 Introduction

The actinobacteria are a large group of Gram-positive bacteria with high G + C% and known to have high biodiversity and chance to acquire a novel species (Otoguro et al. 2009). Several members of phylum actinobacteria produce important secondary metabolites as a promising source of bioactive compounds notably including antibiotics, antimicrobial, and enzyme (Qin et al. 2011). Actinobacteria play an important role in producing natural products that show biological activity by recycling to degrade waste in the environment (Mohanraj and Sekar 2013). In recent years, marine actinomycetes have been shown to produce many new active compounds. Important discoveries of active compounds produced by marine microorganisms have been reported more than 50 years ago. The sea was considered as an unfavorable environment for microbial growth due to high salt content. Instead, land and terrestrial is considered as a supportive environment for microbial growth. Biodiversity of marine microorganisms is an interesting thing to be studied more deeply by researchers. The different and more varied conditions of the marine environment compared to the terrestrial environment apparently affect the characteristics of marine microorganisms including the types of active compounds they produce (Meiying and Zhicheng 1998).

Marine actinobacteria are a potential source of novel bioactive compounds in which the environmental conditions of the sea affecting bioactive properties are entirely different from the terrestrial conditions (Meiying and Zhicheng 1998). Actinobacteria isolated from the marine environment have been developed novel antibiotics and reported producing a variety of enzyme inhibitors, antibiotics, antibacterial, antifungal, and anticancer compounds (Biabani et al. 1997; Maskey et al. 2003; Charan et al. 2004; Li et al. 2005; Sujatha et al. 2005; Fehling et al. 2003; Riedlinger et al. 2004; Imade 2005; Peela et al. 2005; Hughes et al. 2008). A total of 97 actinobacteria strains were isolated from 24 marine samples such as seawater, sea sediments, sponges, and corls obtained from Tamilnadu state, India (Kumari et al. 2013). It was reported that among the actinobacteria group, more than 500 species of *Streptomyces* spp. have been reported and the genus of *Streptomyces* is the majority.

More than 70% of our planet's surface is covered by oceans and the life on Earth comes from the sea. The greatest biodiversity is in the oceans (Donia and Harman 2003). As an archipelagic country, the Indonesia territory is dominated by waters, therefore an exploration of marine microorganisms is recommended, including actinobacteria. Marine actinobacteria may have different characteristics from terrestrial actinobacteria that might produce new bioactive compounds and new antibiotics due to the marine environment being very different from terrestrial conditions. This review will focus on the biodiversity of marine actinobacteria, the role of actinobacteria in the marine environment, and actinobacteria as bioactive compound producers.

7.2 Actinobacteria

Phylum actinobacteria, recognized as prokaryotic organisms, are a large Gram-positive bacteria, aerobic, non-motile, and (70–80%) high guanine-cytosine content in their DNA and phylogenetically related to the bacteria based on the evidence of 16S ribosomal RNA. They are originally considered as an intermediate group between bacteria and fungi.

These actinobacteria microorganisms have hyphal bearing spores of air mycelia which have a diameter somewhat larger than mycelia substrate. Phenotypically it was reported highly diverse and found in most natural environments (Goodfellow and Williams 1983).

Actinobacteria found on terrestrial are known as common genera of actinobacteria which have been mentioned before being different compared to actinobacteria from the marine environment, in which actinobacteria from marine were classified as rare actinobacteria. Therefore, different mediums were used to isolate both terrestrial and marine actinobacteria. Dharmaraj (2011) reported the morphological characteristics of marine actinobacteria, in which the colonies were chalky, folded, and aerobic; and the aerial mycelial color patterns were a white, grey, and yellow series that is different for all the strains. Most strain showed smooth spore surface and rectiflexibiles hyphae, but spiral and retinaculiaperti hyphae are rare. This distinguishes the actinobacteria morphology commonly found in the terrestrial environment. Marine actinobacteria have unique characteristics in terms of chemical and structural features, physiological, to survive in the marine environment (Jose and Jha 2017). In fact, potential sources of new bioactive compounds from several marine actinobacteria for therapeutic applications have not observed in their terrestrial counterparts yet. Figures 7.1 and 7.2 exhibit the differences between terrestrial and marine actinobacteria colonies.

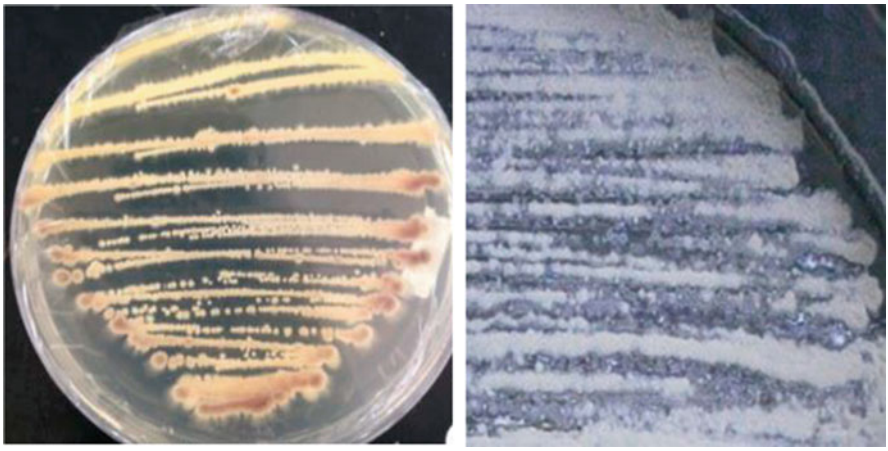

Fig. 7.1 Terrestrial actinobacteria (Rante et al. 2017; Sari et al. 2014)

Fig. 7.2 Marine actinobacteria (Asnani et al. 2016; Sulistyani and Akbar 2014)

7.3 Biodiversity of Marine Actinobacteria

The marine environment is a vast source of acquiring new types of actinobacteria, having the potential to produce novel bioactive natural products. *Actinomadura*, *Actinoplanes*, *Amycolatopsis*, *Marinispora*, *Micromonospora*, *Nocardiopsis*, *Saccharopolyspora*, *Salinispora*, *Streptomyces*, and *Verrucosispora* are some examples of actinobacteria found from the marine environment (Manivasagan et al. 2014)

The role of actinobacteria reflects the marine environment regardless of the production of antimicrobe (Donadio et al. 2010; Meena et al. 2013).

It was reported that actinobacteria take a part in phosphate solubilization, nitrogen fixation, improvement of physical parameters, immobilization of mineral nutrients, mineralization of organic matter, and also protection of environment. The decrease and increase of particular enzyme-producing microorganism may indicate the concentration of natural substrate and the environment conditions (Dastager and Damare 2013; Alharbi 2016).

The first marine actinobacteria species within the genus *Rhodococcus* to be characterized, early evidences supporting the existence of marine actinobacteria came from the description of *Rhodococcus marinonascene* (Helmke and Weyland 1984). It was reported that only 7–8% is the coastal area of the total sea surface and the rest is 60% deep sea, of which is covered by water more than 2000 m deep (Alharbi 2016). Culture experiments of actinobacteria isolated from deep-sea sediments in the earlier studies and more recently have demonstrated that indigenous marine actinobacteria certainly exist in the oceans.

Actinobacteria which have been isolated from marine are genera *Dietzia*, *Rhodococcus* (Teng et al. 2009; Qin et al. 2009; Mehbub and Amin 2012), *Streptomyces* (Bredholt et al. 2008; Duncan et al. 2014), the newly described as genera *Salinispora* (Jensen et al. 2015; Freel et al. 2012; Steinert et al. 2015) and

Table 7.1 The several exploration of terrestrial actinobacteria in Indonesia

Terrestrial actinobacteria	Potency	References
Identification of indigenous *Streptomyces* from soils	Producing antibacterial compounds	Lestari (2006)
Identification of endophytic actinomycetes from the rice plant	Fixing nitrogen	Sari et al. (2014)
Exploration of actinomycetes from the soil of mangrove (*Sonneratia caseolaris*) in Tanjung Api	As antibacterial: *E. coli*	Fatiqin (2015)
Actinomycetes of *Orthosipon stamineus* rhizosphere from the medicinal plant in Makassar city of South Sulawesi Province.	As producer of antibacterial compound against multidrug-resistant bacteria	Rante et al. (2017)
Isolation of actinobacteria from soil samples	Antimicrobial activity (*S. aureus*, *B. cereus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *C. albicans*, *A. niger*, *A. flavus*)	Elbendary et al. (2018)

Marinispor (Jensen et al. 2015; McArthur et al. 2008; Subramani and Aalbersberg 2012), both of which require seawater for growth and have marine chemotype signatures; and *Aeromicrobium marinum* which also has a requirement for salt. In addition, genus *Marinophilus*, *Solwaraspora*, *Salinibacterium*, *Williamsia maris*, and *Verrucosispora* also belong to the indigenous marine actinobacteria (Jensen et al. 2005) (Table 7.1).

7.4 Marine Actinobacteria as Bioactive Compound Producers

Marine actinobacteria have attracted the interest of the research community because of their unique characteristic as potential sources of secondary metabolites or bioactive compounds. Among actinobacteria, *Streptomyces* are an economically important group and they are the source for a wide range of biologically active compounds (Berdy 2005). In addition, *Streptomyces* have been shown to have the ability to synthesize bioactive compounds for antimicrobe, antiparasitic, antifouling, antiinfective, nematicidal activity, plant growth-promoting compounds, enzyme inhibitors, and producing various extracellular hydrolytic enzymes (Alharbi 2016).

Microbial bioactive compounds have been at the frontier in the discovery of novel antimicrobial agents for the pharmaceutical industry. As actinobacteria are prolific producers of secondary metabolites or bioactive compounds with biological activities, therapeutic applications are still waiting to be discovered especially from those produced by actinobacteria (Alharbi 2016). Bull and Stach (2007) reported that marine actinobacteria are new opportunities for the discovery of natural products for new drugs, especially antibiotics. Secondary metabolites are metabolic products that produced at the end of the exponential growth phase, and not essential for vegetative growth from producing organisms but they are considered

Table 7.2 The several explorations of marine actinobacteria in Indonesia

Marine actinobacteria	Potency	References
Marine actinomycetes (*Streptomyces* spp.) isolated from West Banten, North Cirebon, and South Yogyakarta	Antimicrobes: *Escherichia coli*, *Streptococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeroginosa*, *Candida albicans*, *Aspergillus niger*	Sunaryanto et al. (2009)
Marine actinobacterial isolates associated with sponges	Antagonistic potential against fish and shellfish pathogens	Dharmaraj (2011)
Actinomycetes isolates from seaweed (*Eucheuma cottonii*), isolated from Lombok, NTB	As antibiotic producer against *S. aureus* and *E. coli*	Sulistyani and Akbar (2014)
Actinomycetes 9ISP1 isolated from sponge from Randayan Island	Antibacteria: *B. cereus*, *P. aeroginosa*, *S. aureus*, *Aeromonas hydrophylla*, *Vibrio cholera*, *E. coli*, *B. subtilis*, *Salmonella*	Kumala et al. (2015)
Screening of marine actinomycetes from segara anakan	Produce natural pigment and hydrolytic activities	Asnani et al. (2016)

differentiation compounds that provide adaptive roles. The syntheses of secondary metabolites greatly depend on the growth conditions.

Their growth is usually limited by a lack of one major nutrient, such as nitrogen or carbon (Sanchez and Demain 2002). Previous studies in several countries discovered that marine actinobacteria have some potential (Table 7.2).

Actinobacteria continue to be productive for research on marine natural products, with many new characteristics as potential sources of secondary metabolites or bioactive compounds that have prominent pharmacological value. In addition, actinobacteria have the ability to synthesize various different biological actives such as antibiotics, pesticides, herbicides, antiparasitic substances, and enzymes. Various antimicrobial metabolites and antibiotics that are important for the pharmaceutical industry have been produced from the genus *Streptomyces*, *Streptoverticillium*, *Actinoplanes*, *Streptosporangium*, *Streptoalloteichus*, *Saccharopolyspora*, *Streptosporangium Nocardia*, *Actinomadura*, *Amycolatopsis*, *Dactylosporangium*, *Frankia*, and *Micromonospora* spp (Table 7.3 and Fig. 7.3).

More and more new antibiotics are discovered, therefore the opportunity to find new antibiotics and antimicrobials from marine actinobacteria is very necessary. Therefore, the focus of industrial screening has shifted to rare genus markers of actinobacteria which are less exploited such as *Amycolatopsis, Actinoplanes*, *Actinomadura*, *Dactylosporangium*, *Kibdelosporangium*, *Micromonospora*, *Microbispora*, *Planomonospora, Planobispora*, and *Streptosporangium* (Lazzarini et al. 2000).

Claverieas et al. (2015) reported about culturable marine actinobacteria isolated from marine sediment based on 16S rRNA approach, consisting of genera *Streptomyces, Agrococcus, Arthrobacter, Aeromicrobium, Isoptericola, Brachybacterium, Pseudonocardia, Ornithinimicrobium, Tessaracoccus, Dietzia, Flaviflexus, Gordonia, Janibacter, Mycobacterium, Microbacterium, Rhodococcus*, and *Corynebacterium.* Those isolated bacteria demonstrated to have antimicrobial activity.

Table 7.3 Marine actinobacteria with their potential to produce bioactive compounds

Indigenous marine actinobacteria	Potency	References
Micromonospora haikouensi with menaquinones compound	Antitumor	Xie et al. (2012)
Several actinobacteria from the marine environment	Cytotoxic from *Dermacoccus* sp.	Abdel-Mageed et al. (2010)
Streptomyces sp. from marine	2-Allyloxyphenol for antimicrobial and antioxidant	Arumugam et al. (2010).
Several actinobacteria isolated from the marine environment among others *Nocardiopsis* sp. (TP-1161)	Antibacterial	Engelhardt et al. (2010)
Actinobacteria from marine	Antibiotics such as: ß-lactams, aminoplycosides, glycopeptides, lipopetides, asamycins, nucleosides, anthracyclines, peptides, polyenes, polyethers, tetracyclines, and macrolides	Alharbi (2016)
Streptomyces sp. MBT76	Isococoumarins, undecylprodiginine, streptorubin B, 1H-pyrrole-2-carboxamide, acetyltryptamine and fervenulin	Wu et al. (2016)
Marine actinobacteria	Source of compounds for phytopathogen control	Betancur et al. (2017)

Actinobacteria have been revealed to produce antitumor agents and bioactive compounds other than enzymes (Tanaka and Omura 1990) after the discovery compound of actinomycin (Lechevalier 1982). A diverse group of marine actinobacteria is known to produce various types of cytotoxic compounds as anticancer compounds have been reported from marine actinobacteria (Jeong et al. 2006). Various enzymes produced from marine actinobacteria are α-amylase, protease, cellulase, chitinase, keratinase, xylanase, and enzyme inhibitors (Das et al. 2006; Imade 2005). Several enzyme-inhibitor-producing actinomycetes were isolated from various samples collected from the marine environment and characterized useful in medicine and agriculture. They were isolated from sediment sampled from neritic seawater and characterized produces antibiotics against Gram-positive bacteria only in the presence of seawater (Imade 2005).

7.5 Conclusion

In conclusion, there are many genera actinobacteria isolated from the marine environment as sources of novel bioactive natural products. Marine actinobacteria play an important role in several fields such as agriculture and medicine. Streptomyces rank first with a large number to produce bioactive compounds. This study contributed to reporting the biodiversity of actinobacteria from the marine environment, which has potential sources of bioactive compounds.

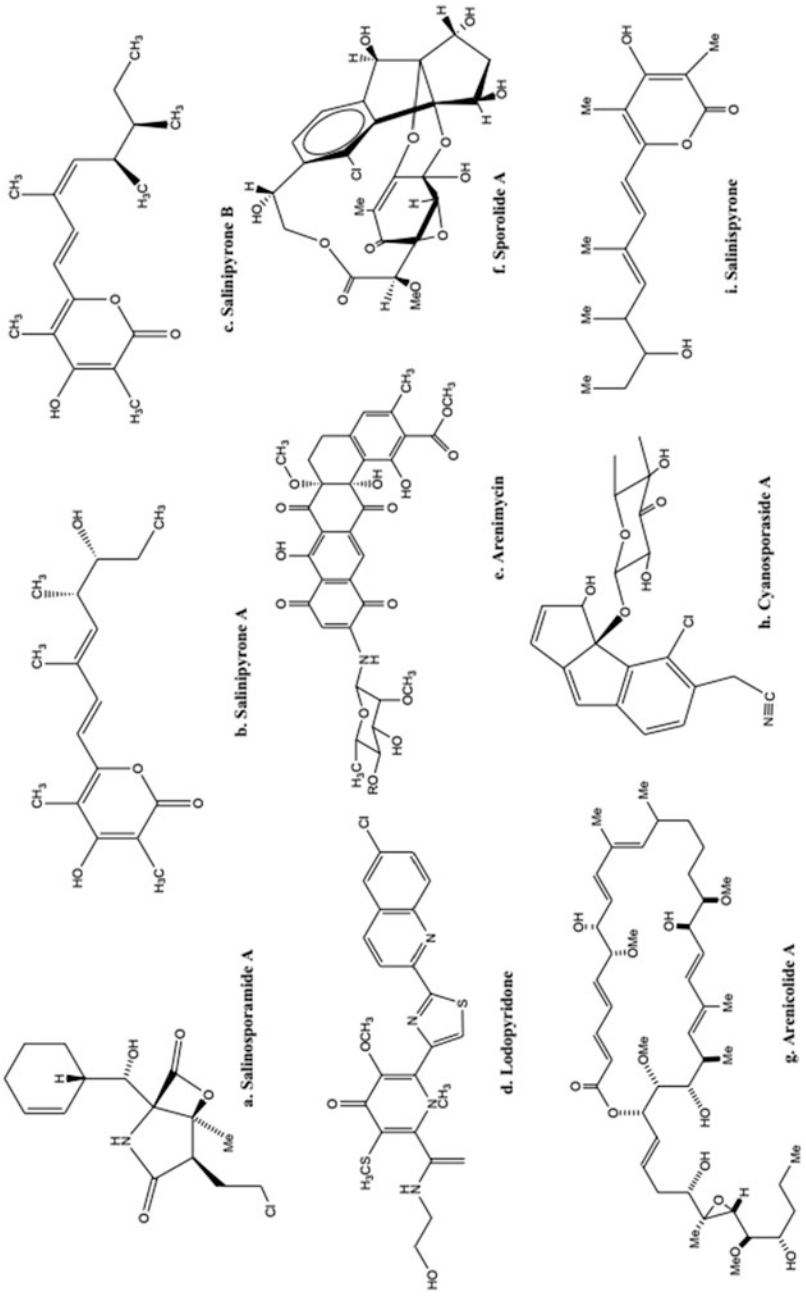

Fig. 7.3 A novel secondary metabolites structured produced by marine actinomycetes (Subramani and Aalbersberg 2012)

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Marine-Derived Fungi: Potential Candidates for Anticancer Compounds

8

Anjana K. Vala

Abstract

Fungi form an integral component of marine ecosystem. They have been observed to play important ecological as well as economical roles. Marine-derived fungi from diverse marine habitats produce a range of metabolites exhibiting various activities having therapeutic and pharmaceutical importance. However, compared to their terrestrial counterparts, fungi from marine world are less explored. Among the metabolites produced by marine-derived fungi, many of them have the potential to modulate activity of enzymes playing key role in growth of tumor and metastasis also. This chapter focuses on anticancer compounds produced by marine-derived fungi associated with diverse habitats.

Keywords

Marine-derived fungi · Anticancer compounds · Secondary metabolites

8.1 Introduction

Cancer is one of the deadliest diseases, and its incidence is increasing worldwide. Global occurrence of cancer is likely to rise to an annual 19.3 million cases by 2025 (Gulland 2014; Gomes et al. 2015). Despite significant advancement in fighting cancer, cancer has been an important health concern (Ruiz-Torres et al. 2017). There has been a growing importance to approaches leading to tumor control, reduction in side effects due to chemotherapy, improvement in quality of life, and prolonged survival of patients (Feinberg et al. 2006; Ruiz-Torres et al. 2017). For the last three

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decades, combinatorial chemistry was the strategy of choice for drug discovery in pharmaceutical industries; however, recently natural products are being given attention for drug discovery. Among top 20 drugs in the market, at least one-third belong to a natural source, chiefly plants, and nearly half of the marketed drugs are categorized as naturally derived or designed on the basis of natural compounds (Paterson and Anderson 2005; Newman and Cragg 2007; Howitz and Sinclair 2008; Ruiz-Torres et al. 2017).

Marine environment is a reservoir of vast biodiversity including plants, animals, and microorganisms. During the course of evolution, the marine world has been a "gold mine" of genetic diversity as well as novel secondary metabolites (Burgess 2012; Shukla and Kim 2016). Though until recently untapped, the marine habitats are now being explored for novel biomolecules due to advancements in research on marine biota, genome mining, and bioassays and natural product chemistry (Vinothkumar and Parameswaran 2013; Shukla and Kim 2016).

Fungi from marine environment have been observed to produce structurally unique and biologically active secondary metabolites including potent anticancer agents (Malaker and Ahmad 2013; Shukla and Kim 2016).

Fungal biota from marine habitats were first reported in France by Duriers and Montagene (1846–1850) (Verma 2011). These biota are not a taxonomic group, but they form an ecological assemblage (Hyde et al. 2000). Fungi in marine environment mainly comprise of *Ascomycota*, *Basidiomycota*, and anamorphic fungi. They can be grouped as temperate, tropical, subtropical, and anamorphic fungi on the basis of their biogeochemical distribution. Obligate marine fungi and facultative marine fungi are the two categories of fungi from marine habitats. While the obligate marine fungi grow and sporulate only in a marine or estuarine (brackish water) habitat, facultative marine fungi have freshwater or terrestrial origin and capability to grow and possibly sporulate also in marine environment (Kohlmeyer and Kohlmeyer 1979; Kohlmeyer and Volkmann-Kohlmeyer 2003; Vala et al. 2016; Vala et al. 2019). The more general categorization of these fungi uses the term "marine-derived fungi" (Bonugli-Santos et al. 2015; Vala et al. 2016; Christophersen et al. 1998; Osterhage 2001; Vala et al. 2019). Nearly all marine habitats have been observed to be inhabited by marine-derived fungi. These biota have been observed to possess diverse capabilities including production of enzyme with anticancer activities (Vala et al. 2004, 2016, 2019; Raghukumar et al. 1994; Vala 2010, 2018 Vala and Sutariya 2012; Vala and Dave 2015). Several pharmaceutical companies are concentrating on bacteria from extreme environment and engaged in bioprospecting marine extreme environments; however, despite their noteworthy capabilities, fungi from such environment are comparatively less explored for their commercial applications (Raghukumar 2008; Vala et al. 2019). This chapter deals with explorations on anticancer compound production by marine-derived fungi. Emphasis is given especially to the compounds playing direct role in bringing about death of cancerous cells.

Fungi from terrestrial habitats have been studied extensively since long and have been source of lead structures for drug development. Marine fungi also have been given importance during the last three decades as a reservoir of new drug leads. It has

been reported that over 1000 new natural products have been obtained from fungi from the marine environment (Rateb and Ebel 2011; Gomes et al. 2015). Many of them have been observed to have unusual carbon skeletons and substitution patterns suggesting unique biosynthetic potential of marine fungal biota (Rateb and Ebel 2011; Bugni and Ireland 2004; Saleem et al. 2007; Gomes et al. 2015).

A range of compounds with potential anticancer activities have been identified due to growing focus on the biochemical potential of fungi from marine environment (Gomes et al. 2015). Over the years, marine-derived fungi have been observed to produce enzymes and other metabolites with anticancer potentials that could be exerted by mechanisms like promoting growth arrest, blocking key enzymes, or stimulating death pathways (Evidente et al. 2014; Gomes et al. 2015; Vala et al. 2018a,b).

Handayani et al. (2018) recently reported cytotoxic activity of marine sponge-associated fungal extracts against various human cell lines; however, chemical nature of the compound has not been identified.

Sekar et al. (2015) have reported yeasts from marine environment as a potential source of anticancer compounds especially inhibiting the lung cancer.

Among various compounds synthesized by marine-derived fungi, enzymes like L-asparaginase, pro-apoptotic metabolites, and some metabolites killing cancer cells without direct pro-apoptotic effects play important role in tackling cancer effectively; some of them have also been observed to exert in vivo anticancer activity (Gomes et al. 2015; Vala et al. 2018a). Table 8.1 shows some of the compounds of marine-derived fungal origin having potential role against cancer.

8.2 Compounds from Marine-Derived Fungi with Anticancer Potentials

8.2.1 L-Asparaginase

L-asparaginase (LA) (L-asparagine amidohydrolase, E.C. 3.5.1.1) is one of the important anticancer compounds. LA of bacterial origin is commercially available and widely used in treatment of certain cancers, especially acute lymphoblastic leukemia (ALL) among children. However, application of this enzyme suffers from certain limitations like hypersensitivity; hence, there has been a growing interest in searching for newer sources of LA (Husain et al. 2016; Vala et al. 2019). Marine-derived fungi have been reported to synthesize LA (Murali 2011; Farag et al. 2015; Vala and Dave 2015; Izadpanah et al. 2018). However, LA production potentials of these mycobiota have not been explored much for their commercial applications. Recently, Vala et al. (2018a,b) examined antiproliferative properties of purified LA produced by marine-derived fungus *Aspergillus niger* AKV-MKBU. The enzyme was observed to be encouragingly effective against the human cancer cell lines, viz., A 549, U87MG, HepG2, JURKAT E6, and bone marrow-derived chronic myeloid leukemia cells. Further studies may lead to some useful revelations. Farag et al. (2015) reported marine-derived *Aspergillus terreus* as

Table 8.1 Compounds of marine-derived fungal origin with anticancer activity/application

Fungus	Compound	Activity/application	References
Penicillium citrinum	Alkaloid	Anticancer compound	Tsuda et al. (2004)
Fusarium sp.	Cyclic tetrapeptide	Anticancer	Ebel (2010)
Apiospora montagnei	Diterpene	Activity against human cancer cell lines	Klemke et al. (2004)
Aspergillus niger AKV-MKBU	L-asparaginase	Activity against human cancer cell lines	Vala et al. (2018a)
Penicillium chrysogenum	Sorbicillactones A and B	Active against human leukemia cell lines	Bringmann et al. (2005)
Bartalinia robillardoides	Taxol	Anticancer	Gangadevi and Muthumary (2008)
Leptosphaeria sp. OUPS-4	Leptosins	Cytotoxicity against human cancer cell lines	Yamada et al. (2002, 2004)
Aspergillus sp. YL-06	Gliotoxin	Activity against human cancer cell lines	Nguyen et al. (2014)
Penicillium citrinum HGY1-5	Dicitrinone B (**30**)	Inhibition of proliferation of multiple tumor types	Du et al. (2010a) and Chen et al. (2014)
Microsporum cf. *gypseum*	Microsporins A (**52**) and B (**53**)	Cytotoxic activity against human colon adenocarcinoma cells	Gu et al. (2007)

Adapted and modified from Raghukumar (2008) and Vala et al. (2019)

good source of LA and confirmed increased production of LA by immobilization of the whole cells. The authors also claimed recyclability of the immobilized enzyme; however, the anticancer potential of the enzyme was not examined by the authors. Hassan et al. (2018) examined anticancer activity of purified LA produced by *A. terreus* against cell lines HCT-116, Hep-G2, and MCF-7 and reported the IC50 to range from 3.79–12.6 μg/ml. A marine fungal isolate *Beauveria bassiana* (MSS18/41) has also exhibited LA production; however, its anticancer activities have not been characterized (Nageswara et al. 2014; Usman 2015).

8.2.2 Metabolites with Pro-apoptotic Effects

Apoptosis, the programmed cell death, is a carefully regulated, energy-requiring process which is characterized by specific morphological and biochemical features (Elmore 2007).

Whether the cells are healthy or cancerous, most cytotoxic cell insults would lead to apoptosis; hence, it is required to differentiate between the direct and indirect pro-apoptotic effects of a test compound (Gomes et al. 2015). A number of marine-derived fungal metabolites with cytotoxic properties exhibit pro-apoptotic effects

against cancer cells and induce apoptosis through the Akt pathway as shown by leptosin C, SZ-685C (21), and ophiobolin O (61) (Yanagihara et al. 2005; Xie et al. 2010; Lv et al. 2015; Gomes et al. 2015).

Indole alkaloid fumigaclavine C (19), produced by a marine-derived strain of *Aspergillus fumigatus*, was observed to display pro-apoptotic effects in human MCF-7 breast cancer cells. The compound also exhibited inhibition of MMP-2 and MMP-9 protease activity in the MCF-7 cells and downregulation of NF-κB cell survival pathway (Li et al. 2013a). Marine-derived strains of genera *Aspergillus*, *Fusarium*, *Nigrospora*, and *Xylaria* have been reported to produce bostrycin (20) (Jiang et al. 2000; Xu et al. 2008; Trisuwan et al. 2010; Huang et al. 2014; Gomes et al. 2015). Chen et al. (2011) reported induction of apoptosis in A549 non-small cell lung cancer (NSCLC) cells by bostrycin (20). The authors related pro-apoptotic effects in A549 NSCLC cells to downregulation of PI3K/AKT protein pathway and upregulation of microRNA-638 and microRNA-923. Sawadogo et al. (2013) reported inhibition of cell proliferation and induction of apoptosis in prostate, gastric, and lung cancer cells by bostrycin produced by marine fungi from the South China Sea.

A prenylated diketopiperazine alkaloid neoechinulin A (18), reported to occur commonly in marine-derived strains of *Eurotium*, has been isolated from a red algae-associated fungus *Microsporum* sp. MFS-YL and has been observed to exert cytotoxic effects against HeLa cells (Li et al. 2008; Gomes et al. 2012, 2015; Kim et al. 2013; Wijesekara et al. 2013). Downregulation of Bcl-2 expression, upregulation of Bax expression, and activation of the caspase-3 pathway were involved in inducing apoptosis in HeLa cells (Wijesekara et al. 2013). *Microsporum* sp. MFS-YL has been observed to produce anthraquinone physcion (29) (parietin) that also exerted pro-apoptotic effects in HeLa cells involving downregulation of Bcl-2 expression, upregulation of Bax expression, and activation of caspase-3 pathway as well as formation of ROS (Wijesekara et al. 2014).

Marine sponge-associated fungi have also been observed to produce potent inhibitor compounds. Dankastatin C, a polyketide tyrosine derivative from a sponge-derived fungus *Gymnascella dankaliensis*, inhibited the P388 lymphocytic leukemia cell line and was found to be equally potent as the common chemotherapeutic drug 5-fluorouracil (Amagata et al. 2013). Sponge-derived fungus *Phoma* sp. produced epoxyphomalin A that could display cytotoxic effect against 12 human tumor cell lines even at nanomolar concentrations (Mohamed et al. 2009).

Mangrove-associated fungus *Penicillium* sp. has been reported to produce new derivatives of shearinine A **(10)**, a class of janthitrem-type indole terpenes (Xu et al. 2007). *Penicillium janthinellum* isolated from marine sediments has been reported to produce shearinine A-related metabolites (**11**, **12**) by Smetanina et al. (2007).

Deep sea-derived fungi have been observed to be potential sources of anticancer compounds; *Penicillium* has been a dominant genus among them. A deep ocean sediment-derived *Penicillium* sp. has been observed to exhibit production of different meleagrin analogs like meleagrin D (**14**), meleagrin E (**15**), meleagrin (**16**), and meleagrin B (**17**) (Du et al. 2009; Du et al. 2010b). While meleagrin (**16**) and meleagrin B (**17**) exerted their effect by arresting the cell cycle through the G2/M

phase and inducing apoptosis in HL-60, respectively, only modest cytotoxic activity against the A-549 cell line was exhibited by meleagrin analogs **14** and **15**. Later, other marine-derived *Penicillium* spp. were also reported to produce meleagrin (**16**) with cytotoxic activity against various cancer cell lines (Shang et al. 2012; Zheng et al. 2013).

Deep sea-derived *Penicillium* sp. F23–2 has been reported to produce sorbicillamines A–E (1–5) displaying cytotoxic activity on HeLa, BEL7402, HEK-293, HCT-116, and P388 cell lines (Guo et al. 2013). Anthranilic acid derivatives, penipacids A-E (1–5) from deep sea-derived *Penicillium paneum* SD-44, have been observed to display inhibitory action against human colon cancer RKO and HeLa cell line (Li et al. 2013b). According to Russo et al. (2015), deep sea-derived compounds fit well in the new approach termed "magic shotguns" hunting for special molecule that broadly interrupts the entire diseases process.

Farha and Hatha (2019) examined bioprospecting potentials of a marine sediment-derived fungus *Penicillium* sp. ArCSPf and reported its significant anticancer activity against MCF-7 breast cancer cells. The secondary metabolite (Z)-octadec-9-enamide (oleamide) was present in the active fraction F2 of extract that exhibited anticancer potential.

8.2.3 Metabolites Killing Cancer Cells by Mechanisms Other Than Direct Pro-apoptotic Effects

A number of metabolites are produced by marine-derived fungi that kill cancer cells without exerting direct pro-apoptotic effects. Members of genera *Aspergillus* and *Penicillium* have been observed to synthesize compounds exerting anticancer activity without direct pro-apoptotic effects. A marine sediment-derived *Aspergillus* sp. SF-5044 produced protuboxepin A (**34**), which was also reported as a co-fermentation product of two algicolous aspergilli (*Aspergillus* BM-05 and BM-05 L). Cytotoxic activity of protuboxepin A (**34**) was attributed to tubulin inhibition (Lee et al. 2011; Ebada et al. 2014; Asami et al. 2012; Gomes et al. 2015). Marine-derived *Penicillium* sp. was observed to produce oxaline (33) (9-O-methyl analog of meleagrin (16)) that displayed cell cycle arrest during M phase and inhibited the polymerization of microtubule protein in vitro (Koizumi et al. 2004). Seaweed-associated *Penicillium pinophilum* Hedgcock produced three hydrogenated azaphilones, viz., pinophilins A (44) and B (45) and Sch 725680 (46) that displayed anticancer activity by the inhibition of DNA replication (Myobatake et al. 2012; Gomes et al. 2015).

Marine sponge-derived fungus *Paraconiothyrium* cf. *sporulosum* produced epoxyphomalins A–E (35–39) displaying cytotoxic activity against various cancer cell lines. Upon detailed analysis, epoxyphomalins A (35) and B (36) were observed to be potent inhibitors of the proteasome complex (Mohamed et al. 2009, 2010). Coral reef-associated fungus *Phomopsis* sp. (strain TUF95F47) was observed to produce phomopsidin (40) that displayed inhibition of microtubule assembly (Namikoshi et al. 1997, 2000).

Inhibitors of $p56^{lck}$ tyrosine kinase have also been produced by marine-derived fungi. Ascosalipyrrolidinone A (**43**) was produced by *Ascochyta salicorniae*, an endophyte of alga *Ulva* sp. (Osterhage et al. 2000). Algicolous fungus *Wardomyces anomalus* produced various xanthones including anomalin A (47), norlichexanthone (48), and 5-(hydroxymethyl)-2-furancarboxylic acid (49) that displayed inhibition of $p56^{lck}$ tyrosine kinase (Abdel-Lateff et al. 2003). Chaetominedione (50), a benzonaphthyridinedione derivative obtained from alga *Valonia utricularis*-associated fungus *Chaetomium* sp., also exhibited significant inhibition of $p56^{lck}$ tyrosine kinase activity (Abdel-Lateff 2008).

Pulchellalactam (51) produced by marine fungus *Corollospora pulchella* ATCC 62554 was observed to be an inhibitor of CD45 protein tyrosine phosphatase. As CD45 protein tyrosine phosphatase plays vital signaling roles in dephosphorylating Src kinases, pulchellalactam (51) symbolized an attractive drug target (Alvi et al. 1998).

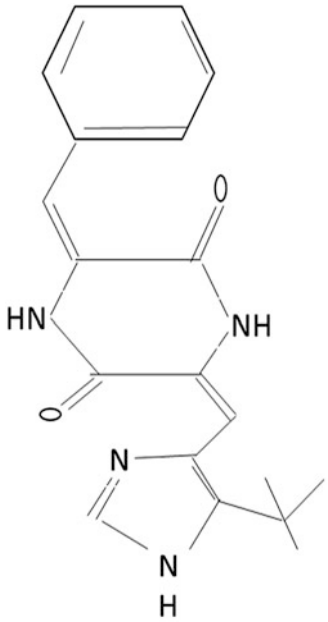
Three linear pentadecapeptides, viz., efrapeptins F (54), G (55), and J (56) produced by marine fungus *Tolypocladium* sp. AMB18, were observed to inhibit the luciferase expression in HT1080 human fibrosarcoma cells. Due to its important functions, molecular chaperone GRP78 is a significant target for development of anticancer compounds. Among the three linear pentadecapeptides produced by *Tolypocladium* sp. AMB18, efrapeptin J (**56**) was also reported to bring about downregulation of the molecular chaperone GRP78 in HT1080 and MKN74 human gastric cancer cells. The death of HT1080 cells was also induced by it due to endoplasmic reticulum stress (Hayakawa et al. 2008; Roller and Maddalo 2013; Shenolikar 2014; Gomes et al. 2015).

8.2.4 Metabolites Exhibiting In Vivo Antitumor Activity and Entering Clinical Trials

In vitro antitumor activity of a number of metabolites obtained from marine-derived fungi has been reported. However, obviously all of them do not enter clinical trials. Some of the marine-derived fungal metabolites have been examined for in vitro antitumor activity. A sea hare-associated fungal strain *Periconia byssoides* OUPS-N133 produced cyclohexenoid metabolites pericosines A (**57**), B (**58**) and D (**59**) which displayed noticeable in vitro cytotoxic activity against murine P388 cells (Numata et al. 1997; Yamada et al. 2007). Among the three metabolites, pericosine A (**57**) showed remarkable in vivo inhibitory activity in mice inoculated with P388 leukemia cells intraperitoneally, and increased survival in mice was observed when administered with 25 mg/kg of pericosine A (**57**) (Numata et al. 1997; Yamada et al. 2007).

Spiroxin A (60), isolated from an unidentified marine-derived fungus, had also been observed to be a potent anticytotoxic against 25 different cell lines. It exhibited in vivo antitumor activity against ovarian carcinoma leading to 59% inhibition after 21 d at 1 mg/kg administered dose in nude mice (McDonald et al. 1999). As under

Fig. 8.1 Plinabulin (7)



clinical situations, ovarian cancers do not develop subcutaneously, assessment of the actual value of the in vivo assay was difficult (Westin et al. 2013).

A halimide-based microtubule-disrupting agent, plinabulin (7) (Fig. 8.1) of marine-derived fungal origin, is the only candidate so far which has reached clinical development. Plinabulin proved promising during preclinical models and has been brought to clinical trials for the treatment of non-small cell lung cancer (NSCLC) (Gomes et al. 2015; Nicholson et al. 2006; Pereira et al. 2019). Protection against development of chemotherapy-induced neutropenia (CIN) by plinabulin has been observed during clinical studies.

A combination of plinabulin with docetaxel proved better for NSCLC patients than treatment with docetaxel alone (Blayney et al. 2016; Mohanlal et al. 2017; Pereira et al. 2019). A global phase 3 trial (DUBLIN-3) was commenced based on these findings to evaluate second- or third-line treatment with this combination in patients with advanced NSCLC with at least one measurable lung lesion. Duration of severe neutropenia with plinabulin versus pegfilgrastim in patients with solid tumors receiving docetaxel myelosuppressive chemotherapy is being assessed in a phase 2/3 trial (Protective-1). Evaluation of combination of plinabulin and nivolumab in NSCLC is also being carried out in additional phase 3 trials (Mohanlal et al. 2018; Pereira et al. 2019).

8.3 Conclusion

Until recently, marine-derived fungi have been overlooked as a source of lead structures. Marine-derived fungi have been observed as a reservoir of potential anticancer agents, and some of the chemicals have exhibited in vivo antitumor activity. However, so far plinabulin (**7**) is the only molecule of marine-derived fungal origin that has reached clinical trials. Further investigations in this line may unveil potentials of many such molecules that can be translated as a tool to combat cancer.

Many of the metabolites produced by fungi from marine environment have been observed to exert anticancer effects by diverse mechanisms including blocking of key enzymes, stimulation of death pathways, or promotion of growth arrest (Evidente et al. 2014; Gomes et al. 2015).

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Part II

Marine Flora and Biomedical Applications

Marine Flora: Source of Drugs from the Deep-Sea Environment

9

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Abstract

Marine floras are highly diverse and productive. Few marine ecosystems are expected to show higher biological diversity than in tropical rain forests. They are chemically different from terrestrial flora and can withstand adverse marine conditions. The production of unique chemicals has diversified further due to the continuous evolution of marine flora with the change of environmental conditions since billions of years ago. They demonstrate a worthy resource for novel potent drugs that might prove to be economical, safer, and useful medicine for dreadful human diseases. They are rich sources of bioactive compounds such as polyphenols and sulfated polysaccharides that have antimicrobial, antioxidant, antitumor, and disease-healing properties. Plant products are widely used since old times as natural medicines and after the exploration of marine floras discovery of marine medicines has also increased. Here, in this chapter, we present the latest developments on drugs originated from marine natural products and their usages.

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9.1 Introduction

Drugs are a boon to ensure the continual of the human race generation after generations. Drug discovery from natural sources is perceived to be much more sustainable and diverse as compared to artificial means. This is because the natural sources undergo continuous change and coevolve in response to the dynamic external environment; as a result, they are the answers to the most complex, resistant, and incurable diseases. Multiple drug resistance in the treatment of cancer and infectious diseases like tuberculosis is progressing at a frightening rate. A number of drugs developed in past years are failing to live up to the dynamic status of the microbes causing these diseases. Thus, with the increasing complexity of the world the diseases get more and more complex and resistant to cures. An exploration for new drugs is the need of the hour. Thus, under these circumstances, discovering the biomedical potential of natural products can serve the purpose. Natural products have provided an endless source of medicine since prehistoric times. A vast array of phytochemicals with potential biological activity have been identified that confer therapeutic and pharmacological effects. In spite of the huge diversification of drug discovery technology, natural products from plants and other biological sources remain an undiminished source of new pharmaceuticals. Amongst plants, marine flora has been an excellent source of a wide range of bioactive compounds with exceptional biological activities (Wratten et al. 1977; Faulkner 2002). The hidden reserves of the marine environment, the ocean provides society with an essential biomedical resource through the rich diversity of marine organisms. Many marine organisms have contributed to biomedicine (novel antibiotics, anti-inflammatory agents, and anti-neoplastic drugs) through the unique molecules they produce (Blunt et al. 2016).

Marine plants have evolved and adapted to life in a largely stable but extremely harsh environment, which has led to the development of many unique chemical features that are not found in terrestrial plants. The marine environment is characterized by high concentrations of halogens, mainly in the form of chloride and bromide salts. Other chemical entities, such as sulfate, are also found in high concentrations. Marine plants use these elements in biosynthetic pathways to produce phytochemicals such as halogenated terpenes, acetogenins, and alkaloids which are unique to the marine environment. Adaptation to wave shock and ocean currents peculiar to the marine environment has resulted in the synthesis of complex polysaccharides (complex sugars) which play a vital role to reduce the surface tension of seawater (Kathiresan et al. 2008). As these constituents are generated and accumulated in response to a set of very unique and distinct environmental conditions and genetic compositions, they emphasize the insurmountable potential

of marine plants. In addition, many of the marine organisms are largely dependent on chemical defense due to lack of protective shield and locomotor organs; they synthesize diverse chemical compounds to discourage predators and competitors and also to paralyze their prey. Moreover, the ocean comprises nearly 72% of the earth's surface and is immensely rich in biodiversity with marine flora comprising cyanobacteria, fungi, microalgae, seaweeds, mangroves, and other halophytes. Together all of them form a great reserve that has a profound potential to be explored in the field of drug discovery. With the advent of the modern chemical and molecular genetics technologies, these reserves can be investigated to procure a number of resources, including medicinal products, cosmetics, foods, industrial chemicals, and other environment-friendly products (Sithranga et al. 2010).

During the late 1960s, pioneering initiatives to extract drugs from the sea began. In earlier times strange episodes of memory loss reported by a fisherman were attributed to a bloom of a dinoflagellate, *Pfiesteria*. Gradually, over a period of time it has all culminated into the announcement of a new cancer-fighting drug isolated from a marine organism, this reemphasizes the potential of the ocean to benefit human health (Javed et al. 2011). Thousands of medicinally important compounds have been obtained from organisms dwelling in the sea with hundreds of novel natural products being added up to the list every single year. The use of marine floras for pharmaceuticals can be traced back to ancient times in many of the Asian countries like India, China. Since the seaweeds especially brown seaweeds are the good reservoir of iodine and hence consuming them as food source assures least incidence of goiter and glandular diseases. Moreover, maritime countries have been utilizing seaweeds as antiheminthic, anesthetics and ointment and also for the curing of cough, wounds, gout, goiter, etc., since ancient times. Many societies, particularly those in the Indo-Pacific region and Asia, have developed important uses for marine algae. As man has become more aware of the unique chemical composition of marine algae, numerous additional products have been developed. Numerous species of marine algae are used in many countries such as China as herbal medicines to treat many maladies, ranging from intestinal problems to sunstroke. During the recent past, many marine natural products have been identified that are in the preclinical or early clinical stage and some are already in the market. Marine flora have recently received attention for exploration because of their diversity and are being appreciated as a rich source of natural chemical compounds for the unearthing of more efficient drugs against complex and incurable diseases (Sithranga et al. 2010). In this chapter, we discuss about the promising use of natural products coming from marine flora as a natural medicine against human diseases but also about their potential to limit infections.

9.2 Natural Products from Marine Algae That Can Be Used as Drugs

Marine algae are a heterogenous group of organisms varying greatly in size from unicellular (3–10 μm) to massive multicellular entities of up to 70 m long. Based on their size, two major types of marine algae have been recognized – microalgae and macroalgae (seaweeds). The class microalgae include mainly blue green algae or cyanobacteria whereas macroalgae comprise brown algae, red algae, and green algae. Organisms belonging to each of this class are great sources of marine natural products that can be utilized as potent leads for new drug discovery.

9.2.1 Potent Natural Drugs from Microalgae (Blue Green Algae/ Cyanobacteria)

Cyanobacteria from the marine environment belong to one of the most primitive and simplest prokaryotic organisms on earth. They can execute photosynthesis and are also adapted to extreme habitats on earth. They are an important component of biogeochemical cycles and represent one of the most crucial components of food chains and food webs in various oceanic ecosystems. Marine cyanobacteria have been explored up to an extent and it has been found to be excellent deliverers of bioactive natural products that can be further explored for its medicinal use for irrepressible diseases such as cancer and AIDS. They are of great interest for the extraction of novel compounds for their application in pharmaceuticals because of their immense biodiversity and their relatively simple growth needs. The use of natural products as an alternative to synthetic chemicals is a renewable, eco-friendly, and much better option, since microbial resistance against conventional antibiotics is growing. However, the use of cyanobacterial compounds as medicine is still under infancy, therefore, further research is recommended in this direction.

There are many cytotoxic compounds obtained from marine cyanobacteria, that are mainly peptides chemically and show inhibitory effect on various cancerous cell lines such as lipopeptides extracted from *Lyngbya* sp. (obynamide, palauimide, lyngbyabellin, ulongamides A, ulongapeptin, apratoxins) (Liu and Rein 2010) and *Symploca hydnoides* (guamamide, micromide, and tasiamide); and deacetyl-hectochlorin from *Bursatella leachii.* Other bioactive compounds extracted from *Lyngbya* spp. (jamaicamides A-C, lyngbyabellins E, dolabellin, aurilides, wewakpeptins, macrolides) were found to restrict the growth of lung cell line of human (Liu 2009; Liu and Rein 2010). Many other lipopeptides of medicinal value have also been isolated and identified from *Anabena torulosa* (laxophycins) and *Hyalidium* (cyclic depsipeptides) in the recent past. An acyl amide, columbamides from *Moorea bouillinii* has also been identified that has a moderate affinity for cannabinoid receptors. *M. producens* produces bioactive compounds such as hectoramide, hectochlorins, and jamaicamides.

In addition to lipopeptides, terpene alkaloids such as bartolosides from *Nodosilinea* species and *Synechocystis salina* have also been extracted. *Nodularia*

spumigena yielded pseudoaeruginosins NS1 and NS2 that are potent trypsin inhibitors. *Okeania* yielded polyhydroxy macrolide, macrolactone, and lipopeptide kurahyne that are antimalarial. Recently, janadolide was identified from *Okeania* sp., which shows activity against *Trypanosoma brucei*. Another cyanobactin, wewakazole, was isolated from *Moorea producens* and it exhibits cytotoxic activity toward human cancer cell lines. In addition, odoamide, a more potent cytotoxic compound, was obtained from a Japanese *Okeania* species, which is active against human cervical cancer cells. Two more cyanobacterial products, coibamide and apratoxin, were also reported which can suppress vascular endothelial growth factor (VEGF and its receptor VEGFR2) expression and show anticancer activity. Antifungal compounds, lobocyclamides, were also isolated from marine blue green algae, *Lyngbya confervoides.* Antimalarial agents such as carmabin, dragomabin, and dragonamide were extracted from *Lyngbya majuscule.* Sulfolipid, an anti-HIV compound from *Phormidium tenue*, was also identified. Hectochlorin, microginin-FR1, largamides, and Microcystin-LR were also identified from cyanobacteria and these could be potent remedies against deadly diseases. Marine blue green algae are also valuable for chromophore phycocyanobilin (PCB) and C-phycocyanin (C-PC) with anti-inflammatory activity. These microorganisms are rich source of vitamins, minerals, and amino acids, and many species are used as a dietary nutritional supplement such as *Aphanizomenon flosaquae*, *Coccopedia*, *Cyanidium caldarium, Spirulina platensis*, and *Synechococcus elongates*. Hopefully, these bioactive metabolites will serve as good candidates to be utilized in medicinal chemistry and future drug discovery. Moreover, these organisms can further be explored at genome and proteome levels using advanced molecular biology tools and techniques, to figure out genes or gene clusters that are involved in the biosynthesis of these natural products.

9.2.2 Potent Natural Drugs from Macroalgae

Macroalgae/seaweeds include members of Rhodophyta (red), Phaeophyta (brown), and Chlorophyta (green) of marine origin. All the species belonging to this class are macroscopic and multicellular. They protect the organisms that are used as food and offer habitat for many marine organisms and also perform photosynthesis and release up to 90% of earth's oxygen. In addition, they are diverse resources for biologically active natural products such as proteins and polysaccharides that can further be utilized for pharmaceuticals.

9.3 Brown Algae (Phaeophyta)

The brown algae comprising the class Phaeophyceae are a large group of multicellular algae, including many seaweeds located in colder waters within the Northern Hemisphere. Most brown algae live in marine environments, where they play an important role both as a source of food and shelter. Most brown algae contain the

pigment fucoxanthin in abundance, giving them characteristic greenish-brown color by masking the effect of other pigments, chlorophyll a and c, b-carotenes and xanthophylls (Bold et al. 1985) and are responsible for the distinctive that gives them their name. Phaeophyceae members are the most complex algae and there are no unicellular or colonial forms in this class. Large brown algae are used as a shelter for some bottom-dwelling animals. They also serve as a substrate for other algae that grow as epiphytes, or plants that grow on other plants. Few macroalgae, *Macrocystis* and *Nereocystis* form a canopy called Kelp forests and another one, *Sargassum* forms floating mats of the Sargasso Sea that provide shelter for many organisms in the sea and allow a high level of diverse organisms to survive. More than 2000 species of brown algae are reported. Some species are important for commercial use because of their medicinal use. Many natural products of medicinal value have been extracted from them (Table 9.1).

9.3.1 Cytotoxic Compounds from Brown Algae

Several cytotoxic compounds with potential anti-cancer activities were isolated and identified from the brown algae during past years such as bifurcadiol from *Bifurcaria bifurcata* (Guardia et al. 1999), Sargol from *Sargassum tortile* (Numata et al. 1991), Leptosins from the fungus Leptsphaeria species that shelter in association with the Sargassum tortile (Takahashi et al. 1994). Terpenoid C was isolated from *Styolpopdium zonale* and its methyl ester shows cytotoxic activity (Dorta et al. 2002). Recently, it was reported that *Stoechospermum marginatum* yields spartane diterpenes, which can induce apoptosis in melanoma cells. Many other cytotoxic compounds have also been isolated from brown algae in the recent past, which are stated in Table 9.1. All these cytotoxic natural products are candidate drugs for cancer treatment.

9.3.2 Antimicrobial Compounds from Brown Algae

A meroditerpenoid, methoxybifurcarenone, was isolated and identified from *Cystoseira tamariscifolia*, which shows a fungicidal effect on three pathogenic fungi of tomato and bactericidal effect on *Agrobacterium tumefaciens* and *Escherichia coli* (Bennamara et al. 1999). Another antifungal compound, deoxy lapachol, was also isolated from *Landsburgia quercifolia* and this compound was cytotoxic to leukemic cells (Perry et al. 1991). Zonarol 140 from *Dictyopteris zonaroides* was reported to be an antifungal compound (Fenical et al. 1973). Extracts from *Dictyota dichotoma* displayed antifungal activity against *Trichophyton mentagrophytes, Candida albicans*, and *Fusarium oxysporuni*. Extracts from seven more species *(Dictyopteris delicatula, Dictyota bartayresiana, D. dichotoma, Padina gymnospora, Sargassum plagiophyllum, Spatoglossum asperum, Stoechospermum marginatum*) also exhibited antifungal activity. Lobophorolide from *Lobophora variegate* exhibits antifungal activity against

Table 9.1 Potent lead compounds for drug discovery derived from marine algae

Name	Drug/active compound	Source organism	Disease/activity
(Blue-green algae) Cyanobacteria	Obynamide, Palau'imide, Lyngbyabellin, Ulongamides A, Ulongapeptin, Apratoxins	*Lyngbya* sp	Cytotoxic (cancer cell lines)
	Jamaicamides, Lyngbyabellins, Dolabellin, Aurilides, Wewakpeptins, Macrolides	*Lyngbya* sp	Lung cancer
	Laxophycins, Hyalidium	*Anabena torulosa*	Antimicrobial, antifungal
	Guamamide, Micromide, and Tasiamide	*Symploca hydnoides*	Anticancerous
	Cyclic Depsipeptides	*Hyalidium*	Cancer therapeutics
	Columbamides	*Moorea bouillinii*	Nervous system-related disorder
	Hectoramide, Hectochlorins and Jamaicamides, Cyanobactin Wewakazole	*M. producens*	Anticancerous
	Bartolosides	*Nodosilinea species*	No strong biological activities
	Microcystin	*Synechocystis salina*	Antibacterial
	Pseudoaer-Uginosins NS1 and NS2	*Nodularia spumigena*	Trypsin inhibitor
	Poly-Hydroxy Macrolide, Macrolactone and Lipopeptide Kurahyne	*Okeania*	Antimalarial
	Odoamide	*Okeania*	Cytotoxin
	Depsipeptides Coibamide	*Leptolyngbya sp*	Tumor growth in a nude mouse
	Coibamide A and Apratoxin	*Lyngbya majuscula*	Anticancer
	Lobocyclamides	*Lyngbya confervoides*	Antifungal
	Carmabin, Dragomabin and Dragonamide	*Lyngbya majuscule*	Antimalarial
	Sulfolipid	*Phormidium tenue*	Anti-HIV compound
	Phycocyanobilin (PCB) and C-Phycocyanin (C-PC)	*Spirulina platensis*	Anti-inflammatory
	Yoshi None	*Leptolyngbya sp.,*	Anti-obesity
Brown algae	Meroditerpenoids, Cystodiones G–L and Cystones	*Cystoseira usneoides:*	Inflammatory inhibitor
	Cadinane sesquiterpene cadinan-4(15)-ene-1b, 5a-diol	*Dictyopteris divaricata:*	Anticancer
	Sesquiterpene	*Taonia atomaria*	Anticancer
	Mozukulin A and B	*Cladosiphon okamuranus*	

(continued)

Table 9.1 (continued)

Name	Drug/active compound	Source organism	Disease/activity
	Disulfides	*Dictyopteris membranacea*	Antibacterial, anti-inflammatory activity
	Sulfoquinovosyldiacylglycerol (SQDG)	*Lobophora species*	Antiprotozoal
	Lobophorenols A–C	*L. rosacea*	Bleaching and necrosis
	Dolastane diterpenes	*Canistrocarpus cervicornis*	Inhibited HIV-1 (anti-HIV)
	Spartane diterpenes	*Stoechospermum marginatum*	Induced apoptosis in melanoma cells (cytotoxic)
Red algae	C15-acetogenins	*Laurencia marilzae*	Antibacterial, insecticidal, antifungal and antiviral activity
	Eudesmane	*L. obtusa*	Antimicrobial
	Brominated eudesmanes (selinanes), brominated cycloeudesmane	*L. Pinnata*	Antibacterial, insecticidal
	Brominated indole-related alkaloids	*Laurencia similis*	Antibacterial
	Obtusol	*Laurencia dendroidea*	Larvicidal (insecticidal)
	12-epoxyobtusallene IV, obtusallene X, and marilzabicycloallenes C and D	*Laurencia marilzae*	Antibacterial
	Bis(2,3-dibromo-4,5-dihydroxybenzyl) ether (BDDE)	*Odonthalia corymbifera*	Antidiabetic agent
	Mycosporine-like amino acids shinorine and porphyra-334	*Porphyra sp.*	Immunomodulatory effect
	Galaxamide	*Galaxaura filamentosa*	Antitumor agents
	Polyhalogenated monoterpene, (-)-anverene	*Plocamium cartilagineum*	Antibacterial
	Bromophenol	*Odonthalia corymbifera*	Antimicrobial
	Palytoxin	*Chondria armata*	Insecticidal
	Meroterpenoids	*Hypnea musciformis*	Anti-oxidative
	Polyhalogenated indoles Halogenated indoles	*Rhodophyllis membranacea*	Antitumor, antimicrobial, antidiabetic, and antioxidant
Green algae	Sesquiterpene chlorellatin A and ergosterol derivatives chlorellatin B	*Chlorella sorokiniana*	Antibacterial and Antifeedant

(continued)

Table 9.1 (continued)

Name	Drug/active compound	Source organism	Disease/activity
	4-Hydroxy-2,3-dimethyl-2-nonen-4-olide	*Ulva. pertusa*	Anti-inflammatory
	Fatty acid esters and carotenoid metabolites	*U. intestinalis* and *U. prolifera*	Antimicrobial
	Dimethylsulfoniopropionate and acrylate	*Ulva sp.*	Antifeedant and Antipredatory
	Astaxanthin and sulfolipids		
	Palytoxin	*Chondria armta*	Insecticidal
	Kahalalide F	*Bryopsis sp.*	Anticancer (phase II))

Dendroyphiella salina, *Lindra thalassiae*, and *C. albicans* (Kubanek et al. 2003). Few sesquiterpenes were isolated from *Dictyopteris divaricata* and *Taonia atomaria*, which were capable of inhibiting bacterial adhesion and barnacle settlement. Extracts of *Dictyopteris delicatula, Padina gymnospora, Sargassum tenerrimum, Turbinaria conoides*, and *Zonaria crenata* of the Phaeophyceae exhibited broad-spectrum activity.

A very important compound, diacetoxy – 8-hydroxy2,6-dollabeladiene, which is a dollabelladiene derivative was isolated from *Dictyota pfaffi* (Barbosa et al. 2004) and it exhibited potent anti-(herpes simplex virus) HSV-1 activity and slight inhibition of HIV-1 reverse transcriptase. Similarly, a diterpene from *D. menstrualis* (Pereira et al. 2002) displayed antiretroviral activity. Dolastane diterpenes obtained from *Canistrocarpus cervicornis* was found to be active against HIV-1 (Bunt et al. 2016). Additionally, many of the antimicrobial compounds have been reported recently (Table 9.1) that can be utilized for new drug discovery against pathogenic strains of viruses, bacteria as well as fungi.

9.3.3 Other Compounds with Medicinal Value from Brown Algae

Dictyopteris membranacea produces a series of six disulfides and two known disulfides that displayed anti-inflammatory activity. Several prenyl toluquinones from *Cystoseira crinite* exhibited potent radical-scavenging effects (Fisch et al. 2003) and a phlorotannin, eckstolonol from *Ecklonia stolonifera* had antioxidant property (Kang et al. 2003). Recently, it was reported that *Cystoseira usneoides* yields meroditerpenoids, cystodiones, and cystones along with eight known meroditerpenes with antioxidant and anti-inflammatory activity. Phlorotannins, phlorofucofuroecko, from *Ecklonia stolonifera* isolated the brown alga *Ecklonia stolonifera* shows antihypertensive activity. Meroterpenes extracted from brown algae possess anti-adipogenic and pro-osteoblastogenic activities and have been found to be active against *Leishmania amazonensis.* Fucosterol from *Pelvetia siliquosa* demonstrated antidiabetic activity. Phloroglucinol and its derivatives isolated from *Ecklonia stolonifera* were reported to be as hepatoprotective agents

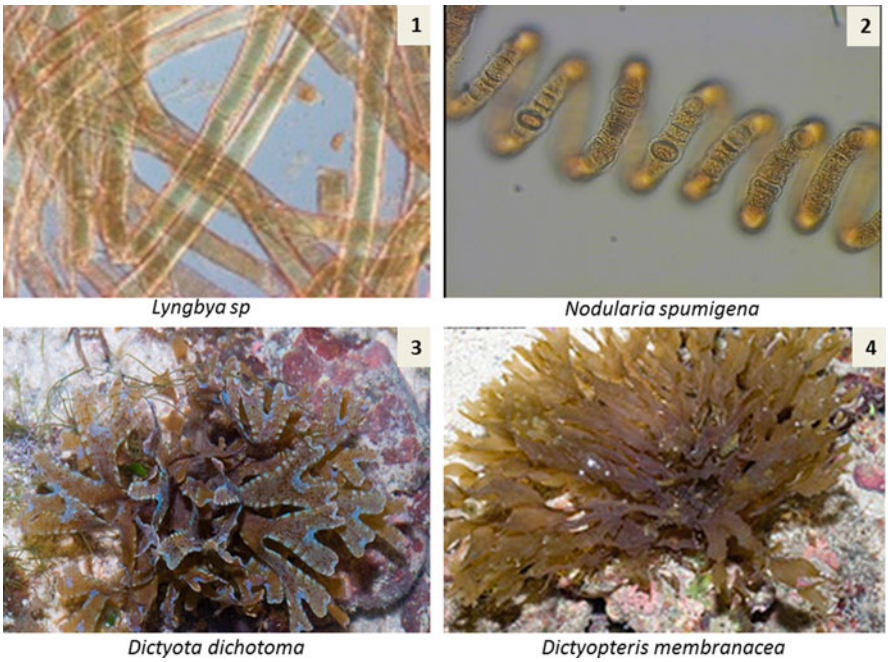

Fig. 9.1 Bioactive compound yielding marine blue green algae (1 & 2) (https://ccsearch.creativecommons.org/search?q=lyngbya&provider&li&lt&searchBy; https://ccsearch.creativecommons.org/photos/ef1e3eca-73a1-4b26-9465-4a74d0a47064) and Brown algae (3 & 4) (https://ccsearch.creativecommons.org/search?q=Dictyota%20dichotoma&provider&li&lt&searchBy; https://ccsearch.creativecommons.org/search?q=dictyopteris%20membranacea&provider&li&lt&searchBy). (All the figures have been taken from Creative commons)

(Kim et al. 2005). In the recent past also, many important natural compounds have been obtained from brown algae and all these compounds can prove to be a good lead compounds for new drug discovery (Fig. 9.1).

9.4 Red Algae

The red algae are one of the largest and oldest classes of algae, comprising over 8000 species. The majority of species are multicellular and mostly live in intertidal and in subtidal zone of the marine environment. The red color of Rhodophyceae members is because of the presence of two pigments phycoerythrin and phycothcyanin in abundance, in addition to chlorophyll a (no chlorophyll b), ß-carotene, and a number of unique xanthophylls (Bold et al. 1985). Red algae are considered to be the most important source of biologically active natural metabolites in comparison to other classes of algae.

9.4.1 Cytotoxic Compounds from Marine Red Algae

A cytotoxic and unique anti-tumor agent, halmon, which is a polyhalogenated monoterpene, was isolated from *Portieria hornemanii* and this compound is at the clinical stage of drug discovery (Fuller et al. 1992, 1994). Laurinterol from *Laurencia okamurai* induced apoptosis and could cause restricted growth of melanoma cells. Triterpenes, 2-acetoxy-15-bromo-6,17-dihydroxy3-palmitoyl-neoparguera-4(19), 9 (11)-diene, teurilene and thyrsiferyl 23-acetate isolated from *Laurencia obtusa* had cytotoxic properties (Suzuki et al. 1985, 1987). Several cytotoxic cyclic monoterpenes were also isolated from *Desmia hornemanni* (Higa 1985) and they show cytotoxic activity against carcinonomas. Furoplocamioid C, perfuroplocamioid, pirene and tetrachlorinated cyclohexane extracted from *Plocumium carttilagineum* were active against human tumor cell lines (de Ines et al. 2004). Sulfur-containing polybromoindoles from *Laurenda brongniartii* were cytotoxic to melanoma cell line (Sun et al. 2006). Few monoterpene aldehydes and sesqueterpenes from *Plocamium corallorhiz* and *Laurencia tristicha* were reported to inhibit the growth of esophageal cell line and HeLa cell line respectively. Thyresenol, a polyether squalene-derived product, was isolated from *Laurencia viridis* that shows potent cytotoxic activity and anticancer property. Dehydrothyrsifero from *Laurencia pinnatifida* induced apoptosis in breast cancer cells ( ).

9.4.2 Antimicrobial Compounds from Marine Red Algae

Sulquinovosyl diacyl glycerol from *Gigartina tenella* shows inhibitory activity against HIV-l reverse transcriptase (Ohata et al. 1998). 2,3,6-Tribromo 4,5-dihydroxybenzyl isolated from *Symphyocladia latiuscula* was shown to be active against HSV. Sulfoquinovosyl diacyl glycerol was isolated from *Caulerpa racemose* and *Ishige okamurai, which* exhibited an inhibitory effect on HSV-2 (Wang et al. 2007). Venustatriol, thyrsiferol, and thyrsiferyl 23-acetate were obtained from *Laurencia venusta* and these bioactive compounds showed antiviral activity on vesicular stomatitis virus (VSV) and herpes simplex virus type 1 (HSV-l) (Sakemi et al. 1986). Sesquiterpene hydroquinone, peyssonol A from *Peyssonnelia* species, was found to possess anti-HIV reverse transcriptase activities (Talpir et al. 1994).

Polybrominated indoles from *Laurencia brongniarti* show growth inhibitory activity against *Bacillus subtilis* and *Saccharomyces cerevisiae.* Bromobeckerelide and chlorobeckerelide from *Beckerella subcostatum* and P-hydroxybenzaldhyde, dichloro-acetamide, and 3,5-dinitriguaiacol from *Marginisporum aberrans* showed antimicrobial activity against *Bacillus subtilis* (Blunt et al. 2016). *L. elata* yielded elatol that displayed growth inhibitory activity against pathogenic bacteria *Staphylococcus* epidermis, *Klebsiella pneumonia, K. pneumonia*, and *Salmonella sp*. Laurinterol, isolaurinterol, allo-laurinterol, cupalaurenol 3, and 2,3,5,6-tetrbromoindol from *Laurencia* species displayed a wide spectrum of antibacterial activity against many pathogenic as well as antibiotic-resistant bacteria.

10-hydroxykahukuene and laurenmariallene from *Laurencia mariannensis* also showed antimicrobial activity. Lanosol enol ether from *Fucus vesiculosus* showed antibacterial and antifungal activity (Barreto and Meyer 2006). Diterpenebenzoic acids, callophycoic acids, and callophycols from *Callophycus serratus* showed adequate levels of antimicrobial, antimalarial, and anticancerous activity (Lane et al. 2007). Cyclic ethers from *Laurencia glandulifera* showed antistaphylococcal activity. Ptilodene, an eicosanoid from *Ptilotafilicina* sp., exhibited antimicrobial activity against pathogenic bacteria. Extracts of *Centroceras clavulatum, Champia parvula, Gelidiella acerosa, Gracilaria corticata, Hypnea musciformis, H. valentiae, Laurencia obtusa*, and *Polysiphonia* sp. showed promising broad-spectrum activity (Debbab et al. 2013).

Among all, red algae (examples: *Centroceras clavulatum, Gelidiella acerosa, Gracilaria corticata, Halymenia floresia, Hypnea musciformis Halymenia floresia, Gelidiella acerosa Gracilaria foliifera, Hypnea musciformis, Hypnea valentiae, Gracilaria corticata, Sarconema filiforme Centroceros clavulatum, Gelidiella acerosa, and Halymenia floresia*) were reported to show the highest antifungal activity. They displayed antifungal activity against pathogenic fungi *Candida albicans*, *Trichophyton mentagrophytes* to a greater extent. (Padmakumar and Ayyakkannu 1997) (Fig. 9.2).

9.4.3 Other Compounds with Medicinal Value from Red Algae

Snyderol, a sesquiterpene from *Laurencia obtuse*, was reported to be active against malarial parasite *Plasmodium falciparum*. Chondriamide and its derivatives were extracted from *Chondria atropurpurea* that showed anthelmintic activity. Parguerene and isoparguerene from *Jania rubens* also exhibited anthelmintic activity (Awad 2004). Ceratospongamide, a cyclic heptapeptide from symbiotic *Ceratodictyon spongiosum* with *Sigmadocia symbiotica,* displayed anti-inflammation activity. Vidalols from *Vidalia obtusaloba* were reported to be anti-inflammatory. In addition, tribromo dihydroxy benzyl cyclohexanone from *Symphyocladia latiussula* possesses antioxidant activity. Bromophenols, isolated from *Polysiphonia urceolata* and *Odonthalia corymbifera,* were potent reactive oxygen species scavengers (Li et al. 2007; Duan et al. 2007). Three meroterpenoids from *Hypnea musciformis* with variable anti-oxidative activities were isolated. Palytoxin with insecticidal activity was isolated from *Chondria armata.* Red algae, *Laurencia dendroidea* produce obtusol, which harbors larvicidal activity against the dengue fever mosquito *Aedes aegypti. Odonthalia corymbifera* yielded dibromo dihydroxybenzyl ether which can serve as an anti-diabetic agent. Shinorine and porphyra-334 isolated from *Porphyra sp.* possess immunomodulatory effects.

Natural products generated by *Laurencia dendroidea* were studied in detail to investigate sterol biosynthesis pathway and cloning and functional characterization of a cycloartenol cyclase was figured out. Additionally, biological activities of natural products coming from *Gracilaria* spp. were investigated. Synthetic analogues of galaxamide initially isolated from *Galaxaura filamentosa* were

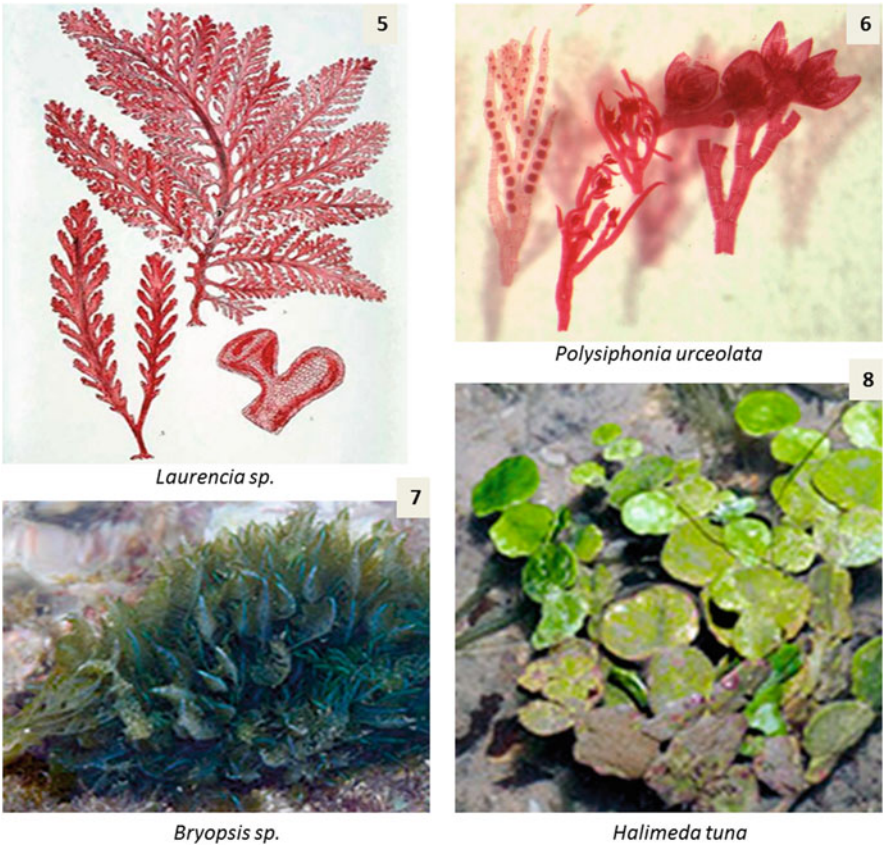

Fig. 9.2 Bioactive compound yielding red algae (5 & 6) (https://ccsearch.creativecommons.org/search?q=Polysiphonia%20urceolata&provider&li&lt&searchBy; https://ccsearch.creativecommons.org/search?q=Laurencia%20dendroidea&provider&li&lt&searchBy) and Marine Green algae (7 & 8) (https://ccsearch.creativecommons.org/search?q=Bryopsis&provider&li&lt&searchBy; https://ccsearch.creativecommons.org/search?q=Halimeda%20tuna%20&provider&li&lt&searchBy). (All the figures have been taken from Creative commons)

designed and proven as potential antitumor agents. Other synthetic studies such as polyhalogenated monoterpene, anverene, epoxyobtusallene IV, obtusallene X, and marilzabicycloallenes were also performed (Blunt et al. 2017). Many other natural products with antimicrobial, cytotoxicity against cancerous cells, and other compounds with medicinal value have also been isolated from red algae in the recent past and they are presented in Table 9.1. They have great potential for being utilized in pharmaceuticals.

9.5 Green Algae

Green algae are not only found in freshwater but also in the intertidal zone and in shallow waters of sea, where there are plenty of nutrients and sunlight. Green algae have characteristic green color due to an equal proportion of chlorophyll a and b (Bold et al. 1985). This class of algae is supposed to be more closely related to terrestrial higher plants as compared to any other class. There are many bioactive natural products reported from marine green algae that have potential usage in pharmaceuticals.

9.5.1 Cytotoxic Compounds from Marine Green Algae

A unique brominated diphenyl methane derivative, Isorawsonol, was isolated from *Arrainvilla rawsonii*, which has antumorigenic and immunosuppressive effects. Communesins, penostatins, cytochalasans, and penochalasins isolated from *Enteromorpha intestinals* showed cytotoxic activity against lymphocytic leukemia cell. Halimedatrial and other diterpenoid metabolites isolated from *Halmida lamouroux* have anticancerous and antimicrobial activities. Depsipeptide kahalalide isolated from *Bryopsis* sp. (Hamann and Scheuer 1993) was found to be active against prostate cancer and HL-60 cell lines and this bioactive compound has been selected for Phase I clinical trials (Dmitrenok et al. 2006).

9.5.2 Antimicrobial Compounds Isolated from Marine Green Algae

A unique diterpene aldehyde halitunal obtained from *Halimeda tuna* exhibited antiviral activity against murine coronavirus (Koehn et al. 1991). Sphingosine isolated from Indian green alga *Ulva fasciata* exhibited antiviral activity against semeliki forest virus (SFV) (Garg et al. 1992). Triterpene sulfate esters, Capisterones, extracted from *Panicillus capitatus* showed antifungal activity against a pathogenic fungus, *Lindra thallasiae* (Puglisi et al. 2004). The extract of *Cladophora fascicularis* yielded dibromophenoxy dibromoanisol, a diphenyl ether that shows antimicrobial activity against *Escherichia coli, Bacillus subtilis,* and *Staphylococcus aureus* (Kuniyoshi et al. 1985). The extracts from green algae, *Caulerpa cupressoides, C. peltata, C taxifolia, Codium arabicum, Enteromorpha intestinalis, Ulva fasciata, and U. lactuca* demonstrated activity against pathogenic bacteria *Staphylococcus aureus* and all the *Vibrio* species. Bioactive compounds extracted from *Caulerpa cupressoides, C. racemosa, Ulva fasciata, and U. lactuca* showed broad-spectrum activity.

9.5.3 Other Compounds with Medicinal Value from Green Algae

Cymobarbatol and its derivatives were isolated from the *Cymopolia barbat,* which possess antimutagenic activity (Wall et al. 1989). Ascosalipyrrolidinones isolated from *Ulva* species and its endophytic fungus *Ascochyta salicorniae*was, showed antiplasmodial activity. Glucopyranosy-lstigmasta diene isolated from *Ulva laetuea* was found to be anti-inflammatory (Awad in 2000). Caulerpals isolated from *Caulerpa taxifolia* was discovered as an inhibitor of tyrosine phosphatase (Mao et al. 2006). *U. pertusa* yielded 4-hydroxy-2,3-dimethyl-2-nonen-4-olide, which can inhibit cytokine production in dendritic cells. From *Chlorella sorokiniana*, few new marine natural products such as, sesquiterpene chlorellatin, ergosterol-derivative (chlorellatin), and lutein were identified. Dimethyl sulfoniopropionate and acrylate were obtained from an unidentified *Ulva species,* which is used as defensive compounds. The carotenoid astaxanthin and sulfolipids isolated from green algae are also tested for biological activities.

9.6 Potent Natural Drugs from Marine Fungi

Marine fungi are the species of fungi that sustain their life in the marine and estuarine environments. They can be either facultative or obligatory. Facultative marine fungi spent most of their life under terrestrial or freshwater conditions and they are capable of living under marine habitats. Thousands of marine fungi belonging to Basidiomycetes, Ascomycetes, and other classes have been reported from the sea and a large number of species are yet to be discovered. Marine fungi can be saprophytic on dead plants and animals or parasitic on mangroves, algae, or other marine plants. Marine fungi are a recognized source of distinct natural products. There are different techniques or substrate for isolation of marine fungi. It is recommended that chemists should collaborate with marine mycologists in order to procure marine fungi from recognized culture collections.

A number of new bioactive compounds have been reported from marine fungi. Several phenolic compounds like aspergilols were obtained from *Aspergillus versicolor*, which can be used as laxatives, antimalarials as well as antineoplastics. Disulfide-bridged dipeptide, diterpene, norditerpene, decalin derivatives, and long-chain peptaibols, trichoderin were extracted from *Trichoderma*. Trichoderin is an anti-tuberculosis aminolipopeptide. *Penicillium* has yielded a wide range of new metabolites, including meroterpenoids, chrysamides, alkaloids, polyketides, and cerebrosides that can serve as a lead compound for new drug discovery. Sponge-derived *Penicillium sp.* led to the production of some macrocyclic polyketides. Several natural compounds such as monoterpenoids, diterpenoids, sesquiterpenes cyclic dipeptides, phenolics, asteltoxins, alkaloids, pseurotin, and chromone were isolated from *Aspergillus sp.* New metabolites isopyrrolonaphthoquinone and sansalvamide A amide were also obtained from the genera *Biscogniauxia.* A number of new metabolites like diterpene glycosides, steroid, cyclopentanone, chlorogriseofulvin, hexaketide and (-)-orthosporin, polyketide-derived linear and

macrocyclic polyesters, coumarins have also been obtained from different marine fungi that are highly beneficial for pharmaceutical industries.

9.7 Bioactive Compounds from Mangroves and Associated Fungi

The term "mangrove" describes trees or shrubs growing in saline coastal habitats, together they form a "mangrove forest" or "mangrove swamp". Plants growing in such an environment usually belong to Rhizophoraceae, Combretaceae, Lythraceae, and Avicenniaceae families. Most of them are facultative halophytes that live in the intertidal zone. Mangroves are well adapted to the conditions that present in marine conditions such as change in humidity, high salt, tides, and biotic stresses like the presence of a huge number of microorganisms and herbivores, etc. Having pneumatophores or prop roots is a very critical adaptation possessed by them, which allow them to do a gaseous exchange and they are capable of excreting and storing in salt glands or hairs.

Because of the constant evolutionary force, mangroves are highly diverse both biologically as well as chemically (Strobel et al. 2004). This chemical biodiversity can be highly useful for the isolation of bioactive natural products that can serve as a lead compound for drug discovery. Moreover, they harbor diverse endophytic fungi as symbionts (Anada and Sridhar 2002) that can also be an important reservoir of diverse chemical compounds (Li et al. 2009; Pang et al. 2008). Mangrove, *Aegiceras corniculatum* has been a rich source of antiplasmodial embelin analogue. *Ceriops tagal* yielded bioactive compound, dolabranes tagalsin. From the seeds of *Xylocarpus moluccensis*, tirucallane and tetranortriterpenes were extracted. Additionally, tetranortriterpenoids and limonoids were extracted from *Xylocarpus granatum* and *X. moluccensis* respectively.

Endophytic fungi also yield diverse potential bioactive compounds that can be suitable for medical and agrochemical applications such as cyclic depsipeptide isolated from *Kandelia candel*-associated fungus showed cytotoxic activity against human breast cancer MCF-7 cells when tested in the MTT assay (Huang et al. 2007). Similarly polyketides isolated from a *Penicillium* sp., a symbiont of *Aegiceras corniculatum* exhibited cytotoxic activities (Lin et al. 2008). Dichlororesorcinol derivatives extracted from *Cosmospora vilior* and endophytic *Eurotium rubrum* exhibited potent antioxidant activity. Endophytic fungus, *Lasiodiplodia* produced polyketides, preussomerin analogues that can be utilized for medicinal uses. Further, *Mucor irregularis* was explored for the presence of secondary metabolites, and rhizovarins were isolated. Nectriacids and epicitreoisocoumarinol are two polyketides isolated from *Nectria* species that showed potent inhibition of α-glucosidase. Neosartoryadins from Neosartorya udagawae exhibited activity against the H1N1 influenza virus. Endophytic *Penicillium* also generated varieties of metabolites such as diketopiperazines spirobrocazine, brocazine, etc., that are crucial compounds for drug discovery. Brocazine displayed cytotoxic activity against tumorigenic cells and strong pathogenic strain of *S. aureus*. Other important

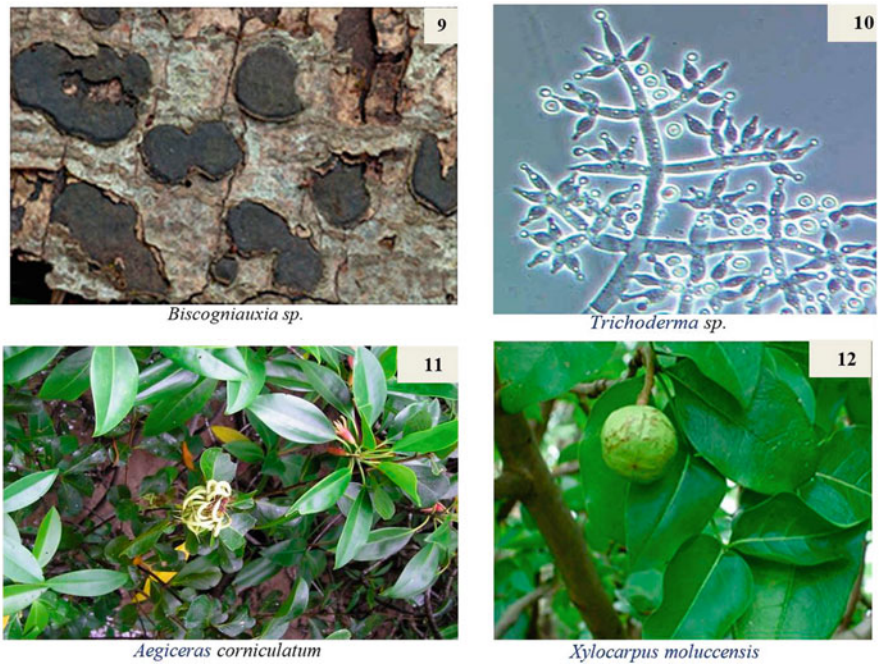

Fig. 9.3 Bioactive compound yielding marine fungi (9 & 10) (https://ccsearch.creativecommons.org/search?q=Biscogniauxia&provider&li&lt&searchBy; https://ccsearch.creativecommons.org/search?q=TrichodermakoningiiOudem&provider&li&lt&searchBy) and Mangroves (11 & 12) (https://ccsearch.creativecommons.org/search?q=Mangroves%20Marine%20plants&provider&li&lt&searchBy; https://ccsearch.creativecommons.org/search?q=Xylocarpus%20moluccensis&provider&li&lt&searchBy). (All the figures have been taken from Creative commons)

metabolites like pestalotiopsis, macrolides, phomopsis, cytochalasins, pseudolagarobasidiu, stemphylium, talaromyces, rhytidhysteron, and chamigrane sesquiterpenes were also extracted from other mangrove-associated fungi (Fig. 9.3).

9.8 Conclusion

Finding natural "eco-friendly" plant products to prevent or treat human diseases will be a better alternative management process in pharmacy. The wealthy and exceptional chemical diversity of natural products has long been a significant reservoir of remedies. Chemical diversity has been associated with biological diversity and the marine environment possesses diverse biota which can provide varieties of chemical compounds that have the potential to be used as medicines. This probability has been an inspiration for the isolation of interesting different chemical compounds, which are unique and extraordinary in nature as compared to the ones that come from terrestrial sources. Marine organisms are subjected to extreme environmental conditions including high salinity, insufficient aeration, hydrostatic pressure,

infection, and predation by other organisms. The adaptations to the harsh conditions can be either physical by conferring motility or chemical. Plants being sessile hugely rely on the chemical defense mechanisms and have evolved phytotoxins and deterrents to enhance their survival to cope up with freely moving predators. Therefore, the potency of the metabolites coming from marine flora is more. Thus, a vast multitude of potential drugs has been isolated from the marine flora with better efficiency and specificity for the treatment of deadly and incurable human diseases. The use of natural products as antibiotics is highly needed as the microbial resistance to these drugs is increasing in humans and animals. Using natural and renewable marine floral compounds is still in its infancy, but considering its importance in the present scenario, further investigation in this direction will surely deliver an effective and sustainable means of disease treatment.

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Edible Seaweeds as Potential Source of Nutraceuticals

10

Sangeeta Saikia, Nikhil Kumar Mahnot, Ravi Kumar Sahu, and Jatin Kalita

Abstract

Edible seaweeds—superfoods of our waterways—are algae, botanically classified majorly into green (Chlorophyta), brown (Phaeophyta) and red (Rhodophyta) groups. They are a rich source of protein, dietary fibre, vitamins, minerals as well as polyphenols, peptides, sterols, PUFA, etc. Seaweeds are a major part of the diet of Southeast Asian countries as well as in parts of Europe, America and Australia. Apart from being a major nutrition source in the human diet, traditional Chinese medicine approves of edible seaweeds having medicinal properties. In recent times, edible algae are being marketed as a rich source of nutraceuticals for their bioactive properties. It has been established that bioactive compounds have a major therapeutic role in the management of a number of human metabolic diseases such as diabetes, cancer, hypertension, etc. Researchers are trying to discover newer bioactive molecules from different sources. Marine life constitutes around 80% of the world biota and edible algae is a major marine flora that can be explored to derive many bioactive compounds and other secondary metabolites for therapeutic applications through optimized harvesting, extraction and recovery for active molecules. The algal bioactive molecules have been widely reviewed to possess antiparasitic, antiinflammatory, anticancer, antioxidant and antidiabetic properties. Thus, opens a vast scope for utilization of bioactive compounds from edible seaweeds for development of nutraceuticals.

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Keywords

Seaweeds · Diet · Nutrition · Bioactive · Therapeutic · Nutraceutical

10.1 Introduction

Edible seaweeds are macroalgae consumed since ancient times by the coastal communities of parts of Asia, Europe, America and Australia. Seaweeds are part of the major diet in many parts of the Asian continent such as Korean peninsula, Japan, China, Vietnam, Philippines, etc. South Korea is one of the largest consumers of edible seaweeds (Sanjeewa et al. 2018).

Botanically, the seaweeds are classified into different taxonomic groups viz. red algae (Rhodophyta), brown algae (Phaeophyta) and green algae (Chlorophyta) based on their pigmentation pattern (Mohamed et al. 2012). Figure 10.1 represents a brief schematic classification of seaweeds with some examples.

In traditional Chinese medicinal system, seaweeds are used to treat a number of ailments. They are rich in minerals like Na, Ca, Mg, K, Zn, Cu, Se, Mb, F, Mn, B, Ni & Co as well as vitamins such as B12, A and K. In addition, they are also abundant in bioactive compounds with therapeutic properties. Researchers are trying to discover newer bioactive from marine sources and seaweeds are one of the important marine sources enriched with numerous bioactive compounds. Thus, the edible seaweeds can be considered as superfoods with lots of nutritional benefits as well as health-promoting properties. In this chapter, an overview of edible seaweeds as potential nutraceuticals and their therapeutic properties is presented.

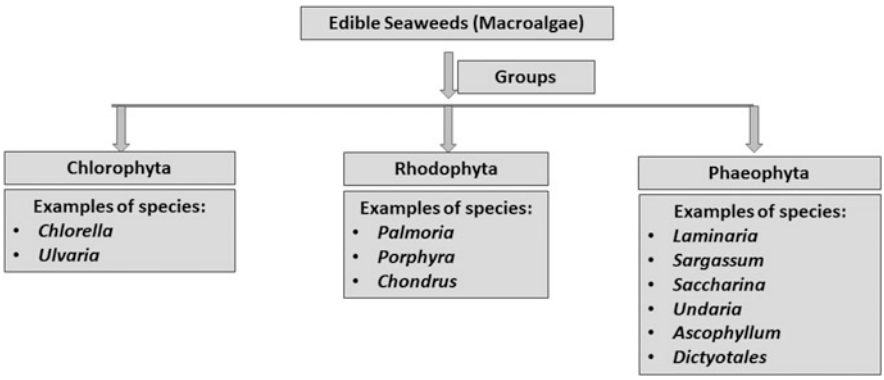

Fig. 10.1 A brief schematic classification of seaweeds with some examples

10.2 Use of Edible Seaweeds in Diets and Traditional Medicines

Seaweeds (macroalgae) use as food and medicine dates to 3000 BC (Doty 1979). Traditionally, Asian countries mainly Japan, South Korea, China, Vietnam and Philippines are the major consumers of seaweeds. Its use as a medicine has been reported in Chinese *Materia Medica* dated 600 BC (Porterfield 1922; Wood 1974). Use of seaweeds in diet can be traced back to fourth century in Japan and sixth century in China (Kılınc et al. 2013). Seaweeds are low in calorie and being a rich source of soluble dietary fibres, proteins, minerals, vitamins, secondary metabolites and polyunsaturated fatty acids (PUFAs) have found their use as a therapeutic food component. Research has reported the presence of seaweeds in the traditional Asian diets has contributed to low occurrences of cancer mainly breast cancer among Japanese and Korean population which is often attributed to the regular consumption of seaweed enriched diet (Lawson et al. 2001). Chinese traditional medicinal system uses *Laminaria* sp. for its anticancer properties (Funahashi et al. 2001). Similarly, Ayurveda also recommended the use of different algae for cancer treatment (Hoppe et al. 1979). The traditional Korean medicinal system suggests a diet enriched with seaweeds for new mothers due to its healing and nutritional properties (Moon and Kim 1999).

Seaweeds like *Saragussum* has been used in Chinese medicine since the sixteenth century for the treatment of goitre. Agar, the most common phycocolloidal extract of red algae, has been reported to be used as a laxative right from the seventeenth century. While during the eighteenth century *Palmaria palmate* extracts have been used to cure fever (Chapman and Chapman 1980).

Cupsosiphen fuvescens is an edible seaweed abundant in the southern coast of South Korea. It is used traditionally as an ingredient for its bioactive properties to treat stomach disorders and hangovers. Apart from that, it is used for its anticoagulant, anticancer and anti-inflammatory properties (Sharma and Rhyu 2014). *Caulesopa lentillifora* is consumed in South Korea, Japan and Philippines and is traditionally used for its antihypertensive, antirheumatic, antibacterial, antidiabetic, anticancer and antifungal properties (Sanjeewa et al. 2018). *Enteromorpha prolifera* is consumed in South Korea either in soup or salad forms. They are very rich in essential fatty acids and exhibits antioxidant, lipid-lowering, anticancer, antiinflammatory and immunomodulatory properties (Shi et al. 2017). Similarly, *Ulva pertusa* is widely distributed in Japan, South Korea and China coastal waters. Traditional healers use this seaweed for urinary tract infection and hyperlipidemia. Additionally, it has low calorific value with adequate dietary fibres, vitamins and minerals. *Porphyra* species commonly known as 'laver' is a popular seaweed consumed widely in Asian countries. Its simple sugar content is low and rich in vitamins and minerals (Fleurence and Levine 2016). Another seaweed, *Gelidium amanrii*, is a Rhodophyta consumed widely in parts of Taiwan, China, Japan and South Korea. It is believed to have plasma lipid-lowering effect (Yang et al. 2017).

Brown seaweeds (Phaeophyta) such as *Sargassum fusiforme* and*Saccharina japonica* are major ingredients in traditional Chinese medicines since ancient times (Peng et al. 2013). *Ecklonia cava* is another brown seaweed used traditionally for its

antiinflammatory properties (Choi et al. 2017) and is abundant in the coastal waters of Japan, China and South Korea (Sanjeewa et al. 2019). In addition to the abovementioned seaweeds, there are many other seaweeds that are considered as a source of food and medicine in different parts of the world.

10.3 Commercial Use of Edible Seaweeds

Seaweeds are used in many pelagic countries as a source of food, in industrial application and as fertilizers. Seaweed cultivation has taken the shape of major commerce in countries like Japan, China and South Korea, with China being the largest producer today followed by South Korea and Japan. Figure 10.2 presents a schematic overview of the various products obtained commercially from seaweeds. Commercial harvesting of seaweeds is done in around 35 countries across the globe in almost all types of environmental conditions (McHugh 2003).

Many seaweeds contain phycocolloids or polysaccharide hydrocolloids which are extracted for various purposes. The three major phycocolloids are alginate, agar and carrageenan. Alginate, an anionic polysaccharide, commonly used as a gelling agent in food and pharmaceutical industry is obtained from brown seaweeds like *Laminaria* and *Undaria* found in the wild. Agar, another gelling agent obtained from red seaweeds mostly from two genera *Gelidium* and *Gracillaria*, is commonly used as a culture medium for microorganisms, a laxative, an alternate for gelatin, thickener, clarifying agent, etc., in food, brewing, cosmetic, pharmaceutical and biotechnology industries (Lee et al. 2017). Carrageenan, used as a stabilizing and gelling agent, is

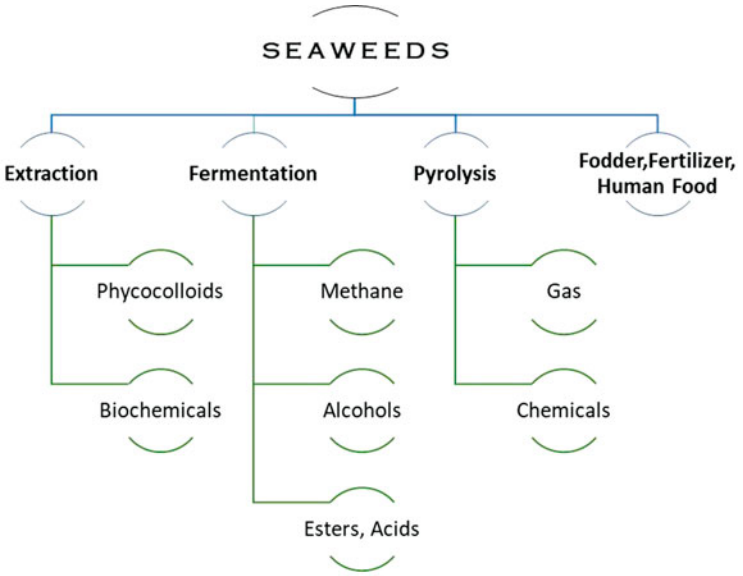

Fig. 10.2 A schematic overview of the various products obtained commercially from seaweeds

also extracted from various genera of red seaweeds. Various types of carrageenan extracted are kappa, lambda & iota carrageenan, each having its own specific applications commercially, be it food, cosmetic or domestic (McHugh 2003).

Seaweeds being a rich source of minerals, antioxidants, vitamins, fibre and iodine have found their platter in the table. Names like Arame, Nori, Wakame, Kelp, Kombu, etc. are used in various forms in the maritime kitchen and are now delicacies, the rest of the world craves for. Edible seaweeds have made its mark in the dietary and nutritional supplement commercial segment and are rapidly expanding with new products with various trade names. Due to their potential health benefits and medicinal values, these are also used vastly in Chinese medicine and have proven good result (Lawson et al. 2001). Fermentation of seaweeds for alcohol production was introduced about 12,000 years ago and archaeological evidences prove that it was mainly used for medicinal purposes and long-term food storage. Modern-day liquor distilleries and breweries have re-adopted those ancient processes to produce potable alcohol used in the spirits industry from seaweeds due to low production cost and abundance of raw materials in the maritime countries (Kraan 2016).

Edible seaweeds contain almost all kinds of nutrients useful for both animals and plants. And it is for this reason that these seaweeds serve as a superior organic fertilizer for plants and agriculture crops. Liquid seaweed fertilizers are used as soil conditioners and effective in nullifying pathogens and pest infestation. Seaweed extracts help increase the nitrogen availability to plants and combat stress conditions to boost plant health (Sumedha et al. 2016). These fertilizers are now commercially available in various e-commerce platforms.

In a nutshell, edible seaweeds have grabbed a major commercial market and are rapidly reaching every household. However, there is a vast scope and immense potential for edible seaweeds in global trade.

10.4 Bioactive Compounds Present in Edible Seaweeds

Seaweeds contain a number of bioactive compounds. Around 4000 bioactive compounds have been reported in seaweeds (450 from Chlorophyta, 1500 from Phaeophyta and 2000 from Rhodophyta). They are generally categorized into polyphenols, polysaccharides, dietary fibre, vitamins, peptides, fatty acid sterols and pigments. Bioactive compounds are secondary metabolites with many functional properties such as antioxidant, antiinflammatory, anticancer, antihypertensive, antidiabetic properties, etc. (MarinLit 2017).

10.4.1 Polyphenols

Polyphenols are secondary metabolites which are generally classified into phenolic acids, simple phenols, coumarins, xanthones, naphthoquinones, flavonoids, stilbenes, anthraquinones and lignins. The major polyphenols present in seaweeds

are phytol (acyclic diterpene), meroterpenoids (sargaquinoic acid, sargahydroquinonoic acid, sargachomenol), plastoquinones (quinone derivative), phenolic acids such as gallic, protocatechuic, chlorogenic, gentistic, vanillic, caffeic, phlorotannins and hydroxybenzoic acid (Fernando et al. 2016). Kazlawska et al. (2010) reported the presence of flavonoids like rutin and catechol in red seaweed, *Porphyra dentata*. The polyphenolic compounds in seaweeds are responsible for antiinflammatory, antidiabetic, antiallergic, anticancer, antioxidant and neuroprotective properties (Koutsavit et al. 2018).

10.4.2 Polysaccharides

Seaweed polysaccharides exhibit may bioactive properties. They are polymer molecules of monosaccharide units linked together by glycosidic bonds. Mainly, fucans and fucoidans, laminaria and alginates are derived from the Phaeophyta group. The Rhodophyta are abundant in carrageenan, porphyrans and agaroids while ulvan is found in the Chlorophyta species. Fucoidans, ulvans and porphyrans belong to the sulphated polysaccharides and have antioxidant, antidiabetics, antiinflammatory and chemoprotective properties (Wu et al. 2016). Alginates are non-sulphated anionic polysaccharides and are used as thickening or gelling agents. It has antiobesity properties. Carrageenans are sulphated galactans and have antioxidant activity and anticoagulant properties (Tang et al. 2017).

10.4.3 Dietary Fibre

Seaweeds are rich in dietary fibre and are a suitable source as prebiotics for human gut health. The total dietary fibre content in seaweeds are in the range of 25–75%, dry matter, which is relatively higher than the dietary fibre content of the most fruits and vegetables (Jime'nez-Escrig and Sa'nchez-Muniz 2000).

10.4.4 Vitamins

Seaweeds are a rich source of vitamins such as vitamin B complex, vitamin C, vitamin E and vitamin K essential for human health. The brown and green seaweeds consist of average 500–3000 mg/kg of vitamin C in dry matter. Similarly, vitamin E content is high in the Phaeophyta group of seaweeds (Watanabe et al. 1999). Vitamin B12 is another vitamin abundant in seaweeds.

10.4.5 Peptides

The seaweed protein content varies according to species. However, highest protein has been reported in Rhodophyta (47%, dry matter) followed by Chlorophyta (26%,

dry matter) and Phaeophyta (15%, dry matter) (Fleurence et al. 2018). Seaweed-derived peptides often exhibit bioactive properties. Generally, bioactive peptides comprise of 3–20 amino acid residues depending on the composition and sequence of amino acids. Lectins and phycobiliproteins are two classes of biopeptides derived from seaweeds. The bioactive peptides are reported to have antihypertensive, anticancer, antiviral, hepatoprotective, antibacterial and antiinflammatory activities (Bleakley and Hayes 2017).

10.4.6 Fatty Acids and Sterols

The average lipid content in seaweeds is around 3% and is rich in monounsaturated and polyunsaturated fatty acids, glycolipids and phospholipids. It contains both ω-3 (eicosapentaenoic acid, docosahexaenoic acid) and ω-6 (arachidonic acid, linoleic acid) fatty acids. Seaweed-derived polyunsaturated fatty acids exhibit antioxidant, antiinflammatory and antitumor activities (Wielgosz-Collin et al. 2016).

Sterols are available both in animal and plant sources. Sterols in plants are generally termed as phytosterols. Phytosterols derived from seaweeds are hydroxylated steroid alcohols with a hydroxyl group (Parish et al. 2008). More than 200 different types of phytosterols have been identified in seaweeds. Fucosterol and its derivatives are the major phytosterols found in brown seaweeds. They exhibit antifungal, antibacterial, antiinflammatory, antitumor, antioxidant and wound healing properties (Hamid et al. 2015).

10.4.7 Pigments

Chlorophyll and carotenoids are the natural pigments present in seaweeds. The Chlorophyta group has chlorophyll as the major pigment but also contains a small amount of β-carotene, violaxanthin and neoxanthin. Similarly, the seaweeds belonging to the Rhodophyta group are rich in lutein and zeaxanthin. The major pigments present in the Phaeophyta group of seaweeds are fucoxanthin, violaxanthin and zeaxanthin. These natural pigments exhibit antioxidant, antiobesity and chemopreventive properties (Lin et al. 2015).

10.5 Therapeutic Role of the Edible Seaweeds

Marine seaweeds are reported to produce approx. 4000 different metabolites (MarinLit 2017) which have been linked to the treatment of various allergies, inflammation, oxidative stress and other lifestyle diseases like cancer, cardiovascular diseases, diabetes, thyroid problems and obesity, lipidemia as well as neurodegenerative disorders. Thus, seaweeds are a source of various nutraceuticals and can be easily added as a functional food ingredient in our daily diet.

The role of seaweeds as therapeutics in various diseases is discussed concisely in the current section.

10.5.1 Cancer Treatment and Prevention

Seaweed components like sulphated polysaccharide fucoidan, fucoxanthin, phlorotannins and peptides have shown potential for chemopreventive therapy (Lowenthal and Fitton 2015; Cho et al. 2012; Peng et al. 2011). Crude extracts and powdered seaweeds have been proved to have antiproliferative activities, antitumor efficacy against various cancer cell lines in different mouse models (Kiruba et al. 2018; Ermakova et al. 2016). Seaweed extracts tend to act against cancer by inhibiting reactive oxygen species (ROS) (Namvar et al. 2012), selective cytotoxicity towards cancer cells (Moo-Puc et al. 2011), inhibition of cancer cell proliferation, (Ferramosca et al. 2016), cell cycle arrest (Yu et al. 2011) as well as DNA media cell apoptosis (Wang et al. 2015). Thus, seaweeds have proved to play a role in preventing cancer occurrence, tumour progression, and have been involved in post-treatment recovery after radio- or chemotherapy treatments.

10.5.2 Maintenance of Cardiovascular Health

Cardiovascular diseases (CVDs) include disorders including hypertension, coronary heart disease, cerebrovascular disease (stroke), heart failure and peripheral vascular disease of which hypertension is one of the major cause of CVDs. Marine algae are rich in polyunsaturated fatty acids (PUFAs), bioactive polysaccharides, essential minerals and vitamins, enzymes, polyphenols and bioactive peptides (Wijesekara and Kim 2010; Pereira et al. 2011) which maintain the cardiovascular health. Alginates from *Undaria pinnatifid* and *Ulva unoi* have been reported to reduce both systolic and diastolic pressures in hypertensive patients (Kumar et al. 2015). Further, seaweed protein and protein hydrolysates have antihypertensive properties (Wijesekara and Kim 2010; Admassu et al. 2018). Phlorotannins from seaweeds are effective antihypertensive and can inhibit angiotensin-I converting enzyme (ACE) activity. Seaweed polyphenols: phlorofucofuroeckol A, dieckol and eckol can inhibit ACE by either following a noncompetitive profile or by sequestering the enzyme metal factor, Zn^{2+} ion (Wijesinghe et al. 2011). Farnesylacetones isolated from seaweed *Sarragassum siliquastrum* (Park et al. 2017) have the ability to block calcium channels to reduce hypertension by controlling both systolic and diastolic blood pressures. Human studies with seaweeds like *Undaria pinnatifida* and *Fucus vesiculosus* have been reviewed to reduce pressure in hypertensive patients and without effecting the blood pressure of normal individuals (Murray et al. 2018). Thus, the addition of seaweeds into daily diets seems to be an advantage in managing cardiovascular diseases; also large clinical studies on humans are to be carried out to further establish the benefits.

10.5.3 Obesity and Diabetes Management

Obesity and diabetes are the major lifestyle inflicted metabolic diseases in the present time. Together obesity and diabetes can lead to other conditions like atherosclerosis, high blood pressure and CVDs. Epidemiological evidence suggests that the consumption of seaweeds leads to a lower risk of metabolic disorders in women of Taiwan and men in South Korea (Yeh et al. 2011; Lee et al. 2010). Lee et al. (2010) observed that increased seaweed consumption can delay in diabetes progression. It has been reported that in Egyptian traditional medicine use of algae *Caulerpa lentillifera, Enteromorpha intestinalis, Spirulina versicolor* and *Ulva lactuca* have been linked to reduction in diabetes mellitus and their efficacy as antihyperglycemic has been proved on mice models (AbouZid et al. 2014). Many seaweed extracts have α-amylase or α-glucosidase inhibitory properties (Yang et al. 2019; Lee et al. 2018). Seaweed phlorotannins can inhibit protein-tyrosine phosphatase 1B (PTP1B) which negatively regulates insulin signalling (Moon et al. 2011). Inhibition of these enzymes usually leads to regulation of blood sugar level.

Obesity management has already been linked to dietary fibre consumption, minerals, polyphenols and omega-3 fatty acids which help to regulate blood lipid levels specifically high-density lipoproteins (HDL), and seaweeds are a good source of the said components. Preclinical studies of *Ecklonia cava* polyphenolic extracts have reported to show downregulation of the obesity-associated inflammatory responses. Alginates from seaweeds have pancreatic lipase inhibitory activity. Fermentation of alginates in the intestine leads to the formation of short-chain fatty acids mostly propionates which can inhibit the incorporation of fatty acids and sterols in hepatocytes by inhibiting acetyl COA synthetase enzyme (An et al. 2013). Although most studies are preclinical the incorporation of seaweeds in diets is potentially beneficial to maintain a healthy metabolism.

10.5.4 Healthy Gut Maintenance

The gut health is basically maintained by the microorganisms present inside it. Their metabolic end products are responsible for the generation of short-chain fatty acids (SCFAs) which have various immunomodulatory activities like T-cell expression modification to reduce susceptibility (TNF-α and IL-1-α), reduction in proinflammatory cytokine production. Sweeney et al. (2012) suggested that seaweeds *L. digitata* and *L. hyperborea* downregulated the expression of cytokine markers IL-1α, IL-10, TNFα and IL-17A in the colon and promoted gut health by the production of SCFAs. Seaweed-derived dietary fibre acts as prebiotics and may possibly promote gut health (Ramnani et al. 2012). Supplementation with marine fucoidans has exhibited an increase in *Lactobacilli* spp. population under in vitro conditions (Sweeney et al. 2012). Research suggests that different seaweeds have different mechanisms to maintain gut health. There is a lack of human studies pertaining to seaweeds and gut health, still there is a great potential to understand and validate the prebiotic properties of seaweeds in human clinical experiments.

10.5.5 Inflammation and Allergy

Inflammation occurs frequently in the human body and is linked to the pathogenesis of a number of diseases like cancer, atherosclerosis, asthma, neurodegenerative diseases, diabetes mellitus, obesity, arthritis, cardiovascular diseases, etc. (Filippin et al. 2008). Thus, the demand for natural antiinflammatory agents is on the rise (Kaboli et al. 2001) due to various side effects of the generally used synthetic anti-inflammatory compounds. Metabolized products of seaweed's polyunsaturated fatty acids mainly eicosapentaenoic and docosahexaenoic acid have antiinflammatory properties (Serhan 2005). Kim et al. (2013) reported that *Myagropsis myagroides* ethanolic extract could be used as therapeutics against neuroinflammatory diseases. Ryan et al. (2011) reported that mineral extract from *Lithothamnion corallioides* as supplement limited the detrimental effects of excessive inflammation in the central nervous system. Phlorotannins extracted from brown seaweed spp. have anti-inflammatory activity (Kim et al. 2018). Chen et al. (2015) reported that the polyphenolic extracts of *Laminaria japonica, Porphyra* sp., *Spirulina platensis, Chlorella pyrenoidosa* and *Scytosiphon* sp. have antiallergic activities under in vitro experiments. Similarly, sulphated polysaccharides from *Porphyra haitanensis* and *Gracilaria lemaneiformis* exhibit antiallergic activities (Liu et al. 2016).

10.5.6 Bone Health

Seaweeds like *Ascophyllum nodosum*, *Laminaria digitata* or *Ulva* spp. has been shown to contain higher calcium content than that of cow's milk. Additionally, the presence of other minerals like magnesium, manganese, copper, zinc and selenium promote osteoblast proliferation and mineralization (O'Gorman et al. 2011). *Lithothamnion calcareum* extract was shown to be responsible for reduced bone mineral loss, improved bone strength and increase in bone mineral density (Aslam et al. 2013). Fucoidans, fucoxanthin and norzoanthamine can reduce bone collagen degradation (Kinugawa et al. 2009). Administration of oyster calcium along with different seaweed extracts has showed increased lumbar spine bone density in elderly women, better calcium absorption, increased bone mineral density and reduced urinary calcium loss (Michalek et al. 2011; Myers et al. 2010; Fujita et al. 2000). Thus, seaweeds have great potential for maintaining bone health and well-defined extensive trials on humans are required for seaweeds to be used as therapeutic treatments.

10.5.7 Antioxidative, Antiparasitic, Antiviral and Antibacterial Activities Exhibited by Seaweeds

Brown seaweeds are commonly reported to be exclusive producers of potent antioxidant molecules—the phlorotannins along with other important compounds such as

fatty acids, terpenoids, carotenoids, sterols and polysaccharides (Peng et al. 2011; Hamed et al. 2015; Shoubaky and Salem 2014). Pinteus et al. (2017) reported that fractions of *Sargassum muticum* showed protection against oxidative stress in human cell line models. Sulphated polysaccharides from *Cyclocarya paliurus* demonstrated reduction of oxidative stress in hydrogen peroxide-induced stress (Wang et al. 2012). Carrageenan extracted from seaweeds has also proved to have antioxidative properties against ultraviolet light inflicted stress (Thevanayagam et al. 2014). Miyake et al. (2014) have suggested the use of algae in neuropsychiatric disorders such as anxiety and insomnia. Ogara et al. (2015) reported, water extracts of seaweeds like *Cystoseira hakodatensis*, *Sargassum horneri*, *Sargassum fusiforme*, *Saccharina sculpera*, *Alaria crassifolia,* etc. can inhibit amyloid β aggregation which is responsible for the pathogenesis of Alzheimer's disease. Protozoal parasites *Plasmodium falciparum* and *Cryptosporidium parvuma* were shown to be inhibited by *U. pinnatifida* fucoidans (Chen et al. 2009). Sulphated polysaccharides of some seaweeds have antiviral activities against herpes viruses (Saha et al. 2012). Also, sulphated polysaccharides from *Adenocystis utricularis, Sphaerococcus coronopifolius* and *Boergeseniella thuyoides* have an inhibitory effect on initial replication of human immunodeficiency virus 1 (Bandyopadhyay et al. 2011). *Gyrodinium impudium*a red algae have been reported to inhibit influenza A virus (Kim et al. 2012). Overall seaweeds have a wide range of application for maintaining human health.

10.6 Prospects as Source of Nutraceuticals for Commercial Production

Use of seaweeds as an alternate source of food, feed, bioenergy, biofertilizer and bioactive compounds for commercial use has increased in the recent time. Since ancient times, seaweeds are used as food with a number of health-promoting properties. Recent researches on the bioactive constituents of the edible seaweeds have validated the use of seaweeds for disease management by traditional healers. The food and pharmaceutical industries are now trying to tap this vast marine resource for the production of different commercially viable products. Overall, the global nutraceuticals demand is increasing and is expected to reach around 385 billion by 2020 (Mordor Intelligence 2015). Marine-derived nutraceuticals are the most sought-after product in the present time. Seaweed-derived nutraceuticals are very diverse in nature. Nutraceuticals derived from *Spirullina* and *Chlorella* spp. dominate the commercial market (Nicoletti 2016) and are available in the form of tablets, powder or in adjunct with other seaweed extracts. The term nutraceutical is an amalgamation of nutrition and pharmaceutical and was coined by Dr. Stephen De Felice in 1989. It refers to functional food or metabolites derived from edible sources that have heath-promoting and disease-preventing properties.

Numerous seaweed-based health supplements are available in the market worldwide. Conventional food supplements are considered as concentrated nutrients for primary metabolism, for example, carbohydrate, protein, and vitamins. Whereas,

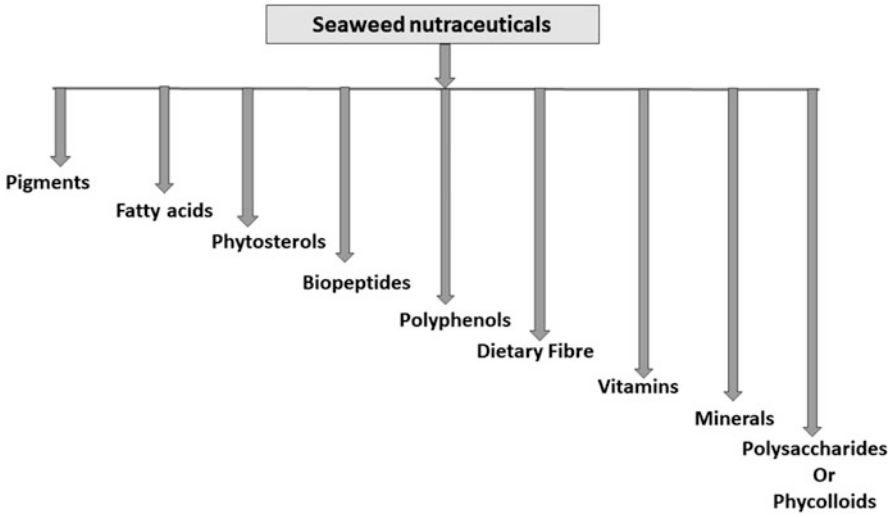

Fig. 10.3 A schematic representation of the prospective nutraceuticals present in seaweeds

seaweed-based supplements are rich in polyphenols, pigments, dietary fibre, bioactive polysaccharides, biopeptides, PUFA and phytosterols (Nicoletti 2016). A schematic representation of the prospective nutraceuticals present in seaweeds is depicted in Fig. 10.3. Additionally, an overview of the applications of the bioactive molecules derived from seaweeds is presented in Table 10.1.

Seaweed polysaccharide such as carrageenan has industrial applications such as in food, pharmaceutical and biomedical industries. Apart from being used as gelling or thickening agents in food, it is used as tablet excipient or encapsulating agents for controlled release drug formulations (Guan et al. 2017). Carageenan extracted from *Stenogramme interrupta* has both anticoagulant and antihyperlipidemic properties (Wijesekara et al. 2011). Similarly, agar is a phycolloid derived from seaweeds and is used as stabilizing and gelling agents in food industries since many years. Additionally, agar has glucose-lowering properties (Hamed et al. 2015). Alginate, another polysaccharide, derived from brown seaweeds such as *Laminaria*, *Ecklonia, Ascophyllum, Durvillacea, Lessonia, Macrocystis, Sargassum* and *Turbinaria* spp. and is extracted either as sodium or calcium salt form (Kraan 2012). It is also extensively used as stabilizing or gelling agents and is bioactive, biodegradable and biocompatible in nature (Zia et al. 2015). Presently, it is also used for controlled release of drugs or as an encapsulating agent. Its major bioactive role is bile acid binding property, antiinflammatory, antitumor and immunomodulatory properties (Lee and Mooney 2012).

The pigments derived from seaweeds have been commercialized as natural dye source as well as antioxidant, antiinflammatory and neuroprotective agents. Phytosterols from seaweeds are used as antiinflammatory, anticancer and antioxidative agents. They are also used as ingredients in supplements for the

Table 10.1 Applications of the bioactive molecules derived from edible seaweeds

Bioactive compounds	Uses	Functional role
Polysaccharides	Gelling and thickening agents, biomedical use, drug delivery agent	Anticoagulant, antiviral, antiinflammatory activities
Fatty acids (PUFA)	Food supplements	Antioxidant, antiinflammatory and antitumor activities, reduced risk of cardiovascular diseases
Phytosterols	Food and pharmaceutical industries	Antifungal, antibacterial, antiinflammatory, antitumor, antioxidant and wound healing properties
Polyphenols	Nutraceuticals	Antioxidant, antihypertensive, anticancer activities, reduced risk of cardiovascular diseases
Pigments	Natural colorant, source of carotenoids	Antioxidant, anticancer, antiinflammatory activities
Vitamins & minerals	Food supplements	Essential for many metabolic processes
Proteins & biopeptides	Gel formation, film -forming and foaming agents, source of protein concentrate and hydrolysates	Antioxidant, anticoagulant, antimicrobial, immunomodulatory, antithrombic, antihypertensive activities
Dietary fibre	Prebiotics for gut microorganisms, bulking and encapsulating agent, low calorific content food items	Glucose and lipid lowering properties, promotes growth of gut-friendly microorganism and production of SCFAs in the colon

management of many nervous system disorders like amyotrophic lateral sclerosis (ALS), Alzheimer's disease, etc. (Luo et al. 2015). The seaweed extracts containing polyphenols such as quinones, flavonoids, phenolic acids and phlorotannins are used as functional food ingredients. It has antioxidant, antiinflammatory, antidiabetic, antitumor and antihypertensive effects (Koutsavit et al. 2018). The bipeptides derived from seaweeds are used commercially in functional food and pharmaceutical industries for their antioxidant, antihypertensive and anticoagulant properties (Bleakley and Hayes 2017). Therefore, seaweed-derived bioactive compounds have immense prospects to be used extensively in the novel nutraceutical formulations.

10.7 Conclusions

Seaweeds or microalgae are a rich source of nutrients and bioactive compounds. Since ancient times, it has been used for food and medicinal purposes by the coastal population. Recent researches on the bioactive constituents of the edible seaweeds have validated the use of seaweeds for disease management by traditional healers. Many technologies have been developed to harness this vast renewable stock for commercial production of food, feed, pharmaceutical, energy, biofertilizer and other

industrially relevant items. Around 4000 bioactive compounds have been reported in seaweeds and researchers are continuously trying to discover newer compounds for better applications. Major bioactives present are polyphenols, dietary fibre, polysaccharides or hydrocolloids, biopeptides, pigments, fatty acids, phytosterols, vitamins and minerals. These bioactives have a number of therapeutic roles in the management of diseases such as hypertension, cancer, cardiovascular disease, osteoporosis, diabetes, etc. Recent increase in interest and discoveries in bioactives from seaweeds has therefore opened a vast scope for potential commercial production of different compounds to be utilized by nutraceutical and pharmaceutical industries.

Conflict of Interest There is no conflict of interest associated with this manuscript.

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Seaweed and Sea Anemones Proteins as a Source of New Pharmaceutical Active Principles

11

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Abstract

Among marine sources, seaweeds and sea anemones are highly attractive for their diversity and complex composition, the major components being polysaccharides, proteins, pigments, phenolic compounds, vitamins, and minerals. Seaweeds and sea anemones could be a source of different proteins

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and peptides with unique structures with interesting biological properties, such as antitumoral, antimicrobial, anti-inflammatory, antidiabetic, and antioxidant, among others. This chapter presents an overview of the marine algal and anemone bioactive proteins and peptides, their extraction and the development of novel carrier systems with potential interest for pharmaceutical applications.

Keywords

Seaweeds · Anemones · Proteins · Peptides · Bioactives · Encapsulation

11.1 Introduction

The biodiversity of the marine environment converts it into an attractive rich source of chemical structures with interesting biological properties and potential for the development of alternative therapeutic products. Abundant research studies on drug discovery from natural sources have been exploring the marine ecosystems for novel bioactive compounds. Particular attention has deserved sedentary organisms, producing a variety of compounds with protecting and defending activities.

Seaweeds show a complex composition, containing polysaccharides, proteins, lipids, minerals, vitamins, etc. Despite their commercial utilization is mainly based on the extraction of the saccharidic fraction for gelling purposes, the protein fraction also represents a sustainable source for the production of peptide-based drugs and functional foods.

Sea anemones have an arsenal of molecules that help them to capture prey or defend from predators. Their venoms are complex mixtures of mostly peptides and small proteins that have been explored for the development of peptide therapeutics (Dutertre and Lewis 2010), based on their actions as ionic channel blockers, neurotoxins, protease inhibitors, and pore-forming toxins (Álvarez et al. 2009; Thangaraj and Bragadeeswaran 2012; Macrander et al. 2016; Logashina et al. 2017; Loret et al. 1994).

This chapter presents an overview of the potential of proteins from anemones and seaweeds as a natural source of active compounds with potent pharmacological properties, the extraction technologies, and their potential for developing novel products.

11.2 Properties

11.2.1 Seaweed Protein Properties

Seaweeds are promising natural sources based on their wide distribution, rapid growth rate, high content of proteins, up to 30–40% of the dry weight, and diverse profile of bioactive compounds, such as phycobiliproteins, phycolectins, and mycosporine-like amino acids (Cian et al. 2015; Admassu et al. 2018).

Bioactive peptides, usually 2–20 amino acids, show activity when released by protein degradation by endogenous and exogenous enzymatic action, processing, or gastrointestinal digestion. The resulting products show a variety of therapeutic actions, such as regulation of mucosal barrier function, prevention of hypertension, enhancing mineral absorption, lowering cholesterol, and showing antihypertensive, anti-inflammatory, immunomodulatory, antimicrobial, antithrombotic, antioxidant, antiobesity, and antidiabetic activities (Holdt and Kraan 2011; Cian et al. 2015; Hayes and Tiwari 2015; Admassu et al. 2018). Short-chain peptides, often carrying polar amino acid residues like proline and with hydrophobic amino acids at the C-terminal tripeptide sequence, have the ability to inhibit angiotensin-I-converting enzyme (ACE), which is a major therapeutic approach in the prevention of hypertension. Antihypertensive peptides are good therapeutic approaches in the management of hypertension (Wijesekara and Kim 2010) without the side effects of other pharmacological drugs (Admassu et al. 2018). Peptides with 2–6 amino acids are absorbed more readily than proteins and free amino acids, although it has been suggested that also larger peptides (10–50 amino acids) can cross the intestinal barrier and show biological action at the tissue level. The influence of the structure on the antioxidant properties has been studied by a number of groups, showing that the most active peptides were those with low molecular weights (Wang et al. 2010; Cian et al. 2015).

Mycosporine-like amino acids (MAAs) are secondary metabolites of low molecular weight (< 400 Da) with ultraviolet-absorbing ($\lambda max = 309–360$ nm) protection (Cian et al. 2015). They have a role in UV protection and have antioxidant activity capable of protecting against the cellular damage induced by high levels of reactive oxygen species (ROS).

Lectins are carbohydrate-binding proteins responsible for lectin involvement in numerous biological processes such as host-pathogen interactions, cell-cell communication, induction of apoptosis, cancer metastasis, and cell differentiation (Cian et al. 2015).

Phycobiliproteins, accounting for half of the total protein content of red seaweeds, are fluorescent proteins covalently linked to tetrapyrrole groups, and found in phycobilisomes. The major are phycoerythrin, phycocyanin, allophycocyanin, and phycoerythrocyanin, and they have applications based on their color and their biological properties, including antimicrobial, anti-inflammatory, neuroprotective, hepatoprotective, immunomodulating, and anticarcinogenic activities (Aryee et al. 2018; Mittal and Raghavarao 2018).

11.2.2 Sea Anemones Proteins and Cancer Treatments

Cancer treatments are mainly based on anti-mitotic compounds that have side effects, because they block the division of both cancer cells and healthy cells. Cancer tissues need to be vascularized by endothelial cells to grow as a tumor and then to spread, forming the metastasis. This vascularization occurring in cancer tissues is called angiogenesis. Almost 50 years ago, the blockage of angiogenesis was

proposed as a cancer therapeutic strategy because angiogenesis is no longer important after embryogenesis (Folkman et al. 1971). Amazingly, there are very few antiangiogenic compounds compared to antimitotic counterparts and only a decade ago antiangiogenic compounds began to be tested in clinical trials. Moreover, the antiangiogenic compounds showed limited efficacy, because they all bind to the vascular endothelial growth factor (VEGF) or the VEGF receptor and tumor cells can trigger different biological ways to have angiogenesis (Carmeliet and Jain 2011). Among antiangiogenic compounds binding to VEGF, the most used in clinical trials is bevacizumab (known as Avastin®), which is a monoclonal antibody. Bevacizumab is costly due to its size and difficulties to have germ free production as a recombinant protein. Furthermore, resistance to bevacizumab is observed due to the upregulation of other redundant angiogenic factors different from VEGF (Kong et al. 2017). The other compounds binding on VEGF are also monoclonal antibodies or Fab fragments. Compounds binding on VEGF receptors are not proteins and have toxic effects that limit their use (Chu et al. 2007).

There is, therefore, a need to have compounds blocking angiogenesis that do not bind to VEGF or VEGF receptor, are easy to produce and have no long-term toxicity. Short-size synthetic proteins (<50 residues) are suitable compounds for this goal. *Anemonia viviridis* (called also *Anemonia sulcata*) has been widely studied and proved to be a remarkable source of low molecular proteins with different pharmacological binding sites on ionic channel receptors (Diochot et al. 2003). Seven short size proteins (42–49 residues) binding on ionic channels were purified and characterized from *Anemonia viridis*. Among them, blood depressing protein 1 (BDS 1) has 43 residues (4708 Da), binds on a specific potassium channel and is commercialized as a synthetic protein to treat heart disease (Diochot et al. 1998). The NMR structure of BDS 1 revealed that the main secondary structure is a triple-stranded antiparallel beta-sheet with no alpha helix (Driscoll et al. 1989). Recently, a partially purified extract of *Anemonia viridis* was reported to affect the growth and viability of selected tumor cell lines (Bulati et al. 2016).

Synthetic proteins <50 residues are now affordable and have the great advantage to make possible a sterile production, which is not the case for monoclonal antibodies such as bevacizumab that required a biological production. The high cost of bevacizumab is not related to the production as a recombinant protein, but to the purification process to have a germ-free pharmaceutical production. A very low molecular weight compound (< 1000 Da) would be certainly less expensive to produce than a synthetic protein. However, a new chemical family of active substance requires now very expensive toxicological studies to have a Drug Master File suitable for clinical studies. Moreover, it turns out that actual preclinical toxicity studies required for clinical studies are not sufficient to guaranty an absence of long-term toxicity. The latter is often due to accumulation in tissues of chemical compounds that cannot be sufficiently degraded. Therefore, a synthetic protein (with a molecular weight < 5000 Da) represents a good compromise between the cost of production and safety issues.

Sea anemones are the best-known source of low molecular weight (< 5000 Da) active ingredients. They all have three disulfide bridges and share sequence

homologies but punctual mutations can totally change their structures and pharmacological properties. With its alpha helix, AS8 appears to have a structure differing from other proteins purified from *Anemonia viviridis* binding on ionic channels. AS8 could be related to the family of the Kunitz-type inhibitors of the elastase-like enzymes that are made of a chain of 40–60 residues stabilized by three disulfide bridges. The first described was the bovine pancreatic trypsin inhibitor (BPTI), and Kunitz-type inhibitors purified from sea anemone have been described (Mourão and Schwartz 2013). The crystal structure of a sea anemone in a complex with an elastase enzyme shows that this sea anemone protein keeps its scaffold made of a two beta strand sheet and a short alpha helix in the complex (García-Fernández et al. 2015). Specific elastase-like enzymes are involved in angiogenesis and, recently, a Kunitz-type inhibitor isolated from a tick was able to interfere with vessel formation (Soares et al. 2016).

Sea anemones produce a wide variety of compounds with different activities, such as neurotoxicity, cytolytic (Oliveira et al. 2006; Pedrera et al. 2014), hemolytic, analgesic, anti-inflammatory (Oliveira et al. 2006; Thangaraj and Bragadeeswaran 2012; Celedón et al. 2008; Stabili et al. 2015), anti-hyperglycemic and anti-diabetic (Lauritano and Ianora 2016) activities. The mucus of anemones also appears as a promising alternative source of antimicrobial lysozyme-like antibacterial agents, which have different modes of action compared with conventional antibiotics (Subramanian et al. 2011; Stabili et al. 2015). Some of these proteins, such as sticholysins, are exclusively found in sea anemones (Pedrera et al. 2014). These cysteine-less proteins, with molecular weights around 20 kDa, have high isoelectric points (>9.5), are water soluble and can interact with membranes and form a stable membrane pore (Álvarez et al. 2009). Table 11.1 summarizes some properties of seaweed and anemone proteins.

Mycosporine-like amino acids are also found in sea anemones, among them are shinorine, porphyra-334, mycosporine-2 glycine, palythine-serine sulfate, stylophora-sulfate, palythine, asterina-330, palythinol, mycosporine-taurine, and mycosporine-sulfate ester. The highest concentration is often found in areas exposed to sunlight. They can also be of dietary origin, present in food or resulting from transformations of dietary MAAs by the action of specific anemone enzymes or bacteria harbored in the anemones coelenteron or ectodermal tissue (Arbeloa et al. 2010).

11.3 Processing Technologies

11.3.1 Seaweed

Among seaweeds, the highest protein levels, up to 50% of dry matter, have been reported for red algae, compared to green (up to 25%) and brown (under 15%) (Cian et al. 2015). Most seaweeds contain all the essential amino acids, show high content of aspartic and glutamic acid, and low content of threonine, lysine, tryptophan, cysteine, methionine, and histidine. The carbohydrate fraction is the most abundant

Table 11.1 Biological properties of representative proteins from seaweeds and anemones

Source	Types	Properties	References
Seaweeds	Phycobiliproteins	Antimicrobial, anti-inflammatory, neuroprotective, hepatoprotective, immunomodulating, anticarcinogenic	Aryee et al. (2018) and Mittal and Raghavarao (2018)
	Phycolectins	Involvement in host-pathogen interactions, cell-cell communication, induction of apoptosis, cancer metastasis or cell differentiation	Cian et al. (2015)
	Mycosporine-like amino acids	UV protection, antioxidant activity	
Anemones	BDS 1, blood depressing protein 1	Antitumoral, treatment of heart disease	Diochot et al. (1998) and Bulati et al. (2016)
	AS8, related to the family of the Kunitz-type inhibitors of the elastase-like enzymes	Angiogenesis, interference with vessel formation	Mourão and Schwartz (2013) and Soares et al (2016)
	Lysozyme-like agents (cysteine-less proteins, sticholysins)	Antimicrobial, antibacterial	Subramanian et al. (2011) and Stabili et al. (2015)
	Mycosporine-like amino acids	Antioxidants	Arbeloa et al. (2010)

and its composition depends on the type of seaweed. Other important constituents are minerals, whereas the lipid fraction is low.

Seaweeds contain a highly structural complex algal cell wall, which difficults the efficient extraction and digestibility of protein fractions (Admassu et al. 2018). The classic extraction methods are limited by the high viscosity and anionic bonding of the cell-wall polysaccharides and glycoproteins. Grinding in liquid nitrogen was proposed, but this approach is costly at the industrial scale and does not provide an efficient degradation of the cell wall. The development of novel extraction methods is key to facilitate the disruption of the cell wall, facilitate access to the seaweed bioactive peptides maintaining high yields and optimal functional properties. Physical, chemical, and enzymatic treatments have been proposed to obtain high protein yields. Among the most efficient are those based on the intensification of the extraction process with ultrasound or pulsed electric fields, as well as the use of pressurized solvent extraction and enzyme-assisted extraction (Wijesinghe and Jeon 2012; Flórez-Fernández et al. 2017; Admassu et al. 2018).

Ultrasound-assisted processes can provide higher protein extraction yields in shorted processing times than conventional extraction with acid or with alkaline media (Kadam et al. 2015; Mittal et al. 2017). Temperature is required to be adequate to avoid undesirable thermal and chemical effects, adequate selection of frequency is recommended, and the use of different alternating frequencies could enhance protein extraction yields (Qu et al. 2013).

The type of enzyme influences the extraction yield, and also the composition and properties of the extracts. The use of carbohydrases alone, including those developed for terrestrial biomass, such as β-glucanases, cellulases, and xylanases, could enhance the protein extraction yield (Fleurence 1999; Wang et al. 2010) or would require the combined use with other polysaccharidase or with other specific enzymes, such as carrageenase or agarase (Fleurence et al. 1995; Denis et al. 2009; Mittal and Raghavarao 2018). Among the operational variables affecting the process are pH and temperature, which should be optimal for both enzyme action and protein recovery, liquid to solid ratio, time and the enzyme to substrate ratio, which should be carefully optimized, since the enzyme costs would be one of the major limitations of this technique. Enzyme-assisted extraction can also be combined with other technologies, such as ultrasound for the extraction of R-phycoerythrin (Le Guillard et al. 2015).

Pressurized hot water extraction of protein was proposed at 120–270 °C to enhance the yields and productivity in an environmentally friendly operation (Gereniu et al. 2017; Pangestuti et al. 2019).

After the extraction process, additional hydrolysis stages are required to enable the release of functional peptides. One of the preferred methods is enzymatic hydrolysis with a single activity or combinations of them, since it can be performed under milder conditions than chemical and physical treatments (Wang et al. 2010; Samarakoon and Jeon 2012). Admassu et al. (2018) have compiled the enzymes, including pepsin, trypsin, papain, chymotrypsin, alcalase, and fungal proteases, used more commonly for hydrolysis of seaweed proteins in producing ACE inhibitory and antioxidant peptides.

The released bioactive peptides are then concentrated and fractionated. Qu et al. (2015) have reported a continuous enzymatic bioreactor with membrane separation in a multistep recycling system and fractionate the hydrolysates according to ranges of their molecular weight. Purification of this protein is performed by different techniques such as ammonium sulfate precipitation and chromatographic techniques (ion exchange, gel filtration, etc.). Usually, a stage with ultrafiltration membranes is proposed for targeting a desirable molecular weight range, and ion exchange, affinity chromatography, and high-performance liquid chromatography are used for purification and enrichment of bioactive components (Harnedy et al. 2017; Cheung et al. 2015). An adequate definition of downstream stages is required to develop cost-effective processing (Hayes and Tiwari 2015) (Fig. 11.1).

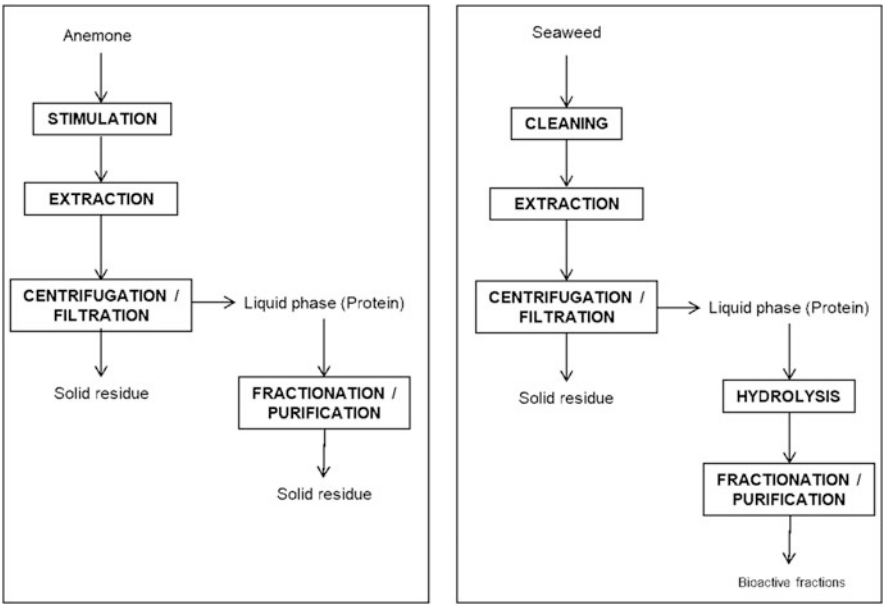

Fig. 11.1 Simplified flow diagram of the processes for the extraction of bioactive protein fractions from (**a**) anemones and (**b**) seaweeds

11.3.2 Anemone

In sea anemones, the venom system is decentralized in all parts of the animal body and is delivered by characteristic stinging organelles or nematocysts, located on body surfaces and in high concentration on tentacles, with functions related to defense against predators and prey capture. These organs possess a high concentration of a diverse range of sizes (12–200 kDa) and protein active principles (Oliveira et al. 2006; Subramanian et al. 2011; Stabili et al. 2015), many of them could not be identified in other animals suggesting that they might be the products of taxonomically restricted genes (Moran et al. 2013).

The mucus produced by the sea anemones is composed in more than 95% by water and the major components of the dry fraction are minerals, more than 60%, protein 29–37%, carbohydrates 5–25%, and less than 0.3–1% lipid (Davies et al. 1990; Stabili et al. 2015).

During stress conditions, the nematocysts can be released from the tentacles. The process can be repeated and the collected nematocysts containing toxins can be filtered, centrifuged, and the supernatant collected for lyophilization (Subramanian et al. 2011).

An easy and ecological method to extract bioactive compounds from sea anemones consists on maintaining and feeding the animals in aquarium, facilitating an increased milking frequency through electrical stimulation, avoiding to kill them and making possible to return to the sea (Oliveira et al. 2006). Most authors have

proposed aqueous, methanol, dichloromethane, and ethanol extracts (Subramanian et al. 2011; Thangaraj and Bragadeeswaran 2012). The crude extracts have to be filtered, evaporated, and lyophilized.

Fractionation in C18 columns was proposed for partial purification of methanol extracts (Arbeloa et al. 2010), or for semipreparative chromatography of the selected fractions from Sephadex G-50 gel filtration (Oliveira et al. 2006). Membrane separation was also proposed for extract purification of anemone mucus extract components before chromatographic techniques (Stabili et al. 2015).

11.4 Novel Pharmaceuticals Formulated with Algal and Sea Anemones Proteins and Peptides

Nature provides compounds with great biological value (Wijesekara et al. 2011; de Jesús Raposo et al. 2015). Terrestrial and marine plants have different properties associated with the exhibited chemical composition and structure, while environmental factors also influence the displayed composition and biological activities (Rioux and Turgeon 2015; Vo and Kim 2013). Marine macroalgae have been used as food and traditional medicines in the Asian countries (Peng et al. 2015), and it is known that they possess some bioactive compounds that are not present in terrestrial biomass (Wijesinghe and Jeon 2012). These algae are composed of complex mixtures of compounds, such as polysaccharides, proteins, pigments, lipids, minerals, and vitamins. The season of collection, the geographical location, and other environmental factors influence the characteristics of the biological compounds of interest. Similarly, the extraction technologies used to achieve these compounds also play a relevant role (Fernando et al. 2019).

According to their pigmentation, seaweeds are classified as red, green, and brown algae. The corresponding main pigments are phycobilin, chlorophyll, and fucoxanthin, respectively (Hamid et al. 2015; Pangestuti and Kim 2011). As part of their complex composition, as referred above, seaweeds can be the source of polysaccharides, which are isolated upon the application of adequate techniques of extraction (Cardoso et al. 2016; González-López et al. 2012; Li et al. 2019). The main algal sulfated polysaccharides are carrageenan, fucoidan, and ulvan, which have been reported to exhibit several biological activities (Stiger-Pouvreau et al. 2016). A review of these materials is available that details their properties of interest, with particular emphasis on drug delivery applications (Cunha and Grenha 2016). Other biopolymers with the algal origin, such as alginate, also have a great interest in the fields of biomedicine and food industry, this being specifically used as a gelling agent, stabilizer, or thickener (Rioux and Turgeon 2015; Draget and Taylor 2011).

In biomedical-related fields, the demand for natural-origin materials is progressively increasing. Properties such as the high propensity for biodegradability and biocompatibility are attractive for pharmaceutical applications, but also for cosmetics and nutraceuticals (Vo and Kim 2013; Wijesinghe and Jeon 2012; Fernando et al. 2019; Lopes et al. 2017; Holdt and Kraan 2011). Nowadays, the

polysaccharides of marine origin with more reported pharmaceutical applications are alginate, chitosan, and carrageenan (Cardoso et al. 2016; Laurienzo 2010).

Drug delivery systems such as nanoparticles and microparticles have been proposed for several biomedical applications, including drug delivery, gene therapy, cell therapy, and tissue engineering (Cardoso et al. 2016; Colliec-Jouault 2015; Manivasagan and Oh 2016). Nanoparticles have reached a position of evidence, owing to several advantages mainly related to the high surface-to-volume ratio (Singh and Lillard 2009). Their use in drug delivery is widely disseminated, having already market representation. The International Organization for Standardization (ISO) defines nanoparticles as particles having at least one dimension below 100 nm (ISO/TS 2015). The definition is, however, not consensual in the area of drug delivery and the term is frequently used for particles with submicron size (<1000 nm) (Cheow et al. 2015; Hyuang et al. 2016). These particles can bear a matrix composed of natural or synthetic materials, where the drugs of interest are dispersed. The report of relevant biological properties of polysaccharides of marine origin renders these materials attractive for biomedical applications (Cunha and Grenha 2016; Venkatesan et al. 2015; Alboofetileh et al. 2019). Their proposal as matrix materials of nanoparticles has, thus, been frequent.

No works refer, so far, the formulation of algal and sea anemones proteins and/or peptides in nanoparticulate form. However, the literature has many reports on the association of proteins/peptides to nanoparticles. Although therapeutically promising, protein-based drugs are very instable and their delivery is extremely challenging, due to inherent physicochemical and biopharmaceutical properties. Therefore, parenteral delivery, which is mediated by injection, frequently represents the unique administration possibility (Alonso 2004; Kammona and Kiparissides 2012). The therapeutic action of proteins and protein-based molecules is not only limited by the potential degradation in biological environments, but is also compromised by limited ability to reach therapeutic sites of action (Kammona and Kiparissides 2012; Antosova et al. 2009; Casettari and Illum 2014). In addition, drug delivery via routes other than the parenteral, such as mucosal routes, faces other major restrictions, including specific mechanisms of defense, the possibility to induce immune reactions at the delivery site and, generally, limitations in the surface area available for absorption (Cleland et al. 2001). The scientific community, thus, directed research efforts toward the development of adequate carriers aimed at delivering drugs through distinct routes of administration, of which nanoparticles are popular representatives. Importantly, nanocarriers have been reported to increase drug absorption by reducing epithelial resistance to drug transport in a localized area or by carrying the drug across the epithelium (Csaba et al. 2006). Additionally, nanoparticles frequently display surface functionality, which offers high potential for the association of protein-based molecules.

There are countless examples of research involving the association of proteins and peptides to nanoparticles. In this manner, a couple of these will be referred as an example, considering that nanoparticulate systems could prove beneficial in potentiating the activity or performance of marine proteins/peptides, particularly those obtained from algae and sea anemones.

As mentioned above, different materials may be used to produce nanocarriers. Polymers and, particularly, polysaccharides, have been one of the most used. The methods that can be applied to produce the carriers are also diverse. The specific structure of proteins defines their exact properties and activities and, therefore, it is crucial to ensure its preservation during the encapsulation procedures. This fragile nature requires the selected methods to not damage the molecule structure, reduce their biological activity, or render them immunogenic (Kammona and Kiparissides 2012). Methods based on the establishment of intermolecular electrostatic interactions, such as polyelectrolyte complexation and ionic gelation, are of the most reported. These are applied when the matrix of nanoparticles is composed of at least one polyelectrolyte, that is, a polymer that exhibits charged groups when in solution, which is the case of the polysaccharides mentioned above. The method relies on the ability of polyelectrolytes to establish stable links with oppositely charged groups (Lima et al. 2012). The fact that electrostatic interaction is the driving force of nanoparticle formation, renders the process very mild and eco-friendly, avoiding the use of organic solvents. Many nanoparticle formulations are reported to be formed by polyanions from macroalgae, along with chitosan. Taking this as an example, a review of nanoparticle preparation methods is available in the literature (Grenha 2012). The mild conditions involved in the method make the association of labile drugs, such as protein and peptides, an easier task (Lima et al. 2012).

Among the polysaccharides from macroalgae, ulvan from green, fucoidan from brown, and carrageenan from red seaweeds are negatively charged owing to the inherent sulfate content. In the last couple of decades, alginate is another anionic biopolymer hugely reported in the biomedical field, being extracted from brown seaweeds (Draget and Taylor 2011; Gepp et al. 2017).

Fucoidan is extracted from the cellular wall of brown seaweeds and has been applied in the elaboration of nanoparticles for drug delivery applications, mainly through electrostatic interaction with cationic materials, chitosan most frequently (Cardoso et al. 2016). Chitosan/fucoidan nanoparticles were reported as an oral delivery system of insulin upon complexation between fucoidan and trimethyl chitosan (Tsai et al. 2019). General applications in oral delivery (Huang and Lam 2011) and the specific delivery of antibiotics (Huang and Li 2014) were also reported.

Nanoparticles based on carrageenan have also been reported, again produced predominantly by complexation with chitosan. Various applications include tissue engineering (Popa et al. 2011) and protein delivery (Grenha et al. 2010; Rodrigues et al. 2015). Some of the authors of this manuscript have reported the effective association of proteins (bovine serum albumin) to different carrageenan-based nanoparticles. The polysaccharide was either complexed with chitosan (Rodrigues et al. 2015) or with aminated pullulan (Dionísio et al. 2013) and nanoparticles of 250–300 nm were obtained that associated the protein with varying efficiency (30–70%). However, despite being Generally Recognized as Safe (GRAS) by the American Food and Drug Administration (FDA) (Rowe et al. 2009), many reports

indicate the inflammatory activity of carrageenan (Ahmad et al. 2018), thus preventing its extended use.

Ulvan is extracted from green macroalgae and its exploration is much more recent than that of fucoidan and carrageenan. From a point of view of nanotechnology applications, its use on nanofiber production (Manivasagan and Oh 2016) and preparation of different types of nanoparticles, metallic and biopolymeric, has been studied (Massironi et al. 2019; Fernández-Díaz et al. 2017), but the association of proteins was not reported so far. Interestingly, ulvan/chitosan nanoparticles demonstrated to possess immunomodulatory properties, by inducing the activation of macrophages from *Solea senegalensis* (Fernández-Díaz et al. 2017).

The authors of this manuscript also proposed the use of polysaccharide-based nanoparticles for oral vaccination. Specifically, both ovalbumin (OVA) and an immunogenic subcellular extract obtained from whole *Salmonella enteritidis* cells (HE) were used as model antigens and locust bean gum/chitosan nanoparticles as carriers. Nanoparticles with size around 200 nm were produced, encapsulating one of the model molecules with an efficiency of 25–30%. The procedure was shown to not affect the structural integrity and the antigenicity of the molecules. The in vivo evaluation of the loaded nanoparticles demonstrated the adjuvant effect of the proposed system when OVA was used as a soluble antigen model, although this effect was not observed when a particulate antigen like HE was tested (Braz et al. 2017).

The potential of using nanoparticles to deliver protein-based drugs and, thus, potentiate their action in some way, has been being demonstrated in countless works. However, lessons learned from the development of protein nanoformulations clearly indicate that the success of the approach not only depends on the system structure, but also on the synergism created with the carried molecule.

11.5 Conclusions and Future Trends

The molecules synthesized by sedentary marine microorganisms could have the potential for novel products with pharmacological applications. Sea anemones contain a variety of bioactive compounds including some toxins, which could have the potential for the production of bioactive compounds of high pharmaceutical and biotechnological interest, i.e., antihypertensive peptides and antimicrobial molecules or immunotoxins against tumoral cells.

Further advances and improvement in extraction and isolation of peptides from seaweed and their purification are expected to comply with the growing demand for novel efficient side effects-free natural derived drugs, as well as the development of cleaner efficient processes.

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Marine-Microalgae as a Potential Reservoir of High Value Nutraceuticals

12

Jeyakumar Balakrishnan, Thiyagarajan Sekar, and Kathiresan Shanmugam

Abstract

Among the innovative entries in the nutrition supplements area, a vital place must be allocated to nutraceuticals encompassing marine microalgae, currently accounting for a great and promptly growing market. The current market values of these marine microalgal-derived bioactive compounds are extremely high. The major products commercially explored include carotenoids, β-carotene, astaxanthin, polyunsaturated fatty acids, polysaccharides, vitamins, sterols, and biologically active molecules. These biomolecules and metabolites possess antibiotic, antiviral, anti-inflammatory, anticancer, and neuroprotective activities. The bioactive compounds have wide array of activities such as inhibition, transcription, activation of key enzymes and transportation, which can revamp several metabolic pathways. This chapter describes the available marine microalgal-derived nutraceuticals and recent research accomplished toward the characterization of bioactive metabolites and use of marine microalgae-based products for various therapeutic potentials.

Keywords

Marine microalgae · PUFAs · Carotenoids · Food supplement · Nutraceuticals

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12.1 Introduction

Algae, a unicellular or multicellular form of organism, are classified as microalgae and macroalgae depending on their cell size (Shivhare et al. 2014). Microalgae are considered as monocellular and can be seen only with a microscope (Mutanda et al. 2011). Microalgae are the earliest life forms on earth with richest biodiversity. Microalgae are able to convert light energy to chemical energy through carbon fixation by means of photosynthesis. Microalgae grow rapidly with limited amount of water and nutrients when compared to other plants (Hannon et al. 2010; Ercin et al. 2012). Moreover, microalgae sequester the atmospheric carbon dioxide that is released by industries. Now it is very well known that marine biotechnology, and its enormous biological diversity have a large impact on the key societal challenges. Microbial sources such as marine microalgae are an alternative to higher plants because of their rich biodiversity, growth rate, and multiple application potential. Hence, it is considered as a sustainable source for future use. Marine microalgal system with its rich biodiversity, namely, the species number ranging from 30,000 described species, extreme evolutionary and phylogenetic diversity has lot of potential to be explored (Guiry 2012; Massana et al. 2006). The diversity of these marine microalgae makes them suitable for bioprospecting and large-scale industrial production of biomolecules. Microalgae (*Nostoc*) were first used by the Chinese for survival of drought some 2000 years ago, but it is only recently that innovative technologies using microalgae have emerged (Priyadarshani and Rath 2012). Nowadays the use of microalgae for biotechnological applications has been increasing for nutraceutical and pharmaceutical purposes (Cadoret et al. 2012). Till date, around 20 microalgae species, from marine, estuarine, and fresh water ecosystems, have been used in biotechnology applications (Chu 2012). There are several challenges to achieve industrial revolution with microalgal biotechnology by identifying desired species with excellent product recovery. For microalgal biotechnology to be successful, understanding the behavior and physiology of microalgal species under different conditions is essential for the specific desired product. This will help develop innovative technologies to reduce the cost of downstream processing involved in microalgal products (Pulz and Gross 2004). Several studies reported that microalgal growth is greatly influenced by environmental factors (pH, light, and temperature) and nutrient factors (nitrate and phosphate) (Khoeyi et al. 2012; Van Wagenen et al. 2012; Huerlimann et al. 2014; Singh et al. 2015). Optimizing these conditions results in improved biomass and reduces the production cost. The currently available products from marine microalgae are carotenoids, β-carotene, phycobilins, astaxanthin, polyunsaturated fatty acids, polysaccharides, vitamins, sterols, and biologically active molecules (Borowitzka 2010; Kyle et al. 1992; Ratledge 2004; Mendes et al. 2009; Singh et al. 2005). The current market values of these marine microalgal-derived bioactive compounds are extremely high and have gained wide interest. It is believed that by 2025, microalgal-derived products could be produced in a large scale with ecological safety (Bhalamurugan et al. 2018). In this chapter, we discuss the available bioactive molecules from marine microalgae

and recent research toward the use of microalgal-derived nutraceuticals in various applications.

12.2 Commercial Bioactive Molecules Derived from Microalgae

In the modern world, people consume high-calorie food items, which cause several health issues like cardiovascular diseases, obesity, and diabetes. These diet conditions are needed to be balanced with enhanced levels of vitamins, minerals, polyunsaturated fatty acids (PUFAs), etc. Many high-value products from microalgae have been identified and marketed as nutraceuticals/pharmaceuticals for several therapeutic applications. The development of microalgal biotechnology paves the way for introducing additional products that have high economic value. The commonly available products from microalgae are listed below and given in Fig. 12.1.

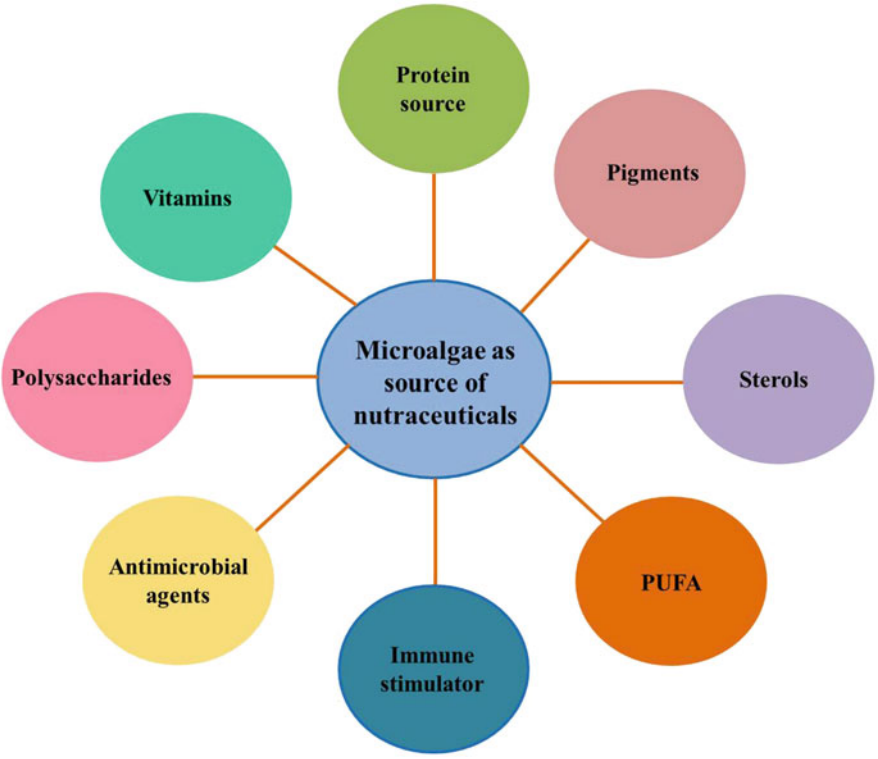

Fig. 12.1 Different value-added products from marine microalgae

12.2.1 Microalgae: An Excellent Food Supplement as a Protein Source

The types of microalgae that were first commercialized as "health food" in Japan, Taiwan, and Mexico were *Chlorella* and *Spirulina* The most important nutritional factor of microalgae is protein-rich biomass with exceptional quality and balanced amino acid content. In this case, *Chlorella* and *Arthrospira* produce high-quality proteins, and their amino acid profile is in accordance with World Health Organization (WHO), Food and Agriculture Organization (FAO), and United Nations University (UNU) guidelines for human use (Becker 2007; Phillips and Williams 2011). Because of its high protein and amino acid content, *Arthrospira* is widely used in the production of human nutrition in China and India under the name *Spirulina pacifica* (Milledge 2011). Furthermore, *Chlorella* and *Arthrospira* microalgal extracts are used in cosmetics as skin and hair care products (Gellenbeck 2012). The usage of microalgae in human diet is less compared to aquaculture, since microalgal biomass contains complex fibers and complex polymers that are easily ingestible and digestible by rotifers and shrimps used in aquaculture (Mišurcová et al. 2010; Taelman et al. 2013). The microalgae like *Chlorella* sp., *Dunaliella* sp., *Nannochloropsis* sp., and *Spirulina plantensis* are used in several industrial applications because of the presence of high protein and nutritive contents (Soletto et al. 2005). Nearly 1000 metric tons of Spirulina is produced in a year for industrial application (Ciferri and Tiboni 1985). More than 20 different countries are manufacturing Spirulina-based products in the market, with USA as the leading country followed by China, Israel, Japan, Mexico, Taiwan, and Thailand (Spolaore et al. 2006).

12.2.2 Carotenoids: An Antioxidant Source from Microalgae

Carotenoid pigments are produced from both natural sources and synthetic chemicals. Using synthetic carotenoid pigments in food ingredients cause potential toxic effects to human and that stimulates the green solutions for natural products in global market that is expected to reach about $1.5 billion by 2020. Safer alternatives to synthetic carotenoid were produced from microbial sources like fungi, bacteria, and microalgae (Dufosse et al. 2005; Yang et al. 2013). Different carotenoid pigments such as chlorophyll, carotenoids, β-carotene, fucoxanthin lutein, zeaxanthin, phycobiliproteins, and astaxanthin have been exploited for their commercial applications in pharmaceuticals, nutraceuticals, animal feed, food, dietary supplements, and cosmetics sector (Ambati et al. 2014; Pangestuti and Kim 2011; Fernández et al. 2010; Rodrigues et al. 2014; Cuellar et al. 2015; Yuan et al. 2011). The demand for carotenoid pigments like astaxanthin, β-carotene, and lutein is highly evolving in the global market and its value is estimated to reach US$1.53 billion by 2021. Astaxanthin has antioxidant activity, which is highly helpful in scavenging free radicals in human body (Kent et al. 2015). The annual market value for astaxanthin is US$2500 per kg and it has been highly used in global market such as salmon feed industry. In future, the requirements of carotenoid need

to be explored, and it is expected to give a turnover usage of US$800 million for animal feed and US$300 million for nutraceutical applications by 2020. The market values of different carotenoid pigments have been evaluated as β-carotene (US $334 million), lutein (US$309 million), and fucoxanthin (500 tonnes). Europe contributes to the major carotenoid production and application in different industrial areas like animal feed, health supplements, and cosmetics, and the leading industries from Europe are L'Oréal, Unilever, Beiersdorf, and Henkel, with a global market for carotenoids.

There is a growing demand for alternative and natural source of carotenoids due to several health issues caused by synthetic carotenoids in food products. Natural sources are known to protect the cells from oxidative damage (Ranga Rao et al. 2013). Carotenoids received a significant attention from the food industry. The use of carotenoids in food products is more valuable to prvent food deterioration during the storage and processing time. The major algal species such as *Haematococcus pluvialis* (*H. pluvialis*), *Dunaliella salina* (*D. salina*), *Chlorella* sp., *Scenedesmus* sp., *Spirulina platensis*, *Botryococcus braunii*, and diatoms are used to synthesize the major carotenoids pigments like β-carotene, lutein, canthaxanthin, astaxanthin, and fucoxanthin (Lamers et al. 2008; Ranga Rao et al. 2010). Microalgae produce high amount of carotenoids especially under nutrient and environmental stress conditions (Sarada et al. 2012).

Dunaliella salina is a unicellular green motile algae found in different habitats like lakes, oceans, and brackish water bodies. They are rich in pro-vitamin A carotenes, which exhibit red color under different stress conditions (Yang et al. 2013). The first commercial β-carotene was produced from microalga *D. salina* in the 1980s by four different companies such as Koor Foods (Nature Beta Technology) in Israel, Western Biotechnology Ltd, Betatene Ltd. in Australia, and Nutrilite in the USA, and its market value was approximately US$300–1,500 per kg. *D. salina* is cultivated in saline conditions that helps to reduce contamination and produce high β-carotene content than other organism (Borowitzka and Borowitzka 1989). β-carotene from *Dunaliella* was first approved in Japan for human use and its total market value was estimated at about US$270 million.

Spirulina platensis was reported to contain high amount of proteins (55–65%) followed by essential fatty acids and pigments (Coca et al. 2015). The carotenoids from *Spirulina* sp. were observed from 0.1 to 0.4 mg/g and the major pigments such as β-carotene, zeaxanthin, and β-cryptoxanthin were isolated and identified (El-Baky et al. 2003; Ranga Rao et al. 2010). The intake of carotenoids from naturally derived microalgae helps to control the photo-oxidative damage of cells, improve the immune system and cell growth, scavenge free radicals, and regulate hormones (Eriksen 2008).

Haematococcus pluvialis, a green alga, is the efficient producer of astaxanthin and carotenes (α, β), which is cultivated in both autotrophic and heterotrophic culture conditions (Sarada et al. 2012). The carotenogenesis of *H. pluvialis* involves the formation of thick- walled aplanospores that helps to accumulate astaxanthin up to 2–4% in its dry weight (Ranga Rao et al. 2010, 2013). Several carotenoid pigments like astaxanthin, lutein, zeaxanthin, carotenes (α, β), and violaxanthin

were identified in the carotenoid biosynthesis pathway of *H. pluvialis* (Ranga Rao et al. 2013). Moreover, the novel astaxanthin has a legal permission to add in the fish food formulation and food ingredient by United States of Food and Drug Administration (USFDA) and several European countries (Pashkow et al. 2008).

Chlorella sp. produces significant quantities of xanthophyll pigments such as lutein, zeaxanthin, and violaxanthin; among them, lutein was found to be the major carotenoid (Del Campo et al. 2004). For high biomass and carotenoid production, the microalgae *Chlorella vulgaris* and *Chlorella protothecoides* are cultivated in both autotrophic and heterotrophic conditions. *Chlorella* sp. has a very rigid cell wall, which needs to be broken down before various industrial applications, and it has been used in several health and nutrition supplement ingredients (Kitada et al. 2009). *Chlorella* sp. is a good source of pro-vitamin A carotenoid that helps in human health.

12.2.3 Marine Microalgal-Derived Polyunsaturated Fatty Acids

Microalgae provide many high value products for human, including long chain polyunsaturated fatty acids (PUFAs) such as γ-linolenic acid, arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which play a significant role in human health and development (Ratledge 2010). The PUFAs are commercialized from different organisms such as *Crypthecodinium*, *Schizochytrium*, heterotrophic thraustochytrids, and *Ulkenia* (Barclay et al. 2010; Wynn et al. 2010). DHA oil is extracted from *Crypthecodinium cohnii* by cell disruption and is stabilized by an antioxidant tocopherol and mixed with 40% w/w oleic sunflower oil (Wynn et al. 2010). The commercial name of DHA product is DHASCO™, and this product is available as infant formula. *Schizochytrium* is used to extract both DHA and EPA oils in commercial market. The unicellular microalga *Nannochloropsis* releases EPA-rich oil and has been commercialized by Aurora Algae. Arachidonic acid is richly present in fungus *Mortiella alpine*, and green alga *Parietochloris incise* also synthesizes high amounts of arachidonic acid (Bigogno et al. 2002). Cyanobacteria *Spirulina platensis* is the best source for γ-linolenic acid production than other organism (Tanticharoen et al. 1994). They are enriched with γ-linolenic acid in total PUFA content, and Spirulina is available in the market as tablet, capsule, and powder forms. PUFA enriched microalgal source is considered as an alternate source to replace the fish and animal oil. Several strategies have been implemented to improve the lipid production through isolation of new species, strain selection, and genetic engineering, and culture optimization favors the PUFA-rich oil production at the economic level. The market price of algal omega-3 oil is about US$ 80–160, which is higher than fish oil products. Fish oil is the major supplement of EPA and DHA in the global market compared to algal oil, but algal oil is estimated to exceed 135,000–190,000 tons in future. Among different EPA- and DHA-rich fish oils, cod liver oil (30% EPA+DHA), tuna oil (20–24% EPA+DHA), and salmon oil (15–20% EPA+DHA) are the dominating source in the global market. The pharmaceutical, nutraceuticals, and functional food applications are

accounting to 72% of the global market volume and of which nutraceuticals have the largest module with 59% of the global volume. At present, two omega-3-based pharmaceuticals products are approved in the pharmaceuticals industry, with the yearly sales of US$1.5 billion, for example, triglyceride reduction product Omacor/Lovaza produced by Pronova Biopharma. The food market is going to be the highest growing field in the upcoming years, especially in Western countries and Asia. DSM is a well-known company for its omega-3 production in the world; they are undertaking algal derived EPA and DHA. Especially for the addition of EPA and DHA in infant formulated milk powder companies like Pfizer, Mead Johnson Nutritionals, Daone, and Abbott Nutrition. Other than pharmaceuticals and food industry, algal products are highly required in animal feed formulation. The animal feed market acts as an alternative source for algal oil that is similar to fish oils. The major reason to add the algal oil in animal feed formulation is efficient final meat production. The usage of algal derived products in animal feeds clearly indicates the less cost than other sources, and potential quality issues in EPA and DHA enriched aquaculture feed that gives remarkable effects for human health by the fish consumption. The salmon feed industry utilizes nearly 100, 000 tons dry algal biomass per year. The use of algal feed in aquaculture industry has a remarkable output with enriched EPA and DHA content in fish meat.

12.2.4 Microalgal Polysaccharides: An Incipient Product in Cosmetic Industry

Microalgae are the potential producers of different polysaccharides, but they have been poorly investigated for commercial application. There are different types of polysaccharides produced from microalgae, especially from unicellular red algae such as *Porphyridium* sp., *Porphyridium cruentum* (*P. cruentum)*, *Porphyridium aerugineum*, and *Rhodella reticulata* (Arad and Levy-Ontman 2010). Many cyanobacteria also synthesize polysaccharides, but their commercial acceptance and availability are not found in the global market, as cheaper alternative sources, such as macroalgae (carrageenan, fucoidan, agar) and higher plants (guar gum, xanthan gum), are available (Whistler 2012). However, few microalgal polysaccharides have been found in different areas of cosmetics and nutraceutical applications. Different microalgal strains excrete up to 20 g/L of exopolysaccharides (EPAs) into their environment, and sometimes, they are associated with a cell envelope called cell-bound polymer. Exopolysaccharides (EPS) are the heteropolysaccharides composed of sugars like glucose, galactose, and xylose and significant amounts of few other monosaccharides (Delattre et al. 2016). Nearly 90,000 tons of seaweed polysaccharides are produced per year and have been used in hydrocolloids and cosmetic and pharmaceutic industries (Kraan 2012). The market for polysaccharides will not be exhausted, but the launch of new microalgae biopolymers encourages innovative development in industrial applications.

12.2.5 Microalgal-Derived Sterols as Functional Foods

Microalgae produce different phytosterols such as sitosterol, stigmasterol, and brassicasterol based on the taxonomical affiliation of algae (Volkman 2003). The presence of sterol content in microalgae varies in relation to alteration of culture conditions (Fabregas et al. 1997). Algal phytosterols have many in vivo bioactivities that support in many food and pharmaceutical applications. The annual requirement of phytosterol is about US$300 million due to its high importance in pharmaceutical industry (Sioen et al. 2011). Higher plants are the major industrial source of phytosterols, but microalgae including Chlorophyceae, Rhodophyceae, and Phaeophyceae are also found in commercial applications. At present, phytosterol contents are found in different sources in United States Department of Agriculture's National Nutrient Database such as 8.09–15.57 g/kg in corn oil (0.809%–1.557% of oil weight), 19.7 g/kg (1.97%) in wheat germ oil, and 32.25 g/kg (3.225%) in rice bran oil. The phytosterol from different microalgae such as *Phaeodactylum tricornutum, Nannochloropis gaditana*, and *Isochrysis galbana* ranged from 7 to 34 g/kg (0.7–3.4%) (Ryckebosch et al. 2014). By changing the cultivation condition of *Pavlova lutheri*, nearly 5.1% dry weight of phytosterol was achieved. This level of extracted phytosterol in algal dry weight biomass is equivalent to plant oil extraction, and microalgal phytosterols have many advantages when used in various industrial applications. Annual microalgal oil reaches from 19,000 to 57,000 L oil per acre and it depends upon the different algal species, which is more than 200 time higher performance than vegetable oils (Demirbas and Demirbas 2011).

Commonly, ergosterols were present in microalga *Chlorella vulgaris* (Yasukawa et al. 1996) and *Dunalliella tertiolecta* having the LPS-induced anti-inflammatory activity, the inhibition of proinflammatory cytokine (TNF-α) production and COX-2 expression is LPS-induced response (Caroprese et al. 2012). Different phytosterols, including ergosterol peroxide, 7-dehydroporiferasterol peroxide, and 7-oxocholesterol, were isolated from *Chlorella vulgaris*, and *Dunaliella tertiolecta* showed anti-inflammatory activities against tumor promoter 12-O-tetradecanoylphorbol-13-acetate. The combination of two phytosterols of 7-dehydroporiferasterol with ergosterol from *Dunaliella tertiolecta* has the potential to suppress the proliferation of concanavalin A (ConA)-stimulated ovine peripheral blood mononuclear cells (PBMCs). Most of the microalgal extracts contain different phytosterols and secondary metabolites, which act as anti-inflammatory agents for human.

12.2.6 Bioactive Molecules from Microalgae

Microalgal extracts have several bioactive properties which have been studied since the past five decades including antioxidative, antibiotic, antiviral, anticancer, anti-inflammatory, antihypotensive, and other activities (Klein et al. 2012; Ohta et al. 1995; Le and Desbois 2017; Kim et al. 2012; Robertson et al. 2015; Liu et al. 2011). Several drugs are developed from the extracts of microalgae and cyanobacteria in

pharmaceutical industry (Borowitzka 1995). Most of the marine organisms including algae have many biologically active compounds by themselves, of which, few are approved for commercial application and few are under clinical trials (Mayer et al. 2010). There are nearly 70 producers involved in Chlorella cultivation and the largest company is Taiwan Chlorella Manufacturing and Co. (Taipei, Taiwan); they turn around 400 tons of biomass per year. The German company Klotz produces about 130–150 tons of biomass per year in a tubular photobioreactor condition. *Chlorella* sp. production exceeds US$38 billion in a year, because of its several health benefits (Barrow and Shahidie 2007). In humans, the intake of Chlorella extract increases hemoglobin concentration, lowers the blood sugar levels, and helps during malnutrition and ethionine intoxication. Chlorella extracts have β-1,3-glucan, which acts as an immunostimulator and a free radical scavenger and also helps to reduce blood cholesterol. Variety of food items were prepared from Chlorella such as intake of plankton soup for leprosy patients helps to increasing the weight, energy, and health. Japanese researchers have developed different food items such as powdered green tea, soups, noodles, bread and rolls, cookies, ice cream, and soy sauce from *Chlorella ellipsoidea*.

12.2.7 Microalgae: A Rich Source of Vitamins

Microalgae are natural rich sources of different types of vitamins including A, C, E, B1, B2, and B12. *Haslea ostrearia* produced significant amounts of vitamin E (tocopherols) and *P. cruentum* yielded high quantities of vitamin A (β-carotene), vitamin E, and vitamin C (Mus et al. 2013). *Dunalliela salina* is a well-known organism, which contains many compounds in its extract including vitamin, A vitamin E, pyridoxine, riboflavin, nicotinic acid, biotin, and thiamine (Hosseini Tafreshi and Shariati 2009). The cyanobacteria, *Spirulina* sp. contains vitamins A, B1, B2, and B12, which contribute to a significant amount of vitamin production for many industrial applications.

12.2.8 Potential Antioxidant Compounds in Therapeutic Applications

Microalgae contain different compounds such as carotenoids, vitamins, flavonoids, and tocopherols, which have high antioxidant properties that help to prevent hazardous effects of free radicals. Antioxidants from microalgae prevent the cells from oxidative damage. Reactive oxygen species (ROS) and nitrogen reactive species (NOS) attack the biomolecules like DNA, proteins, and lipids and cause several diseases including Alzheimer disease, coronary arteries disease, diabetes, cancer, obesity, and stroke (Ngo et al. 2008). Antioxidants are produced from both natural and synthetic sources, and the use of synthetic chemicals causes several side effects when compared to natural antioxidant compounds (Pena-Ramos and Xiong 2001). Natural antioxidant compounds are abundantly found in medicinal plants, and also

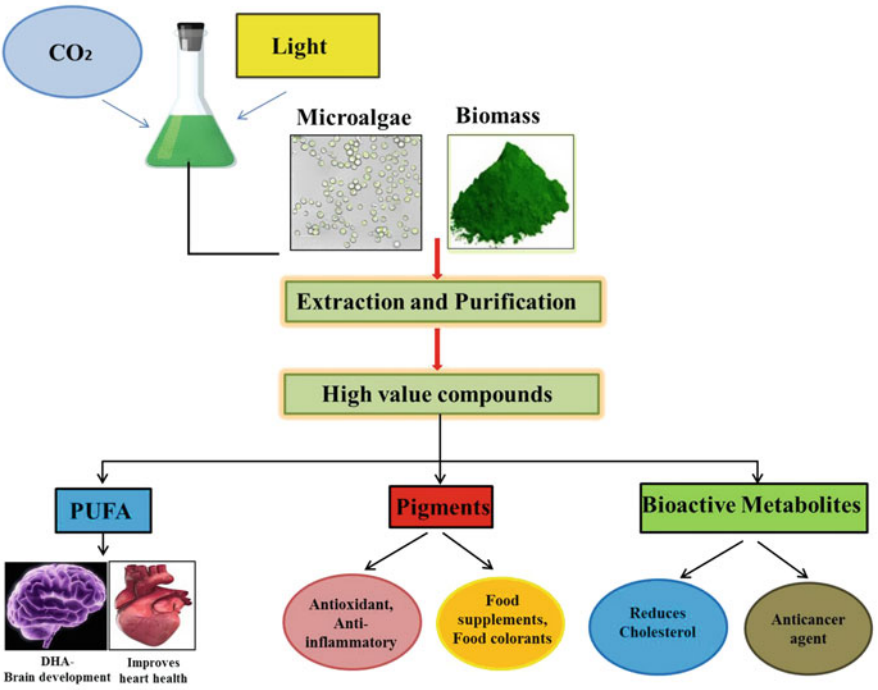

Fig. 12.2 Multiple health benefits of microalgal-derived metabolites

microalgae are one of the rich and potential sources to produce biologically active antioxidant compounds, which are more economic and have strong antioxidant effects (Cornish and Garbary 2010). Most of the pigment derivatives of microalgae, such as chlorophyll A, fucoxanthin, β-carotene, and phycoerythrin, have antioxidant properties. Microalga *Undaria pinnatifida* produces relatively high amounts of fucoxanthin, and its derivatives of auroxanthin have strong free radical scavenging activity (Sachindra et al. 2007). Fucoxanthin contains two hydroxyl groups in a ring structure, which are considered as active moieties for free radical scavenging. Fucoxanthin has higher antioxidant effects than β-carotene. The pigment compound phycobiliprotein is commercially used in many nutraceutical industries as a natural dye agent, and phycobiliproteins isolated from different microalgal species are shown to possess antioxidant properties. Fucoxanthin was extracted from many microalgal species that have a significant role in cancer treatment. Fucoxanthin inhibits the human blood cell proliferation and tube formation of HUVECs (human umbilical vein endothelial cells). Fucoxanthin helps to enhance the production of arachidonic acid and docosahexaenoic acid content, which prevent diabetes. The schematic representation of microalgal-derived metabolites and their multiple health benefits are given in Fig. 12.2.

12.2.9 Antimicrobial Compounds from Microalgae

Microalgae and their products act as effective anti-microbial agents in the crude or purified form. The first microalgal antibacterial compound was extracted and identified from the green microalga *Chlorella* sp., which helps to inhibit the growth of both gram-positive and gram-negative bacteria (Washida et al. 2006). The antifungal compounds like okadaic acid and ciguatoxin were identified from microalgae *Prorocentrum lima* and *Gambierdiscus toxicus*, which showed high antifungal activities. Furthermore, the antimycotic compound and its activity was reported in dinoflagellate *Amphidinium.* The lipid metabolites from *Chaetoceros lauderi* prevent the growth of several bacterial strains. *Microcystis* (*M. aeruginosa*) possesses several toxic metabolites in its crude extract, which showed strong antifungal and antibacterial activities (Khalid et al. 2010). Green microalga *Dunaliella salina* showed antibacterial activity against several organisms including *Staphylococcus aureus* (*S. aureus*), *Aspergillus niger*, *Escherichia coli*, *Candida albicans*, *Psuedomonas aeruginosa.* The crude extracts and multiple substances found in the *Dunaliella* sp. supported the growth inhibition of *Klebsiella pneumonia* and showed antibacterial activity against *S. aureus*.

12.3 Future Perspectives

The sustainability of value-added products derived from microalgae depends on sustainable and environmentally friendly technologies. Microalgal biotechnology is still in its infancy, since many products from microalgae have not yet reached their commercial application. It is considered to become a multibillion industry in the upcoming years, as it is ecologically safe to produce value-added products. The boon in microalgal biotechnology will lead to the development of high-value bioactive molecules, bio polymers, bio plastics, cosmetics, paints, and cosmetics. Due to their bioactive potential, microalgal-based products provide tremendous openings in the worldwide market. This will reduce the usage of toxic chemicals and provide green economy for the society. Execution of economically feasible processes coupled with industrial scale up is important for the commercial production and to reduce the capital and operating cost of microalgal-based products. For effective extraction of bioactive compounds from microalgae, large amount of biomass is a prerequisite. The research should focus on improving this concern to attain higher biomass. Furthermore, extraction and purification strategies need to be improved for efficient product output. In addition, bioprospecting is important to identify the preferred microalgal strains with higher growth rate and to obtain high product yield at low operating cost. This continued development of microalgal-derived products will lead to high market demands and develop new openings in worldwide market. Based on the literature study, much emphasis has been placed on obtaining biodiesel form microalgae over the past few decades. The exploration of microalgae is limited to other applications, particularly pharmaceutical, nutraceutical, and cosmetics. Therefore, research regarding the development of microalgal-derived products needs to be

carried out to prove the therapeutic potential of microalgal-derived bioactive compounds.

12.4 Conclusion

There is ample evidence to confirm the key roles of bioactive compounds produced by microalgae in various biotechnology applications, especially in the field of nutraceuticals and pharmaceuticals. The microalgal-derived metabolites stimulate immune system and help in the prevention of various diseases, which assists in their utilization in various foods and pharmacological and medical applications. Only few species have been used in microalgal biotechnology so far and several species need to be explored for new metabolites. Apparently, there is a need for further study of the metabolites/bioactive compounds and their activities in the in vivo model to prove their therapeutic potential. In conclusion, the application of microalgal-derived compounds is still in its infancy in the nutraceutical and pharmaceutical industries, and further developments will be required to commercialize these products in future.

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Synthetic Biology Tools for Microalgae

13

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Abstract

Microalgae can provide a platform technology to synthetic biologists for the production of pharmaceutical biocommodities. Based on their diversity, the main pathways for a range of plant-derived compounds are innately present across the multiple phyla of microalgae. Current efforts focus primarily on overexpression of genes involved with lipid biosynthesis but the doors are opening for a whole range of therapeutics, including pigments, terpenes, recombinant proteins, and RNAi products. A variety of open source tools are expanding into the academic space for a whole range of molecular tasks. This chapter focusses on heterologous photoautotrophic production of pharmaceuticals in microalgae.

Keywords

Synthetic molecules · CRISPR/Cas system · Markers in microalgae · Marine biotechnology

13.1 Introduction

Massive scale farming projects are a necessity for our everyday lives, and it is through this industry that we obtain all the necessary resources to support human life on the planet. A constant challenge that stands in the way of this, however, are the geographical requirements to support an ever-growing worldwide population. As

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the biotech industry and world population grow, there is a greater demand for bioproducts that also compete with large areas of arable land, for example, biofuels.

Microalgae provide a new outlook to produce bioproducts, which can be farmed at large scale on non-arable land, with high yields and in almost any type of water. Recent advances in molecular biology are seeing some microalgae develop into platforms similar to that of yeasts, like *Pichia pastoris* and *Saccharomyces cerevisiae*, but with photoautotrophic machinery. Microbes grow faster than regular crops and lend themselves to automation, the entire chain of production can be automated, and it can be envisaged that very little intervention is needed in the future once an operation is running and regularly checked. The microalgal molecular biology world is dominated by seven main species from a variety of phyla, including genera *Chlamydomonas*, *Chlorella*, *Dunaniella*, and *Haematococcus* from Chlorophyta as well as *Nannochloropsis*, *Phaeodactylum*, and *Thalassiosira* from the stramenopiles (heterokonts), alveolates, and rhizaria (SAR) supergroup. There are additional examples, but these genera represent a large proportion of the current body of knowledge and the latest techniques.

Two of the major traits attracting researchers and industry toward synthetic biology in microalgae is the pre-existing photosynthetic machinery, enabling photoautotrophic production at the microbial scale and with this come many of the pathways associated with or derived from tools in the molecular machinery that make up many unique plant systems. For instance, terpene production systems for phytol and pigments are not present in most yeasts and when they are present, they are usually specific to a few compounds, for example, in *Phaffia rhodozyma*. Microalgae and plants tend to produce many different carotenoids/xanthophylls for accessory antenna pigments, nonphotochemical chlorophyll fluorescence quenching (NPQ), etc., and have large sets of genes devoted to these purposes. The intrinsic production of these compounds means preoptimized systems for production already exist and need not be recreated in yeast (Galanie et al. 2015).

Tools to enable synthetic biology in microalgae, especially *Nannochloropsis oceanica*, already exist in their infancy; however, there is still a plethora of work to be done. Genomes of *Nannochloropsis* sp. are non-complete and require polishing to find more useful tools and expression strategies. Very few endogenous inducible and constitutive promoter terminator systems have been elucidated and standard parts-based construction systems, like golden gate assembly (MoClo), have not been introduced. A full set of molecular tools will enable a new era of synthetic biology in microbial photoautotrophs, potentially change the global chemical supply chain, and enable food security in the future.

Figure 13.1 provides an overview of the three main methods for transforming foreign DNA into microalgae: biolistic, conjugation, and electroporation. Table 13.1 shows examples of how CRISPR-associated systems have been used in microalgae. The following paragraphs highlight the various approaches and strategies that can be used to enable microalgae as powerful metabolic engineering biofactories.

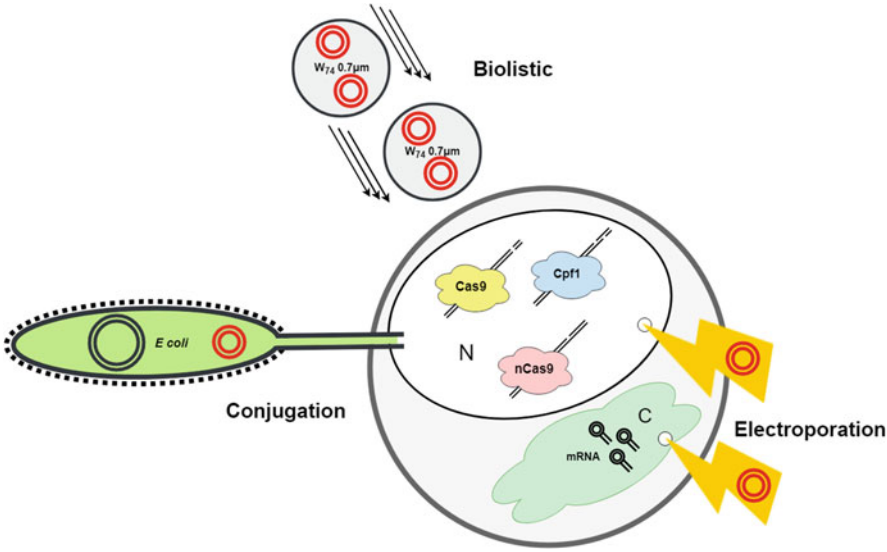

Fig. 13.1 Demonstrates the three methods for transforming foreign DNA into microalgae: biolistic, conjugation, and electroporation. Plasmid DNA is represented by red double line circles, *N* identifies the nucleus, and *C* identifies the chloroplast. Site directed nuclease activity can be seen in the nucleus where cut patterns for each nuclease are demonstrated. Finally, micro RNA production is displayed in the chloroplast

13.2 Synthetic Biology Toolkit to Metabolically Engineer Microalgae

13.2.1 Signal Peptides

The ability to predetermine the localization of enzymes is key to synthetic biology in eukaryota to rationally assign synthesis pathways the ability to direct enzymes to various organelles and cellular spaces. A good example of localization signal exploitation is Cas9; the bacterial origin of the enzyme does not require Cas9 to be transported to any nucleus. Earlier attempts at gene editing with Cas9 without localization signaling to the nucleus displayed very low levels of transformation efficiency (Jiang et al. 2014) In order to drive transformation efficiency up, a nuclear localization signal (NLS) is employed to direct the enzyme to the nucleus for it to function as an endonuclease and have access to gDNA (Jakočiūnas et al. 2015). As there are no NLS elucidated in microalgae, a molecular biology favorite, the SV40 NLS from simian virus, is often used. The SV40 NLS can be applied to both, the C and N terminals, of a protein and in some cases an NLS is included on both ends to enhance the efficiency of nuclear import (Cong et al. 2013; Nymark et al. 2016) A bioinformatics pipeline has been established for predicting localization signal peptides in heterokont algae, HECTAR, and has been used extensively in

Table 13.1 Examples of CRISPR-associated systems in microalgae, includes all current examples

Species	Transformation method	Transformation package	Codon optimized	NLS	Modification type	Selection marker	Targets	Reference
Chlamydomonas reinhardtii	Electroporation	Plasmid	Y	N	Indel	Endogenous resistance to rapamycin on successful modification of target	FKB12	Jiang et al. (2014)
	Electroporation	RNP + Linearized plasmid as donor	N/A	N	Indel + NHEJ	Phenotype/ fluorescence	CpFTSY and ZEP	Baek et al. (2016)
	Electroporation	RNP	N/A	Y	Indel	Tryptophan auxotrophic selection on successful transform or phenotype	MAA7, CpSRP43, ChlM	Shin et al. (2016)
	Electroporation	Plasmid	Y	N	Indel + HDR	Mutate ALS gene for sulfometuron methyl resistance + ARG7 auxotroph repair	ARG7, ALS, FKB12	Jiang and Weeks (2017)
	Electroporation	Plasmid	Y	Y	Indel + HDR	Hygromycin B	*COP1/2, COP3, COP4, COP5, PHOT, UVR8,*	Greiner et al. (2017)

							VGCC, MAT3, aCRY	
Phaeodactylum tricornutum	Bombardment	Plasmid + Selection plasmid	Y	Y	Indel	Zeocin	CpSRP54	Nymark et al. (2016)
	Bioconjugation	Episomal plasmid	Y	Y	Indel	Zeocin	Urease	Slattery et al. (2018)
	Bombardment	Plasmid	Y	Y	Indel	Zeocin	PHO4, VTC2	Stukenberg et al. (2018)
	Bioconjugation	Episomal plasmid	Y	Y	Indel	Zeocin	Myb	Sharma et al. (2018)
	Bombardment	RNP	N/A	?	Indel	Endogenous resistance/ auxotroph generation	PtUMPS, PtAPT, PtAureo1a	Serif et al. (2018)
Thalassiosira pseudonana	Bombardment	Plasmid	Human	Y	Indel	Nourseothricin	Urease	Hopes et al. (2016)
Nannochloropsis oceanica	Electroporation	Plasmid	Y	Y	Indel	Hygromycin	NR	Wang et al. (2016)
	Electroporation	Episomal plasmid	Y	Y	Indel	Hygromycin	NR	Poliner et al. (2018)
Nannocloropsis gaditana	Electroporation	Editor strain + sgRNA + cassette	Y	Y	NHEJ	Blasticidin and hygromycin	18 transcription factors	Ajjawi et al. (2017)
	Electroporation	Editor strain + sgRNA + floxed cassette	Y	Y	NHEJ + Lox scar	Blasticidin, hygromycin, zeocin	Many	Verruto et al. (2018)

RNP ribonucleoprotein, *NHEJ* non-homologous end joining, *HDR* homology directed repair, *Indel* insertion or deletion, *NLS* nuclear localization signal

Nannochloropis for the determination of localization signals. Several localization signals have been elucidated in various *Nannochloropsis* for targets such as endoplasmic reticulum, mitochondrion, periplastidal compartment, and the stroma.

13.3 Auxotrophs, Knockout Mutants, and Endogenous Markers

Many microalgae are auxotrophic for vitamins, particularly B group vitamins (Croft et al. 2005, 2006); this is believed to be part of a coevolution and symbiosis with bacteria associated with microalgae. Based on available genomes of algae there is a clear division of the species within the kingdom; around half are able to produce methionine without the usual B12 cofactor, akin to fungi and land plants utilizing a B12-independent methionine synthase (METE). The other half of the kingdom are auxotrophic for vitamin B12 and require supplementation. B12 autotrophy seemingly correlates with the presence of intact copies of METE; both *Volvox carteri* and *Gonium pectorale* are dependent on B12 and have pseudo copies of METE (Helliwell et al. 2011). *Nannochloropsis* sp. contains two separate genes for methionine production: a B12-dependent METH gene and a METE B12 independent gene. It is likely that the metabolic preference is for the cofactor-based METH gene and METE is present only as a backup for vitamin-devoid scenarios (Jinkerson et al. 2013). In *Chlamydomonas reinhardtii* and *P. tricornutum*, both METH and METE genes are present; in culture conditions where B12 is present, METE transcription is repressed, which Helliwell et al. (2014) infer to be a mechanism for METE gene loss. The METE promoter has been used as a riboswitch-controlling gene expression in the absence of B12 in *C. reinhardtii* (Ramundo and Rochaix 2015). No work has been conducted to create mutant auxotrophic strains by repression or knocking out of the METE gene. A loss of function mutant would be useful for a positive selection as no antibiotic resistance gene is required because the -METE strain can grow on +B12 media. Transformations, including repair of function to METE, would create a strain selectable via growth on -B12 media.

One of the few examples of utilizing a knockout mutant has been to modify the chloroplast genome without need for a resistance marker. In this scenario, Charoonnart et al. (2019) use a loss of function psbH mutant of *C. reinhardtii* that cannot photosynthesize and requires supplementation with acetate to survive. The psbH mutant has a streptomycin resistance marker in place of psbH and its function is restored by transformation with a cassette including the gene of interest (GOI) package and psbH. Transformants with restored psbH function and containing GOI are selected for by growth on media devoid of acetate and streptomycin; as such, persistent use of antibiotic selection is not required for maintaining the transformation.

13.4 Episomal/Extra-chromosomal Replicating Vector

A long-term strategy for hosting large pathways in microalgae is the use of extra-chromosomal vectors; this is a plasmid harboring a centromere from yeast and an autonomous replication sequence that also allows for replication in heterokonta. This technique was first applied in diatoms to *P. tricornutum*. In order to maintain the episome, a constant positive selection is required in the host alga; this is achieved with an antibiotic resistance gene and for maintenance in histidine-auxotrophic yeast, HIS3 is included (Karas et al. 2015). A similar system has also been developed for *Nannochloropsis oceanica*, employing a similar CEN ARS fusion from *S. cerevisiae* and a resistance marker for maintenance in *Escherichia coli* and *N. oceanica* (Poliner et al. 2018). In this system, the episome houses a Cas9-nlux fusion and an sgRNA scaffold. As a proof of concept, Poliner et al. (2018) used the system to knock out native nitrate reductase (NR), which allows for negative selection on NH_4^+ media. Once the Cas9/sgRNA has performed the editing task, the episome can be cured from the newly transformed line by growth on NH_4^+ medium without antibiotic selection agent hygromycin. Transformants that have been cured of their episomes will no longer have luminescence from Nlux or confer hygromycin resistance. Episomal transformation systems provide a solid base for further work with multiplex gene editing and will, no doubt, be employed in the future for marker-less stacking of knock-ins and knock-outs in heterokonts.

13.5 Cas9 and Beyond

Key to understanding the toolbox nature of the CRISPR/cas system lies within the domain architecture of the Cas9 enzyme (Chen et al. 2017). The homing activity of Cas9 is granted by the recognition domains REC and a C-terminal domain (CTD). Targeting of a DNA sequence occurs when an sgRNA binds to the REC domain that undergoes a conformational change to form a Cas9-sgRNA complex (Ribeiro et al. 2018). This allows the CTD domain to begin searching DNA strands that contain a protospacer adjacent motif (PAM) site. If a PAM is found, then REC will try to hybridize the sgRNA with complementary nucleotides to the 3′ end of PAM. If complementarity is perfect then the nuclease domain, HNH, cleaves DNA at the target strand and RuvC cleaves the non-target strand at the complementary base (Chen et al. 2014; Jinek et al. 2012). In the ideal scenario, this results in a double-stranded break (DSB) with two blunt ends and the eradication of a base (Jinek et al. 2012). This basic Cas9 engineering strategy has been demonstrated feasible in *C. reinhardtii* (Baek et al. 2016; Greiner et al. 2017; Jiang et al. 2014; Shin et al. 2016) and in several heterokonts, diatoms *P. tricornutum* (Nymark et al. 2016) and *T. pseudonana* (Hopes et al. 2016), and two eustigmatophytes from the same genus, *N. oceanica* (Poliner et al. 2018; Wang et al. 2016) and *N. gaditana* (Ajjawi et al. 2017; Verruto et al. 2018).

13.6 *Chlamydomonas reinhardtii*

Jiang et al. (2014) first demonstrated the vector-based expression of codon-optimized Cas9 and sgRNAs in microalgae on the model platform, *C. reinhardtii*. The system did not employ a true selection marker gene and instead targeted FKB12, which, if mutated correctly, would confer resistance to rapamycin. Very low efficiency was reported where only one transformant with rapamycin resistance was generated after 16 independent rounds of transformation via electroporation with $>10^9$ cells. This study also revealed a potential toxicity of Cas9 protein when constitutively expressed. In addition, the shortcomings of this experiment was very likely due to the absence of an NLS explained above. Shin et al. (2016) and Baek et al. (2016) both proposed a solution to Cas9 toxicity from constitutive vector-based expression in the same month. Both systems employed electroporation-based transformation with premade Cas9 ribonucleoproteins (RNP) and synthetic sgRNAs. Baek et al. (2016) targeted photosynthesis components CpFTSY and ZEP that were identifiable through a reduced chlorophyll phenotype. Streaked plates of transformants were grown out and visually assayed to identify transformants of the visually identifiable CpFTSY mutant, and fluorescence imaging of chlorophyll was used to find ZEP mutants with overly dramatic quenching. No information was given as to the stability of these transformants and if they reverted with no selection pressure. The strategy proposed by Shin et al. (2016) also involved screening without the use of selection markers by phenotype alone, but also offered some additional selection strategies. Firstly, the authors were able to knock-in a cassette into the DSB target site via non-homologous end joining (NHEJ). By co-transforming *C. reinhardtii* with a linearized plasmid, DSBs were able to be repaired utilizing the linearized plasmid as a donor sequence, which had no potential flanking homology to the break site. Secondly, Shin et al. (2016) were able to demonstrate that for transformants with no supplied donor fragment, DSBs were always repaired with in-frame indels of three base multiples. Thirdly, by employing an NLS on the C-terminus of the Cas9 protein, a more efficient transformation rate was achieved.

Pioneering authors Jiang et al. (2014) proposed an entirely new system, utilizing a gene within a gene to express both Cas9 and sgRNA concurrently. Jiang and Weeks (2017) encoded the sgRNA scaffold within an intron in the Cas9 cassette with no NLS, and the sgRNA matures when the intron is removed from mRNA. The new sgRNA scaffold sees increased successful mutations in transformants, increasing efficiency from 1 positive transformant out of 16 independent rounds of transformation in Jiang et al.'s (2014) original work to 13 positive transformants in four rounds of transformation when FKB12 was targeted with the new hybrid construct. To further confirm effectiveness of the hybrid system, function was restored to an argininosuccinate lyase ARG7-auxotrophic strain of *C. reinhardtii*. The repair was facilitated by homologous recombination, whereby the plasmid containing the hybrid expression system was co-transformed with a phosphorylated ssDNA donor fragment with DSB flanking homology and wild-type copy of ARG7. Finally, the gene acetolactate synthase (ALS) was targeted to cause a single-base A-C

mutation that confers resistance to herbicide, sulfometuron methyl, by once again providing a short ssDNA with flanking homology and the desired point mutation. For the last example in *C. reinhardtii*, Greiner et al. (2017) deployed a Cas9 from *Staphylococcus aureus*, with a different PAM recognition, NNGRRT, compared to the standard *Streptococcus pyogenes* Cas9 NGG site. Direct comparison of transformation efficiency between SpCas9 and SaCas9 indicate that the mesophilic *S. aureus* Cas9 performs significantly better when coupled with a tailored cell recovery system (16% vs 3%).

13.7 Diatoms

Extensive work with the Cas9 system in diatoms has further increased the understanding of efficient expression of Cas9 in algae. Nymark et al. (2016) were the first to report a successful system deployed in *P. tricornutum* where a codon-optimized NLS-fused Cas9 construct was housed in a plasmid along with sgRNA scaffold. This time, the authors also included a positive selection marker housed within an additional plasmid. Both Cas9/sgRNA plasmid and selection marker plasmid were linearized and co-transformed on tungsten beads. The issue with a system like this is that only 30–40% of transformants will only contain the selection marker plasmid (Falciatore et al. 1999); however, this likely resulted from size restrictions when delivering whole plasmids biolisticly. During biolistic transformation, there is a physical limit to the size of DNA that can be fired from the gene gun into a cell of a given size. Shearing may occur during particle acceleration or impact (Krysiak et al. 1999a, b; Twyman and Christou 2004). The next advancement saw Slattery et al. (2018) utilize the pKSdiaCas9_sgRNA plasmid from Nymark et al. (2016). The authors assembled a new plasmid from pKSdiaCas9_sgRNA for episomal hosting with CEN6-ARSH4-HIS3 centromere and autonomous replication region, ShBle for zeocin resistance, and oriT for maintenance and a bioconjugation-based transformation. The authors also created an additional episome from this new episome with an I-TevI homing endonuclease fused to the N-terminus of Cas9, called TevCas9. With dual nuclease sites, the fusion protein can cleave an additional PAM site 5′-CNNNG-3′. The addition of this extra nuclease should enable 33–36 bp deletions, as this is the usual span between target PAM sites of both nucleases (Wolfs et al. 2016), However, both Cas9 and TevCas9 had almost identical editing efficiency (~60%) and only one transformant was detected with a large deletion between PAM sites. The addition of selection markers and autonomous replication ability greatly increased the overall efficiency of the system and transformants were able to be cured of their episomes. Interestingly, transformants were demonstrated to be stable for at least nine generations.

Also utilizing Cas9 form pKSdiaCas9_sgRNA, Stukenberg et al. (2018) created a vector with a resistance gene, ble, conferring resistance to zeocin. Unlike the previously described system, no autonomous replication features were added, meaning the plasmid would fade without selection. To negate any potential cytotoxic effects of Cas9, the expression of the enzyme was tied to the endogenous nitrate

reductase promoter and terminator. Although the promoter is leaky, expression remains attenuated enough to avoid Cas9 toxicity. Transformation is achieved biolisticly in this system with the entire expression construct on one linearized plasmid. Despite the size of the plasmid, efficient transformation was still achieved.

Sharma et al. (2018) from the same research group as Nymark et al. constructed their own episomal expression system using pPtPuc3 (Karas et al. 2015) and pKSdiaCas9_sgRNA, named PtPuc3m diaCas9_sgRNA for bioconjugation into *P. tricornutum*. Sharma et al. (2018) performed a direct comparison to the Nymark et al. (2016) system and found that the frequency of transformants expressing the desired modification was lower in general for bioconjugation compared to bombardment, 33–50% vs 25–33%, respectively. The total number of transformants with resistance to the selection marker zeocin was greater than tenfold.

Finally, Serif et al. (2018) present a DNA-free system for *P. tricornutum* transformation where Cas9-sgRNA complexes are directly bombarded with high editing efficiency. Authors were able to select mutants with targeted modification in a gene with no phenotype by simultaneously targeting a gene for knock-out, such as PtUMPS, which is responsible for native uridine synthesis, and PtAPT, which is responsible for adenine scavenging, which causes an autotrophy. Of the selected dual gene transformants, 19/29 contained the two desired modifications and of these, 10/29 were biallelic; additionally this method has an overall transformation efficiency of 10^{-6}.

Apart from *P. tricornutum*, the other diatom that receives some molecular attention is *Thalassiosira pseudonana*. To our knowledge, there has only been a single strategy devised for this species by Hopes et al. (2016). In this case, a plasmid-based biolistic transformation was employed and the designed vector contained two sgRNA sites, human codon-optimized Cas9 and a selection marker for nourseothricin. Employing two sgRNAs increases target site specificity, and precise control of the deletion length is possible as the two blunt ends from both cleavage sites repaired via NHEJ with the small fragment between sites was deleted (Garst et al. 2016).

13.8 *Nannochloropsis* sp.

The first published use of Cas9 in *Nannochloropsis* sp. was reported by Wang et al. (2016), using an electroporated plasmid with *Nannochloropsis oceanica*. The plasmid was designed with a *N. oceanica* IMET1 codon-optimized Cas9 fused to an NLS, sgRNA scaffold, and a hygromycin resistance gene, driven only by endogenous promoters and terminators. NR was chosen as the target gene for modification, as disruption enabled a dual selection mechanism via growth on NH_4Cl and hygromycin. A total of 300 mutant colonies were picked from selection media and of these, only two were identified with correct mutations.

The next development was by Ajjawi et al. (2017) in *Nannochloropsis gaditana* and involved the generation of a custom strain expressing codon-optimized Cas9 and blasticidin deaminase (BSD) as well as a non-optimized turbo green fluorescent

protein (GFP). All three genes were driven by endogenous promoters and terminators and combined with Gibson assembly into a minimal backbone pUC-vector then transformed via electroporation. The Ng-Cas + editor line was then selected for by growth on blasticidin+ solid media. In order to perform a gene disruption, synthetic sgRNA was synthesized in vitro and then co-transformed with a PCR-amplified expression cassette containing hygromycin resistance. Transformants were then switched to hygromycin media and in resistant colonies the sgRNA target site was cleaved and repaired by NHEJ using the expression cassette as the donor fragment. By utilizing an editor strain already expressing Cas9, the authors demonstrated that the efficiency of transformation with sgRNA and donor fragment was exceedingly high. Almost all colonies that grew on secondary hygromycin plates had the intended NHEJ-facilitated insertion.

Poliner et al. (2018) then demonstrated an improved transformation efficiency system in *N. oceanica* by utilizing an episomal Cas9 and sgRNA expression with the ability to remove the episome after completion of the desired modification. Like the work by Slattery et al. (2018) and Sharma et al. (2018) in *P. tricornutum*, a vector was constructed with the *S. cerevisiae* CEN/ARS6 centromere and automatic replication sequence to enable hosting in *N. oceanica*. A reporter gene, nanoluciferase NLUX, was fused to Cas9 and flanked by C- and N-terminal NLS. Expression was driven with an endogenous bidirectional promoter, RIBI. Transcription of sgRNA scaffold was driven by the same RIBI promoter and was flanked by hammerhead and hepatitis delta virus self-cleaving ribozymes to enable transcription of sgRNAs by protein expressing promoters. The efficiency of Poliner et al. (2018)'s system like others is largely dependent on the sgRNA design. When primed with well-designed sgRNA, no wild-type sequences were detected at the target site of screened transformants.

Members of the same lab group as Ajjawi et al. (2017) published a newer system involving reusable selection markers facilitated by CRE/LOX recombinase (Verruto et al. 2018), theoretically enabling unlimited stacking of modifications. The initial random integration plasmid was built much the same as described in the initial publication by Ajjawi et al. (2017) with the key inclusion of Cre recombinase driven by endogenous NR promoter. The Cre cassette required an intron inserted to avoid Cre recombinase activity during plasmid replication in *E. coli*. This plasmid was termed pSlice'n'excice and was used to generate an editor line of *N. gaditana*, named $NgCas9^{+}Cre^{+}$. To obtain disruptions in desired genes, a lox-flanked ("floxed") cassette with either hygromycin or zeocin resistance markers and a turboGFP reporter is PCR-amplified and transformed with sgRNAs for the desired target. Mutants are screened for with respective selection markers, and upon PCR confirmation of cassette insertion at target site and disruption, colonies are transferred to Cre induction media ($+NH_4Cl$). The Cre recombinase induction causes the floxed cassette to be removed and left with a lox scar site still disrupting the target. Unlimited stacking can be achieved by repeating this process with different targets. The authors do not provide a maximum for the number of stackable disruptions.

13.9 Extensions

By manipulating the nuclease domains of Cas9, tools with new abilities have been unlocked, for instance by mutating the D10A residue in the RuvC domain, Cas9 loses the ability to cut the non-target strand (Jinek et al. 2012). RuvC-deficient Cas9 is referred to as nCas9 or a nickase, due to only removing a single base target strand. If two sgRNAs are utilized and two PAM sites are present within close proximity (<20 bp on opposite strand) and a nickase is used, then the two single nicks will cause an instability in the DNA strand causing a single break with long sticky overhangs. If a donor fragment with homology to both sides of the break is supplied, then repair will be facilitated by homologous recombination; this enables high target specificity and even lower off target specificity. Unfortunately, nickases have not been demonstrated to date in microalgae, however CRISPR interference has been demonstrated in *C. reinhardtii*. By mutating the H840A residue of the HNH nuclease domain along with the D10A mutation in RuvC, Cas9 becomes completely nuclease-deficient and is known as deadCas9 or dCas9. Upon binding to DNA, dCas9 becomes a steric hindrance to polymerase and can stop expression if targeted mid gene or decrease expression if targeted to the promoter region. In *C. reinhardtii*, a dual vector approach is taken with dCas9 expressed from one vector along with hygromycin resistance and the sgRNA scaffold from the other along with paromomycin resistance. Both plasmids are co-transformed and then selected by growing out on media with hygromycin and paromomycin. Using this strategy, it was possible to repress the activity of rfp transgene consistently by ~90% for at least seven generations. By incorporating polymerase recruiting signals with dCas9, it is also possible to upregulate gene expression; this is referred to as CRISPR activation and has not been reported in microalgae to this date.

Additionally, Cas9 alternatives have been elucidated and even tested in algae, recently cpf1 or cas12a has entered the spotlight as a potentially more accurate homing nuclease. Cpf1 is a smaller enzyme and requires a smaller guide RNA to that of Cas9, it requires a T-rich TTTN PAM site, and cuts ~18 bases 3′ of the PAM. Ferenczi et al. (2017) demonstrated the use of cpf1 in *Chlamydomonas* using recombinant cpf1 RNPs and an ssDNA donor fragment for HDR repair. Because of the sticky overhang cutting nature of Cpf1, HDR is suited for repair of brakes, meaning that with only 1 sgRNA it is possible to knock-in an entire fragment at the break site.

13.10 RNAi Technology

Production of RNAi molecules for therapeutic applications in microalgae is still in its infancy and to date examples are mainly for use in crustaceans as antiviral agents (Charoonnart et al. 2019; Saksmerprome et al. 2009). Conceptually, microalgae are to be used as a production system for dsRNA, which in turn are applied as a feed to an at-risk population that would normally feed on microalgae in the hope that the dsRNA will mature into RNAi constructs and silence the pathogen. Issues arise,

however, when RNAi machinery is present at the sight of production and the dsRNA is prematurely processed by a ribonuclease III (Bally et al. 2018). One way dsRNA degradation can be avoided is by expression in the chloroplast that is devoid of RNAi machinery, such as Dicer-like RNase III-like endonucleases and Argonaute family proteins. The lack of these core RNAi enzymes allows dsRNA to accumulate at high levels in the plastid.

Attempts to produce RNAi to silence yellow head virus (YHV) in shrimp in *C. reinhardtii* have had mixed success rates. The strategy employed an RNAi targeting the RNA-dependent RNA polymerase gene of YHV (Saksmerprome et al. 2009) and has been transformed into both the nucleus and the chloroplast genome of *C. reinhardtii*. Transient nuclear expression showed only mild improvement over positive control shrimp and shrimp fed on wild type alga (22% vs 5%), likely attributable to degradation of dsRNA by endogenous nuclear and cytosol RNAi enzymes (Somchai et al. 2016). More recently, Charoonnart et al. (2019) expressed the same dsRNA cassette in the chloroplast genome of *C. reinhardtii* and the survival rate of treated shrimp increased to 50%. This marked performance gain between the two transformation systems is attributed to the state of dsRNA degradation and the RNAi enzyme lacking chloroplast, providing a hospitable environment for dsRNA to accumulate.

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Part III

Marine Fauna and Their Role in Medical Science

Malacology and Pharmacology: An Integrated Approach with Special Emphasis on Marine Realm

14

Devanshi Joshi and P. C. Mankodi

Abstract

Marine environment has tremendous potential to meet the demands of pharmacological industries rendering large array of bio-active compounds. This chapter deals with the role of molluscs in amelioration of various physical impairments and disorders. The phylum mollusca is the second largest invertebrate phylum, comprising 80,000 existing species. Their extraordinary adaptive radiation has not only rendered the phylum with taxonomic diversity but has brought forth considerable morphological, ecological, physiological and behavioural variation. This implies their extensive physiological plasticity and hence potential biochemical productivity. This truth is conjectured to be well excogitated by our ancestors and reflected through the vogues and rituals involving large to small molluscan shell usage. Moreover, presence of various mollusc shells is deep rooted in many healing practices like Ayurveda. As per the recent discoveries, the phylum mollusca indeed serves as a treasure of bio-active compounds such as anticancer, antimicrobial, antifungal, anti-inflammatory and analgesic compounds. Furthermore, molluscs have also proven a potential source of remedies to cure cardiovascular diseases producing compounds like glycosaminoglycan. Hence, the phylum seizes essential attention of pharmacologists, malcologists and conservationists, too.

Keywords

Analgesic compounds · Anticancer · Antifungal · Anti-inflammatory · Antimicrobial · Molluscs

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14.1 Introduction

Marine molluscs have adequate potential to enrich pharmacopoeia by availing a number of remedies to cure a vast array of disorders. Hence, it is required to explore them carefully in order to decipher their ecological and biological role and thereby the bio-active molecules responsible for those functions.

The molluscs are successful survivors of a number of ecological niches. The excellent adaptive radiation achieved by molluscs has enriched the animal kingdom with enormous diversity. Molluscs hold the position of phylum in the taxonomic categorisation, comprising six different classes. It is second to the largest phylum on earth following arthropods. The diversity of the Molluscs gets reflected in a variegated manner, that is, the sluggish slugs to the agile octopus, the brilliantly ablaze nudibranchs to the dusty dull cephalopods, the butter soft slugs to the rock hard conches, pearl producing Pearl-oysters to ink releasing octopus and sepia, the tiniest snail to the giant clams, mountain dwelling gastropods to the benthic bivalves, the gentle grazers to the brutal hunters, all have been classified under a common roof – the Mollusca. Besides morphological and behavioural diversities, they exhibit a vast variety of ecological functioning and niche occupation. All the varieties together constitute about 100,000 species which have been recorded and described; moreover, potentially 100,000 species are yet to be described (Strong et al. 2008). The phylum is divided into six classes, that is, (i) Monoplacophora, (ii) Amphineura, (iii) Scaphopoda, (iv) Gastropoda, (v) Bivalvia and (vi) Cephalopoda (Ruppert et al. 2004).

Monoplacophora: This class includes primitive, exclusively marine mollusc having a single, cap-shaped shell and bilateral symmetry. It was thought to have become extinct, however after 1952, live species of monoplacophora were dredged out from deep ocean bottom, for example, Neopilina.

Amphineura: It includes bilaterally symmetrical marine molluscs, having two lateral and two ventral nerve cords, and being commonly divided into the Polyplacophora and the Aplacophora, for example, Chiton, Chaetoderma and Neomenia.

Scaphopoda: The members of this class have a very typical, tusk-shaped conical shell, open at both ends and the anterior portion is wider than the posterior one, for example, Siphonodentalium.

Gastropoda: The Gastropoda is the largest living class among molluscs and includes about 30,000–70,000 living species inhabiting marine, fresh water and terrestrial habitats. Their name indicates the position of the ventral foot situated after the head region. The asymmetrical bodies due to torsion and detortion may or may not be covered by shell, for example, slugs, snails, limpets, conches.

Bivalvia: A typical class of mollusca having the body covered in two lateral shells (valves). Their body is laterally compressed having muscular foot, for example, mussel, oysters.

Cephalopoda: The body of these exclusive marine molluscs is bilaterally symmetrical with a well-developed head. As per the class name, muscular appendages are

attached to the head, surrounding the mouth. The shell, if present is internal, for example, sepia, octopus.

Apart from the immense diversity and abundance of extant species, the phylum is having formidable palaeontological records since the Cambrian period. Rostroconchia and Helcionelloida are the marine mollusc classes, conjectured to be extinct and ancestors of present-day bivalves and gastropods, respectively (Runnegar and Pojeta 1974; Clarkson 1998).

14.1.1 Ecology and Threats

The molluscs, as a result of proficient adaptation to various habitats, occupy marine, fresh water and terrestrial habitats. They might be pelagic, benthos, substrate creepers or sedentary one. Considering the trophic level standpoint, they may be herbivore, predators, filter feeders or even parasites (endoconchidae). The excellent camouflage exhibited by cephalopods is one of the escape mechanisms from predators and has much importance from an ecological as well as a bio-chemical point of view. Sacoglossan sea slugs contribute some more diversity to the feeding styles of molluscs, that is, they simply suck the algal sap using a single row of radula (Jensen 1997; Williams and Walker 2014).

Being one of the diverse taxa of the marine realm, molluscs need serious attention and conservation measures. This can well be illustrated by the example of oyster (belonging to phylum mollusc), that is, oyster populations of the Chesapeake Bay that once filtered the entire estuary once a week, now filter it only once a year because of stock depletion from overfishing and diseases. This proves that threats to marine resources are many and varied.

As discussed earlier, molluscs have occupied varieties of habitat and further microhabitat for different stages of their life cycle. Thus, understanding their habitat preference becomes an essential aspect of their conservation biology. A study suggests the prevalence of a gastropod *Thylacode* sp. is associated with the coral species *Porites lutea*, that is, 66% of the population was found embedded in the live coral colonies wherein 60% solely were found associated with the colonies of *Porites lutea* (Joshi and Mankodi 2016). The *Porites lutea* is under threat due to delayed recovery from bleaching stress (Joshi et al. 2015). Thus, the magnitude of threats on the coral species may affect the survival of such gastropods. Another such case is of fresh water Uninoid, whose larva leads a parasitic life on the fish host. Therefore, the future survival or threats are the functions of multi-fold changes in the wetlands. Pollution may directly affect the bivalve or indirectly through associated species. As a whole, habitat destruction, pollution, alien species introduction (which may induce habitat alteration, asymmetric competition from fast-growing and dispersing alien species predation on native species, and diseases), climate change, over exploitation, etc., are the major threats periled on molluscs (Peters et al. 2013).

14.2 Background and Rationale of the Approach

Malacology signifies the study of various aspects of molluscs such as taxonomy, ecology, biology, evolution and physiology of molluscs. Malacology also includes geological and paleontological studies, wherein the molluscan shells provide a crucial source of paleo-environment traces and thereby configuring past climate as well as assist to predict future ones. Terrestrial molluscs play a typical role in agriculture and veterinary by spreading diseases like schistosomiasis. As far as malacology and pharmacology are concerned, in the marine environment many chemicals have been identified playing a defensive role, but for almost all of them the sensory mechanisms of action are still to be explored (Hay 1996).

In order to overcome the side effects of anti-cancer, anti-microbial and anti-inflammatory drugs, the search for potent remedies have been shifted towards natural sources, especially marine ecosystems. Marine environment has tremendous potential to meet the demands of pharmacological industries rendering a large array of bio-active compounds. Additionally, the bio-active natural compounds produced by marine animals and plants differ significantly from the terrestrial ones. The metabolites have been developed by the marine organisms during their evolutionary history which avail them chemical communication, ease of reproduction and escape from predators in the marine environment. The discovery of more than 3000 novel chemical compounds from marine environment serves as a significant example of its being a potential source of bio-active molecules. Out of all the marine phyla, mollusca being the successful survivor of sea ought to be the rich and diverse source of bio-active compounds. Hence, the rest of chapter will discuss the varieties of bio-active compounds, their ecological and biological role in molluscs as well as the most important segment of the chapter, that is, various applications of such bio-active compounds to ameliorate a number of physiological disorders in human.

14.3 The Chemical Defence and Bioactivity

Molluscs are well known for their chemical defence mechanisms and possession of many bioactive compounds. Such compounds are produced in their body either to escape from the predator, to repel predators, or to envenom the prey. *Hapalochlaena* spp. (blue-ringed octopuses) serve as one of the classical examples of marine predation as its venom is potential enough to kill 26 adult human individuals within a few minutes. The venom, in fact, is composed of tetrodotoxin, histamine, taurine, octopamine as well as neurotransmitters like acetylcholine and dopamine. The tetrodotoxin (TTX) leads death, not directly by affecting heart or brain as it does not cross the blood-brain barrier but it affects the nervous system by blocking the sodium (Na+) channels of the neurons. The TTX functions as a fatal neurotoxin by capping the sodium (Na+) channel receptors and thereby disrupting the nerve impulse transmission in myelinated peripheral nerves. This condition results in the paralysis of voluntary muscles including the diaphragm and chest wall, consequently

leading to respiratory failure (Sheumack et al. 1978). It is noteworthy that tetrodotoxin is found to be 1200 times more toxic than cyanide (Hwang et al. 1989). Moreover, the blue-ringed octopus is immune to its own venom; however, no antivenom has been discovered to cure its toxicity spread in humans, which may be the area of marine pharmacological prospects (Sheumack et al. 1978, 1984; Yotsu-Yamashita et al. 2007).

Apart from the cephalopods, many gastropods have also been studied to demonstrate their chemical defence, the majority of which is produced/mediated via skin, mucus or digestive glands. The skin and mucus are found to have distasteful and deterrent compounds in order to deter the predators. Such compounds include terpenoids, especially sesquiterpenoids and diterpenoids (Avila et al. 1991; Avila 1995; Kamiya et al. 2006). Moreover, gastropods do exhibit this defensive mechanism for their reproductive success, that is, they use it to protect their egg mass or capsule with some anti-microbial compounds (enzyme) like L-amino acid oxidase present in their albumen glands (Kamiya et al. 2006; Iijima et al. 2003; Cummins et al. 2004). Such chemicals deter the predator as well as microbial infections and allow the gastropods to achieve reproductive success. Clinically, these compounds are found to induce symptoms such as apoptosis, oedema, haemorrhage and platelet aggregation (Li Lee et al. 2014). Indeed, such L-amino acid oxidases induce oxidative deamination of L-amino acids and produce many products like hydrogen peroxide, ammonium ions, α-keto acids and carboxylic acids which are destructive to animal tissues.

Another proficient example of chemical defence is exhibited by sea hare (*Aplysia californica*), an opisthobranch belonging to the class gastropod. It shows excellent panoply of prey-predator mechanisms in order to produce chemical defence against spiny lobsters (*Panulirus interruptus*). The standard mode of chemical defence in sea hare is inking. Whenever attacked by spiny lobsters, the sea hare releases ink, however it is noteworthy that the secretion is a mixture of two different glands the ink gland (secretes ink) and the opaline gland (its secretion is a transparent-to-whitish liquid that polymerizes and becomes highly viscous upon contact with water). Both these glands co-secrete the secretions and they are mixed in the mantle just before their release. These compounds are potential to evoke a variety of responses in the predator such as stimulation of feeding, dissuade or aversion. One such compound identified from the secretion is taurine, which is present in opaline (Kicklighter et al. 2005). Such compounds give rise to a very peculiar behavioural response in the spiny lobster termed as 'phagomimicry'. This implies that the spiny lobster gets attracted towards the secretion of sea hare or the other substrates covered by the secretion and tries to feed upon such false food. Thus, finally, the sea hare escapes from its predator by fooling it. This very much resembles the distraction of the predator by the reptiles wherein they remove and detach their (vibrating) tails.

14.4 Molluscs and Various Pharmacological Applications

14.4.1 Anti-microbial Potential

In spite of being micro, microorganisms play a 'macro' role in human life. They show both beneficial as well as adverse impacts to humans. A typical human body contains 1×10^{13} body cells, yet harbours an estimated 1×10^{14} bacterial cells. It has been estimated that the sea water column contains on average 10^5–10^6 microorganisms/ml (Whitman et al. 1998). These microorganisms play an important role to maintain a healthy state of our body. Fermentation is an important phenomenon carried out by microorganisms in many food preparations and industries as well. Participation of microbe in carbon and nitrogen cycle shows their crucial role in ecology. But the other side seems to be very dreadful when we come across plague, tuberculosis, AIDS and many more – the fatal diseases caused by pathogenic microbes. Epidemics create a great loss to the country's economy and public health. Not only animals but also plants become victim of various minor to fatal diseases of microbial origin. However, the invention of antibiotics has opened up new doors to cure such diseases. It is reported that, on an average, two to three antibiotics derived from microorganisms are launched each year (Clark 1996). Antibiotics have been a proven boon since a long time but some of them produce side-effects like dizziness, hearing loss, kidney damage, etc., which again triggered scientists to find out another way to overcome the microbial infections. In recent decades, scientists also realize that the effective life span of any antibiotic is limited (Cowan 1999). Therefore, again the anti-microbial drugs with no side-effects are in demand. Hence, the search for potent anti-microbial drugs has been shifted to marine environments.

The marine realm, brimming with an enormous density of microbes, also compels the other animals and plants to produce anti-microbial compounds in order to maintain their continual, disease-free survival. As far as molluscs are concerned, the maximum anti-microbial peptides have been obtained from the members of class bivalve followed by gastropods. A Caenogastropod from family Babyloniidae *Babylonia spirata* was extracted to obtain anti-microbial potential and it showed significant effectiveness towards the human pathogen, that is, *Klebsilla pneumonia*, *Proteous mirabilis*, fungi like *Aspergillus niger*, *Kendida albicans*, etc. (Kumar and Rawat 2011). Several gastrointestinal (GI) tract infections are very common and hazardous caused by *Salmonella typhi*, *Escherichia coli* and *Aeromonas hydrophila.* A species of the aforesaid genus *Babylonia*, that is, *B. zeylanica*, is capable of producing anti-bacterial substances against the GI tract–infecting bacteria. A number of pelecypodes have been studied to find out the presence of anti-microbial peptides (AMP). Such studies have revealed the occurrence of such AMPs in *Mytilus galloprovincialis* and the blue mussel *M. edulis* (Li et al. 2011). Besides this, a number of body parts (opercula, ink, egg mass) or whole body of molluscs have been extracted to derive desirable anti-microbial compounds. *Nerita albicilla*, *N. oryzarum*, *Chicoreus virgineus*, *Rapana rapiformis* and *Chicoreus ramosus egg masses* showed inhibitory effect to a broad spectrum (40) of bacteria (Ramasamy and Murugan 2005).

14.4.2 Anti-inflammatory Potential and Analgesic Compounds

A Neogastropoda family Muricidae was studied to isolate and trial the pharmacological applications of their bio-active compounds. The study revealed that extracts of many muricids provided various compounds like indoles, choline esters and indirubin (Benkendorff et al. 2015). Such compounds were found to show anti-inflammatory activity against leukemic monocyte macrophage cells of mice and microglia of rat brain (Benkendorff et al. 2015). Derivatives of Isatins, a bio-active compound derived from hypobranchial glands of muricids, can act as effective anti-inflammatory agents. In ancient times, the muricid ash (burnt flesh) was used to cure swelling of the parotid gland. *Purpura persica* belonging to family Muricidae provided significant anti-inflammatory activity in albino rats (Santhi et al. 2011). The extracts of *Perna canaliculus* have provided omega 3 polyunsaturated fatty acids which showed anti-inflammatory properties. Lyprinol (and a stabilized lipid extract of *Perna canaliculus*) has been proven to prevent inflammatory bowel disease as well as arthritis and asthma (Tenikoff et al. 2005).

Purpura persica belonging to family Muricidae is a thick large gastropod. It was explored to obtain analgesic compounds and satisfactory results were achieved using 100–200 mg/kg dose of the purified chloroform extracts of the gastropod (Santhi et al. 2011).

14.4.3 Anti-cancer Potential

Cancer has become a major concern of public health nowadays. The extent of its peril suggests that 21–29% of death in various countries is caused by cancer. It is also the leading cause of death in many countries. The major types include cancer of breast, prostate, lungs, uterus, skin, colon and rectum. In India, the trends of cancer onset and death are described in Table 14.1. As per the current increasing rate of cancer, the number of new cases per year is expected to rise to 23.6 million by 2030. The voraciously rising cancer statistics agitates an urgent need to come up with effective ameliorations. Despite the discoveries of some drugs and therapies, the major cancers remain refractive to such remedies (WHO 2011). Hence, novel solutions are always in high demand to cure the dangerous disease. Due to the drug resistance as well as side effects of the synthetic drugs, research for new anti-cancer drugs has been shifted to natural sources and one such promising source of compounds is marine environments (Newman and Cragg 2012).

- *Sepia esculenta* is a cephalopod belonging to family Sepiidae. Its distribution was recorded from China, Japan, Taiwan, Korea, Vietnam, etc. The species produces

Table 14.1 The cases of cancer and death caused by it at different time periods (NCI 2007)

Year	Cases reported	Death
2012	3,016,628	465,169
2013	2,820,179	491,598
2014	2,820,179	491,598

ink as a part of common defence mechanism of cephalopods; however, the sepia ink contains oligopeptide which is biologically active. This sepia ink oligopeptide (SIO) was tested against many cell lines such as of DU-145, PC-3 and LNCaP prostate cancer cell lines. Based on certain time periods and dose amount, it showed significant effectiveness to inhibit the cell growth of the aforesaid cell lines and is able to induce apoptosis in those cells. However, the population trend and distribution patterns of this *Sepia esculenta* are not known presently. Hence, in order to proceed further towards the pharmacological benefits of the species, it is more crucial to resurrect the ecological and biological aspects of the species (Huang et al. 2012).

- Apart from this, a sea slug was explored in the search of the anti-tumour potential, that is, *Elysia rufescens*, which resembles a nudibranch however belongs to clade sacoglossa (sap-sucking sea slug) of the class gastropod. The slugs provide a bio-active compound Kahalalide F which is a depsipeptide and an excellent anti-tumour agent. It is used to block the cancer growth by changing the membrane properties of lysosome and mitochondria. It is also capable of inhibiting the function of genes involved in DAN replication and cell proliferation. Thus, the compound can serve as a significant anti-cancer agent (Hamann and Scheuer 1993). A European and Mediterranean sea slug *Pleurobranchus forskalii* was explored to obtain a bio-active compound Keenamide A (Wesson and Hamann 1996).
- A gastropod species *Dollabella auricularia* (a sea hare) recorded in the Indian Ocean has been found to have several bio-active compounds, that is, Dolastatin 10 and Dolastatin 15. These compounds are anti-proliferative and can actively cease mitosis at various stages by inhibiting the microtubule formation and inhibiting the tubulin-dependent hydrolysis of GTP. They were clinically trialled for breast cancer, liver cancer, solid tumours and some leukaemia. The anti-mitotic property of such compounds indicated their significant effectiveness over various target cells (Poncet 1999). Moreover, the dolastatins (dolastatin 10 and dolastatin 15) were found to have similar chemical properties to symplostatin 1. Symplostatin 1was tested to cure several drug-insensitive tumours in vivo. Symplostatin 1was found to be effective against the mammary tumour and colon tumour. Likewise, the dolastatins can also offer new hopes in the direction of potent anti-cancer agent production from marine sources (Luesch et al. 2001).
- Many other anti-cancer compounds such as spisulosine ES-285, ulapualide-A, various alkaloids, etc., have been extracted from various marine molluscs like *Spisula polynyma (bivalve)*, *Turbo sp. (gastropod)*, *Dicathais orbita (gastropod)*, etc. (Luesch et al. 2001).

14.4.4 Other Applications

The extracts of a gastropod *Rapana venosa* showed wound healing properties and anti-inflammatory activity. The skin burns in Wistar rats were especially

significantly recovered using lipid extract of *R. venosa.* Additionally, the extract is found to contain Vitamin E, sterols, polyunsaturated fatty acids and aromatic compounds. These kinds of results were also derived using amino acid abstract of the same species, wherein the skin healing occurred at least 10 days faster in rats treated with the amino acid extracts compared to untreated controls. Indole derivatives of some muricidae are known to have a broad range of pharmacological activities (Benkendorff et al. 2015). The cardio protective effect of *Sepia pharaonis* liver oil was studied on the isoproterenol administrating rats and derived positive results (Sherief et al. 2004).

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Alkaloids from Marine Ascidians (Tunicates) and Potential for Cancer Drug Development

15

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Abstract

Tunicates are deliberated to be a rich source of alkaloids, with unique and diverse chemical structures. There are a number of alkaloids derived from marine ascidians and reported to have a variety of pharmacological activities. More than 300 such alkaloids from ascidians have been reported across the globe. In recent years, cancer has become a complicated disease encountered by developing and developed countries around the world. The marine ascidian-derived alkaloids play a potential role in cancer and other diseases. So far, two ascidian-derived alkaloid compounds are commercially available and employed as drugs for the treatment of various cancer in the USA. In other countries, few compounds are under clinical and preclinical trials. This chapter provides detailed information on alkaloids from marine ascidians and the development of these as a promising application in future as anticancer drugs.

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15.1 Introduction

Cancer is a malignant disease involving abnormal cell growth with a potential to spread to the nearby body tissues in the human body (Hinck and Näthke 2014). Cancer is one of the major issues of global health responsible for about 7.6 million deaths (13% of all deaths) worldwide, which is expected to rise to 13.1 million by 2030 as reported by the World Health Organization (2019). The American Cancer Society (ACS) estimated around 1,762,450 new cancer cases and 606,880 cancer deaths in 2019, with 4830 new cancer patients admitted for treatment and 1660 cancer deaths per day. According to WHO, seven out of ten deaths are caused by cancer in Asia, Africa, and South and Central America (WHO 2017), posing a challenge for developing countries to improve strategies for surveillance, early diagnosis, and improving the life of cancer patients (Jimenez et al. 2018).

Lung, pancreas, breast, and colorectal cancer are among the leading cancer types, leading to deaths in the USA, as reported by ACS 2019. Lung cancer is most commonly diagnosed in men, and breast cancer is most commonly identified in women. In India, 317,928 deaths occurred due to tobacco consumption and smoking in 2018. Oral and lung cancer account for over 25% deaths in males. Researchers and doctors have classified cancer as follows based on where it is initiated: (i) carcinomas that begin on the surface of internal organs, (ii) sarcomas that begin in the supported and connected tissues, (iii) leukemias that affect the blood vessel, (iv) lymphomas starting in the lymphatic system, and (v) brain tumors (Berman 2004). The possible primary symptoms include mysterious weight loss, abnormal bleeding, prolonged cough, and lumps. The different key factors mainly involved in the development of cancer include genetic mutations, alcohol consumption, smoking, tobacco usage, obesity, physical inactivity, poor nutrition, exposure to radiation, carcinogenic chemicals, and environmental factors (Fig. 15.1) (Colditz and Wei 2012). High or low level expression of some biomolecules in blood, urine, and other body fluid are used to diagnose cancer. Further imaging techniques of X-ray, CT(A computerized tomography) scan, nuclear scan, ultrasound scan, MRI(Magnetic resonance imaging), and PET(A positron emission tomography) scan are used to identify the cancerous tissue growth. Biopsy tissue test is the most common medical test used to diagnose all type of cancers (Ahmed and Abedalthagafi 2016).

15.1.1 Current Therapies for Cancer

Cancer treatment includes surgery, radiation therapy, chemotherapy, hormone therapy, precision medicine, immunotherapy, targeted therapy, and stem cell therapy. In

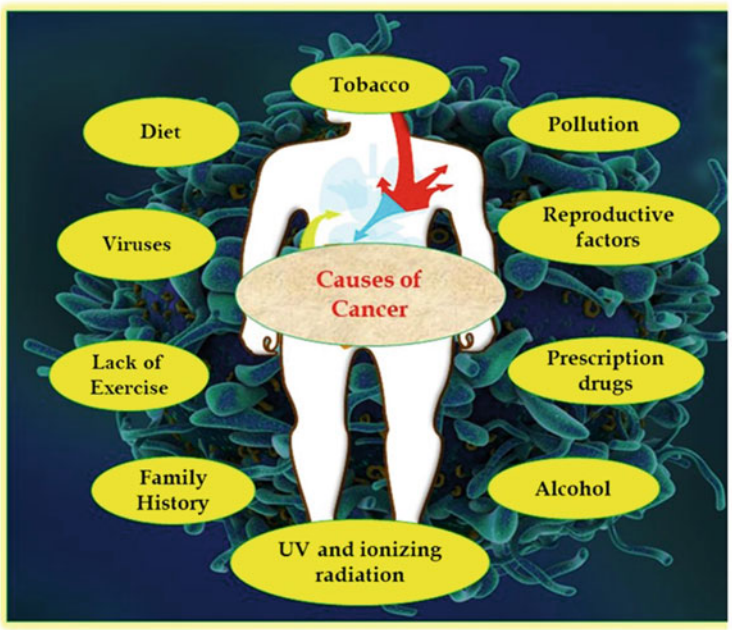

Fig. 15.1 The major environmental factors involved in cancer in humans

these cancer therapies, commonly three treatments have been widely used for cancer treatment worldwide. "Chemotherapy" is term first coined by a German scientist Paul Ehrlich (Arruebo et al. 2011). In the 1960s, radiotherapy and surgery for solid tumor treatment followed by chemotherapy were preferred for better treatment for cancer. Currently, the most commonly used treatment of cancer is chemotherapy. However, this requires continuous new inventions of anticancer drugs from vast resources as future candidates for pharmaceutical development. Chemotherapy has fewer side effects that damage the cancer as well as normal cells (Pearce et al. 2017). The identified side effects for chemotherapy include emotional state, disturbance in the quality of life, bone marrow, skin, mouth, hair, and intestines. For instant treatment of cancer, surgery is preferred to remove the obvious cancerous tissue, particularly in case of solid tumors. It also leads to several side effects like pain, fatigue, appetite loss, swelling around the site of surgery, numbness, bleeding, and infection. Another effective treatment, that is, radiation therapy, is an alternative to surgery or chemotherapy. Radiation therapy is the advanced treatment for cancer, which uses high-energy x-rays to treat or shrink the tumor (Huang et al. 2017). Radiation therapy also has some common side effects such as skin problems, fatigue, hair loss and loss of appetite, nausea, vomiting, and low blood cell counts. Immunotherapy is an attractive strategy among other cancer treatments. It induces the anticancer properties by utilizing the body immune mechanisms. Inhibitors of immune checkpoints, engineered T-cell therapy, and identification of novel tumor antigens are new strategies of cancer immunotherapy (Zhang and Chen 2018).

ipilimumab (CTLA-4 inhibitor), nivolumab, pembrolizumab (PD-1 inhibitors), and PD-L1 antagonists of durvalumab, atezolizumab, and avelumab are checkpoint inhibitors approved by the U.S. Food and Drug Administration (US FDA) for cancer treatment. Tisagenlecleucel (Kymriah) and axicabtagene ciloleucel (Yescarta) are blocking agents of CD19-targeted CAR-T cells used for the treatment of B cell lymphoma, approved by US FDA (Li et al. 2018). Due to the side effects of current cancer treatment therapies, researchers are now focusing on the natural resources and options with minimal side effects for humans as well as being environmentally friendly. In context to the same, current scientists are searching for natural products from marine sources to treat the cancer. This chapter highlights the biologically active alkaloids from marine ascidians and their biological roles, which has a promising future for drug development against cancers.

15.2 Marine Environment

The ocean covers more than 71% of the earth and is one of the largest unexplored wealthy resources covered with more organisms such as plants and animals (Cragg and Newman 2013). The marine environment has a great diversity and has extremely potent compounds that are not found in the terrestrial resources (Malve 2016). In the past two decades, a number of potential compounds, which have biological properties against various diseases, were newly introduced from the marine environment (Mehbub et al. 2014). The marine environment has several bioactive groups like phenolics, pigments, enzymes, polyunsaturated fatty acids (PUFA), polysaccharides, proteins, peptides, and other secondary metabolites from prokaryotes, micro- and macro-algae, seaweeds, crustaceans, sponges and other invertebrates as well as various vertebrates, which seem to be promising as the future candidates for the food industry and pharmaceutical applications (Singh et al. 2017). The first marine drug cytarabine (Cytosar-U®), derived from the Caribbean sponge *Cryptotheca crypta* and approved by the U.S. Food and Drug Administration (FDA), reached the market in 1969. Other six marine-derived natural products passed the clinical trials and are approved as drugs such as ziconotide (Prialt®), eribulin mesylate (Halaven®) and four for antiviral, antihypertriglyceridemia, and anticancer activities (Mayer et al. 2011). In the recent past, 21 out of 23 new moieties from the marine have entered into several clinical trials as anticancer agents; the remaining two compounds were assessed for neurological disorders (schizophrenia and Alzheimer's disease) and chronic pain (Choudhary et al. 2017).

15.3 Marine Ascidian Alkaloids

Ascidians are filter-feeding species and mostly diverse group of the subphylum Tunicata. Worldwide, more than 3000 species were found in the shallow waters to deep sea (Arumugam et al. 2018; Watters 2018). Ascidians are divided into three orders based on the structure of branchial sacs, with class *Ascidiacea*

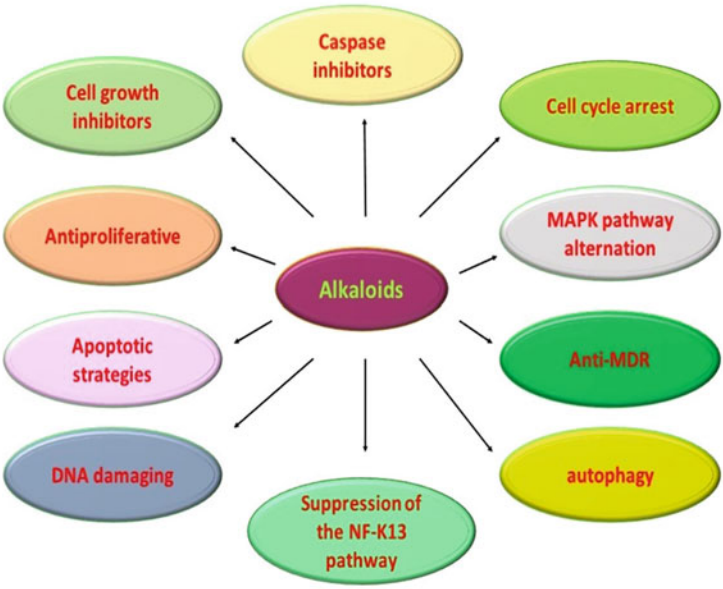

Fig. 15.2 Alkaloids involve different signaling pathways in cancer

Stolidobranchia (5 family) and *Phlebobranchia* (11 family) in both solitary and colonial forms and *Aplousobranchia* in colonial forms (13 family) (Shenkar and Swalla 2011; Shenkar et al. 2019). In foreign countries, Japanese and Koreans consume the ascidian *Halocynthia roretzi* (common name of sea pineapple) as a traditional food. Some other countries like Chile, France, Italy, and Greece also consume raw ascidians and use them as seafood (Lambert et al. 2016). Several researchers have reported that the ascidians have a diverse group of bioactive compounds such as polyketides, cyclic peptides, depsipeptides, alkaloids, and other secondary metabolites (Chen et al. 2017). Several reviews have reported that the ascidian families Didemnidae, Polyclinidae, and Polycitoridae have potential profile for natural products against various diseases (Arumugam et al. 2019). Marine alkaloids and pyridoacridine alkaloids have numerous biological properties and act as sources for anticancer agents (Ibrahim and Mohamed 2017). Ecteinascidin (trabectedin ET-743), trade name Yondelis, was derived from the *Ecteinascidia turbinata* (Rinehart et al. 1990) and FDA approved it for the treatment of soft tissue sarcoma. Another important compound Aplidin® (dehyrodidemnin B, plitidepsin), isolated from the *Aplidium albicans*, used as orphan drug, is marketed by Pharma Mar (Madrid, Spain) (Chen et al. 2018; Watters 2018). The different types of alkaloids reported from ascidians include pyridoacridine alkaloids, carboline-based alkaloids, indole-based alkaloids, tyrosine- and phenylalanine-derived alkaloids, lysine-derived alkaloids, protoalkaloids, tetrahydroisoquinoline alkaloids, dimeric

steroidal alkaloids, oxazole alkaloids, and imidazole alkaloids (Menna et al. 2011; Jin 2006); their biological role in anticancer cancer is summarized in Fig. 15.2.

15.4 Techniques of Extraction and Characterization of Ascidian Alkaloids

Various techniques have been adopted for the extraction, purification, and characterization of ascidian alkaloids. Recently, some advanced techniques have been used to isolate the alkaloids and advanced instrument facilities are used by the researchers. The common methods used for extraction of alkaloids are polar and non-polar solvent-based extraction (Bontemps et al. 2010). The purification of alkaloids is followed by column chromatography (CC), silica gel flash chromatography (Verbitski et al. 2002), and high-performance liquid chromatography (HPLC) (Zhang et al. 2016). The characterization of alkaloids is usually carried out by UV (ultra-violet) spectroscopy, (Infrared spectroscopy(IR) (Ibrahim and Mohamed 2016), Electrospray ionization mass spectra (ESIMS), Liquid chromatography electrospray ionisation mass spectroscopy(LC/ESIMS) (Urban et al. 2002), One-dimensional nuclear magnetic resonance(1D NMR) and Two-dimensional nuclear magnetic resonance spectroscopy (2D NMR) (Bontemps et al. 2010), and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) (Rajesh and Annappan 2015).

15.5 Ascidian Sources for Drug Development

The first process starts with collection of samples and identification of the collected marine ascidians. The sample availability places should be easy to reach and place should be easily accessible for collecting the samples. Advanced underwater equipment will provide new possibilities for the collection of samples from unexplored depths. In recent years, molecular genetic analyses have been made available to modify the species and cultivation techniques made available to produce enough quantity for extraction and other purification process. In drug development, the following steps are commonly followed for marine organisms. The lack of drug development process or quantity is the problem for the development of drugs. Recent advanced techniques or processes have solved this problem by marine biotechnology advances like chemical synthesis/semi-synthesis/modification aquaculture/mariculture/fermenter cultivation, genetic engineering, enzymatic synthesis or modification. Currently, 20 marine-derived drugs are in clinical trials worldwide (Lindequist 2016; Mayer et al. 2017).

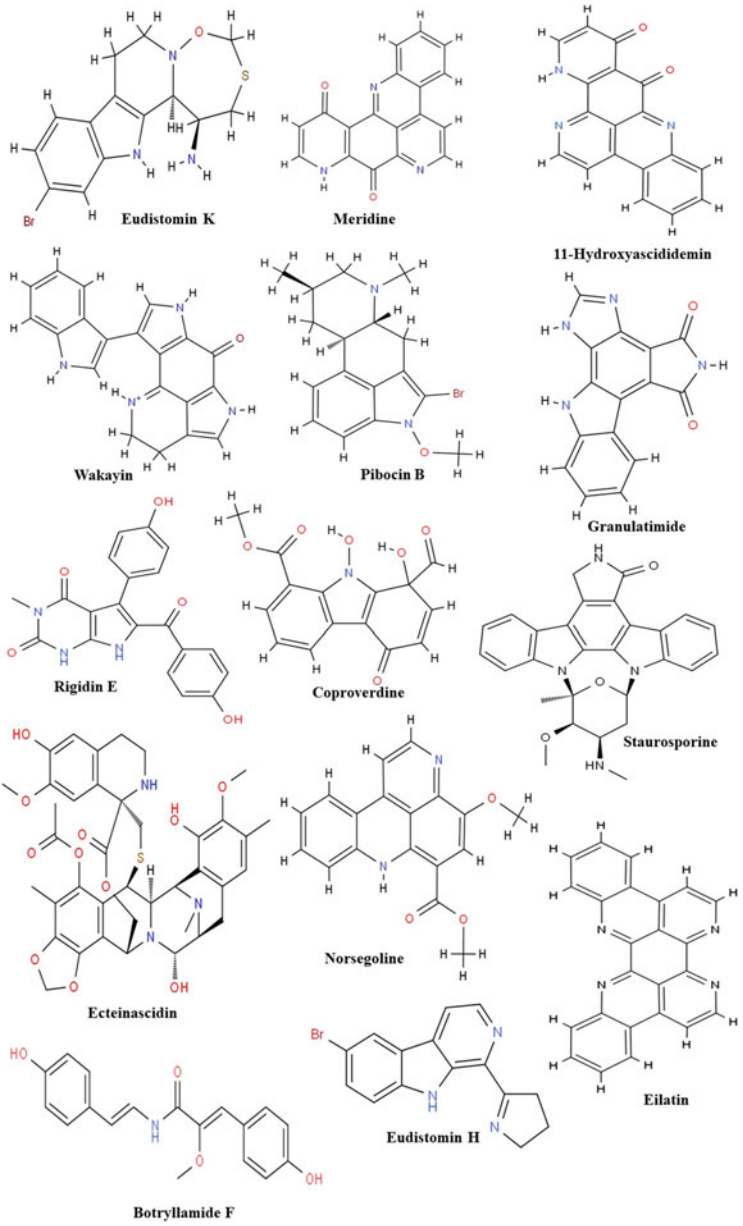

Fig. 15.3 Some important chemical structures of alkaloids derived from ascidians

15.6 Anticancer Properties of Alkaloids Derived from Ascidians

Bioactive nature of marine ascidian-derived alkaloids plays an imperative role in cancer drug identification and development. Alkaloids have been reported to have creditable potential to interact with cellular ingredients, which inhibits the cancer growth. The different type of alkaloids reported from the ascidians are summarized (Fig. 15.3). Lake and his co-workers reported the alkaloid Eudistomin-K (1) from the Caribbean ascidian *Eudistoma olivaceum* and it has shown antitumor activity in different cell lines of A549, HCT-8, L-1210, and P-388, with moderate activity showing the IC_{50} of P-388 reported as 0.01 μg/Ml (Lake et al. 1989). cystodytins A–K (2-12) are iminoquinone alkaloids, which were isolated from *Cystodytes dellechiajei* in Okinawan. These cystodytin derivatives have been shown to have potent cytotoxic activity and more powerful Ca^{2+}-releasing activity in the sarcoplasmic reticulum (Kobayashi et al. 1988, 1991).

Grossularine-1 and grossularine-2 are first naturally occurring ascidian alkaloids from *Dendrodoa grossularia*, which inhibit the cytotoxic effect and cell accumulation in the G1-phase on L1210 leukemia human cell line (Moquin-Pattey and Guyot 1989). Meridine (15) has been isolated from *Amphicarpa meridiana* and 11-Hydroxyascididemin (16) is derived from *Leptoclinides* sp. (South Australia and Truk Lagoon). These two pentacyclic pyridoacridine alkaloids show cytotoxic activities, which inhibit the cancer growth (Schmitz et al. 1991). Copp and his coworkers reported pyrroloiminoquinone alkaloid. Wakayin, (17) isolated from marine ascidian *Clavelha* sp., inhibits the topoisomerase II enzyme and has in vitro cytotoxic effect on human colon cancer cell line (Copp et al. 1991). Especially 11-hydroxystaurosporine (18) from ascidian *Eudistoma* sp. is a Protein Kinase C (PKC) inhibitor, which has better activity compared to staurosporine alkaloids (Singh and Majik 2016). Six different alkaloids such as segoline A-B (19-20), segoline B (21), isosegoline A (22), norsegoline (23), debromoshermilamine (24), and eilatin (25) were isolated from Red Sea purple tunicate, *Eudistoma* sp., and these alkaloids inhibit the cell proliferation and morphological differentiation of cancer cells and are involved in the pathway of Cyclic AMP(cAMP) signaling in the cancer cell lines (Shochet et al. 1993) (Table 15.1).

The tetrahydroisoquinoline alkaloid, ecteinascidin (ET-743, trabectedin) (26) was first isolated from the ascidian *Ecteinascidia turbinata* (Sakai et al. 1992). It has been commercially approved as an anticancer drug in the USA and Europe for soft tissue sarcomas and ovarian cancer under the trade name Yondelis® (Watters 2018). The dimeric disulfide alkaloid, polycarpine dihydrochloride (27) and its derivatives are derived from ascidian *Polycarpa clavata*, collected in Western Australia, and polycarpine dihydrochloride is reported to confer strong cytotoxicity against the human colon tumor cell line HCT-116 at IC_{50}: 0.9 μg/ml (Kang and Fenical 1996). Rashid and his team reported new β-carboline alkaloids: three identified as 2-methyleudistomin D (28), 2-methyleudistomin J (29), and 14-methyleudistomidin C (30), eudistomins C-E (31-33) and J-L (34-36) isolated from marine ascidian *Eudistoma gilboverde*. 14-methyleudistomidin C showed high cytotoxic activity against four different human cancer cell lines of LOX (melanoma),

Table 15.1 Some important alkaloids derived from ascidians and their biological role in cancer cell lines

Species	Compound	Cancer Cell lines	IC_{50} Value	Mechanism	Country	References
Eudistoma vannamei	2-hydroxy-7-xostaurosporine	HL-60	25.97 nM	Antiproliferative activity	Taíba Beach, Brazil	Gompel et al. (2004)
		Molt-4	18.64 nM			
		Jurkat	10.33 nM			
	3-hydroxy-7-xostaurosporine	K562	144.47 nM			
		HCT-8	58.24 nM			
		MDA MB-435	57.9 nM			
		SF-295	28.68 nM			
Eudistoma toealensis	7-hydroxy-staurosporine	MONO-MAC-6	27.6 nM	Inhibitors of protein kinase C	Taíba Beach, Brazil	Schmitz et al. (1991)
Eudistoma sp.	Pibocin B	Ehrlich ascites carcinoma	ED_{50} 25 μg/mL	Cytotoxic activity	Vladivostok, Russia	Makarieva et al. (2001)
Eudistoma viride	Eudistomin H	HeLa cell line	53 μg/ml	Induce the apoptosis	Tuticorin, India	Tardy et al. (2004)
Eudistoma sp.	Rigidin E	HCT 116	0.75 μM	Cytotoxic activity	Papua New Guinea	Shochet et al. (1993)
		A431	1.08 μM			
Aplidium meridianum	Meridianin E	PTP; Hep2	22.0;1.0 μM	Inhibition of protein kinase; anti proliferative	South Georgia Islands	Rashid et al. (2001)
		U937; LMM3	9.8; 11.1 μM			
Cystodytes dellechiajei	Cystodytin A-B	L1210	0.22 μg/ml	Anti-proliferative effect	Okinawa	Bruneton (2008)
	Cystodytin C		0.24 μg/ml			
Amphicarpa meridiana	Meridine 6	Murine leukemia (P388)	ED_{50} 0.344 μg/mL	Cytotoxic effect and inhibition of topoisomerase II	South Australia	França et al. (2014)

(continued)

Table 15.1 (continued)

Eudistoma sp.	Segoline A	N1E-115, NIL8 and NIL8- HSV cells	16–20 μM	Anti-proliferative effect and affect the camp signalling pathway	Red Sea	Singh and Majik (2016)
	Segoline B					
	Isosegoline A					
	Norsegoline		6–32 μM	Anti-proliferative effect		
	Debromoshermilamine		0.12–0.16 μM			
	Eilatin		0.05–0.2 μM			
Didemnum proliferum	Shishijimicin A	3Y1; HeLa P388	2.0; 1.8 μM	Cytotoxic activity	Amakusa islands	Copp et al. (1991)
			0.47 μM			
Didemnum sp.	Bengacarboline	A26 cell line	0.9 μg/mL	Inhibition of topoisomerase II	Fiji Islands	Kobayashi et al. (1991)
Dendrodoa grossularia	Grossularine	L1210	10.0 μg/mL	Cytotoxic activity	Coasts of Brittany	Jin (2005)
Clavelha sp.	Wakayin	HCT116	0.5 μg/mL	Inhibition of topoisomerase II	-	Gul and Hamann (2005)
Didemnum granulatum	Granulatimide	p53- MCF-7	1 – 1.8 μM	Inhibition of checkpoint I kinase	Brazil	Schupp et al. (2001)
	Isogranulatimide					
Lissoclinum cf. badium	Lissoclibadin- 1	HCT-116	4.0 μM	Induced cell death via apoptosis	Indonesia	Facompre et al. (2003)
		SK-MEL-28	6.3 μM			
		HT-460	7.6 μM			
		THP-1	7.1 μM			
Aplidium glabrum	3-demethylubiquinone Q2	JB6 Cl41 mouse cell line	11.4 μM	Induction of p53-independent apoptosis	Far Eastern	Copp et al. (1991)

Ciona edwardsii	Iodocionin	L5178Y PC-12 lymphoma	7.75 μg/mL	Cytotoxicity effect	Bay of Naples	Segraves et al. (2003)
Polycitorella sp.	Iheyamines A and B	P388, A549, HT29	1 μg/mL	Cytotoxicity effect	Island of Iheya, Okinawa	Moquin-Pattey and Guyot (1989)
Didemnum sp.	Asciddemin	A431	11 and 87 μM	Cytotoxicity effect	–	Guittat et al. (2005)
Didemnum candidum	Euseynstelamide B	MDA-MB-231	5 μM	Cytotoxic, causing G2 arrest	–	Liberio et al. (2015)
Riterella tokiada	Ritterazines A-M	P388	ED_{50}: l4.2, 0.17, 102.3,17.5,3.8,0.81,0.81, 17.8,15.3,14.0,10.4,11.1,16.7 μM	Cytotoxicity effect	–	Imperatore et al. (2014)

OVCAR-3 (ovarian), COLO-205 (colon), and MOLT-4 (leukemia) with IC_{50} value < 1.0 μg/ML (Rashid et al. 2001). The novel alkaloid coproverdine (37), derived from the unidentified New Zealand ascidian, has shown moderated activity against different murine and human cancer cell lines of P388, A549, HT29, MEL28, and DU145 with IC_{50} values of 1.6, 0.3, 0.3, 0.3, and 0.3 μM, respectively (Urban et al. 2002).

Bengacarboline (38), β-carboline alkaloid derived from the Fiji Islands of ascidian *Didemnum* sp., have shown a potent cytotoxic effect on in vitro A26 cell line human tumor panel and inhibit the topoisomerase II activity (Foderaro et al. 1997). Sasaki et al. (1999) reported the bisindole pigments – Iheyamines A-B (39-40) – derived from ascidian, *Polycitorella* sp., and both compounds exhibit moderate cytotoxicity in in vitro cell line studies. Schupp and his co-workers reported alkaloids of staurosporine(41) and their eight subderivative alkaloid analogues such as 3-hydroxystaurosporine(42), 4′-N-demethylstaurosporine (43), 3-′-demethoxy-3′-hydroxystaurosporine (44), 3-hydroxy-3′-demethoxy-3-′-hydroxystaurosporine (45), 11-hydroxy-4′-N-demethylstaurosporine(46), 11-hydroxystaurosporine(47), 4′-N-methylsta-urosporine (48), 3-hydroxy-4′-N-methylstaurosporine (49) from *Eudistoma toealensis* (Schmitz et al. 1991). These compounds were studied on the MONO-MAC-6 cell line; they inhibited the cancer growth and are known to be strong inhibitors of Protein Kinase C (Schupp et al. 2001).

The unique structural feature N-O-methylindole alkaloid, pibocin B(50), derived from Japan ascidian *Eudistoma* sp., shows moderate cytotoxic activity against mouse Ehrlich carcinoma cells (ED_{50}:25 μg/mL) (Makarieva et al. 2001). Shishijimicins A-C (50-52) are β-carboline alkaloids, which are isolated from *Didemnum proliferum*, are potent antitumorigenic agents (Oku et al. 2003). Davis and his co-workers reported pyrrolopyrimidine alkaloids, rigidin (53), rigidin E (54), and 1-methylherbipoline (55), isolated from a Papua New Guinea Sea-Squirt *Eudistoma* sp.; these three alkaloids reported moderate inhibition of cancer growth in the human p53 colon carcinoma cell lines and A431 epidermoid carcinoma cell lines (Davis et al. 2003). The sponge alkaloid fascaplysin (56) was reported to be isolated from *Fascaplysinopsis Bergquist* sp.; later, this compound, together with its 3-bromoderivative, was isolated from ascidian *Didemnum* sp. These sponge alkaloids have an excellent cytotoxic effects against murine C38 CFU cell lines and the human H116 versus the CEM cell lines (Segraves et al. 2003, 2004; Menna et al. 2011). The brominated 3-(2-aminopyrimidine)-indole alkaloids, meridianins A-G (57-62), which are purified from South Georgia Island Ascidians *Aplidium meridianum*, have shown anti-proliferative effects, inducing apoptosis and inhibiting various kind of protein kinases such as cyclin-dependent kinases, casein kinase-1, and glycogen synthase kinase-3, in NT2 teratocarcinoma cells(Gompel et al. 2004). The marine alkaloid lamellarin D (63) represented sophisticated cytotoxic effects against tumor cells and was proposed as a potential antitumor agent for targeted topoisomerase-I cancer therapy (Tardy et al. 2004). Lamellarin series of alkaloids represent an important potential source for development of anticancer agents, meanwhile few aspects of mechanisms of action of lamellarin analogues (topoisomerase-I

and etc.) used in biotechnological and pharmaceutical industries are known (Facompre et al. 2003; Marco et al. 2005). The pyrrole hexa-cyclic alkaloids, lamellarin and derivatives of lamellarin-ζ (64), lamellarin-η (65), lamellarin-ϕ (66), lamellarin-χ (67), lamellarin-K (68), lamellarin-I (69), lamellarin-J (70), lamellarin-K triacetate (71), lamellarin-L triacetate (72), lamellarin-F (73), and lamellarin-T diacetate (74) are obtained from *Didemnum obscurum* from Gulf of Mannar, India, and it has been reported that lamellarin-ζ, lamellarin-χ, lamellarin-L triacetate, and lamellarin-F compounds have demonstrated better activity in colorectal cancer cell lines (COLO-205) (Reddy et al. 2005). The heterocyclic aromatic alkaloids, granulatimide (75) and isogranulatimide (76) are isolated from the methanolic extract of marine ascidian *Didemnum granulatum*. These two bioactive compounds act as inhibitors for G2-specific cell cycle checkpoint (specifically inhibition of Checkpoint 1 kinase) and demonstrated anti-tumor potential in p53 cancer cell line on molecular level (Henon et al. 2007). Lissoclinidine B (77) is a type of alkaloid isolated from Papua New Guinea ascidian *Lissoclinum* cf. *badium*, which inhibits the ubiquitylation and degradation of p53 in cancer cell lines (Clement et al. 2008).

Botryllamide A-J (78-87), brominated tyrosine alkaloid derivatives, isolated from ascidian *Botryllus* sp., focused on a new class of inhibitors on ABCG2 human membrane transport protein and have a significant role in cancer-multidrug resistance activities (Henrich et al. 2009). Tyrosine-iodinated derivative alkaloid, iodocionin (88), identified from Mediterranean ascidian *Ciona edwardsii*, showed significant cytotoxic effects against lymphoma cancer cell lines (Aiello et al. 2010). Takada and his coworkers reported the biological cytotoxic activities of botryllamide F (89) and G (90) derivatives and a potential therapeutic agent on cancer treatment (Takada et al. 2010). Two important staurosporine alkaloid derivatives of 2-hydroxy-7-oxostaurosporine (91) and 3-hydroxy-7-oxostaurosporine (92) are isolated from the Brazilian Endemic ascidian *Eudistoma vannamei*. The mixture of these bioactive alkaloids has antiproliferative effects against the leukemia cancer cell lines, inducing a distinguished and persistent G2 arrest in sub-toxic concentrations, while at toxic concentrations, the treated cells underwent apoptosis (Jimenez et al. 2012). The brominated indole alkaloid, eudistomin H (93), isolated from HPLC fraction of *Eudistoma viride*, could play an important role in cervical carcinoma cancer cells at IC_{50} 0.49 μg/ml at 0.5 μg/ml concertation (Rajesh and Annappan 2015). Tatsuta and his coworkers 2017 reported on Indonesian ascidian, *Lissoclinum* cf. *badium*, and they isolated the polysulfur aromatic alkaloids of lissoclibadins 1 (94), 3(95), 4(96), 7(97), 8(98), and 14(99). These aromatic alkaloids inhibit and control the cancer cell growth on four different human solid cancer cell lines: HeLa-S3 (cervix adenocarcinoma), HCT-15 (colon adenocarcinoma), MCF-7 (breast adenocarcinoma), and NCI-H28 (mesothelioma). Lissoclibadin-1 alkaloid was observed to be having more cytotoxic potential through in vitro and in vivo studies and also promotes the apoptosis through an intrinsic pathway with caspase-9 and caspase-3 activation on caspase-dependent pathway in HCT-15 cell line. Lissoclibadin-1 confirmed the suppression of tumor growth in nude mice in in vivo studies (Tatsuta

et al. 2017). These are the important ascidian-derived alkaloids, which were reported to have potential anticancer properties across the globe.

15.7 Ascidian-Derived Alkaloids for Future Cancer Drug Development

The potential anticancer agents from marine ascidians have a great success that span over decades with mechanisms of action and target commiserating with cancer types affecting human society (Isah 2016). The present great success is achieved through screening of the ascidians for natural products with anticancer properties and come with limitations on their use for human cancer chemotherapy due to cytotoxicity and other side effects.

In future, pharmaceutical companies not only will depend on high-throughput screening and combinational chemistry, but other technological advancements will also be involved in the future discovery techniques of natural product discovery like genomics, metabolomics, proteomics, metagenomics, structure-function drug design, recombinant DNA(Deoxyribonucleic acid) technology, genome mining, semi-synthesis, and combinatorial synthesis (Demain and Vaishnav 2011). In recent years, scientists have developed advanced techniques for screening methods and machinery identification of structural analysis, metabolic engineering and synthetic biology for developing the new natural products discovery and the future development of anticancer compounds of alkaloids from marine ascidians.

15.8 Conclusion

Marine ascidian-derived alkaloids are reported to have several biological activities, especially anticancer. Tunicates alkaloids have unique chemical structures and more numbers of alkaloids are clearly investigated. More than 90 ascidian alkaloids were discussed in the present chapter, and these alkaloids can inhibit different types of cancer like breast, lung, brain, colon, and other cancers. Recently, advanced molecular approaches and drug discovery process have been invented by scientists, and they will be helpful to develop new alkaloids and therapeutics. It is clearly indicated that the marine ascidians are a promising future candidate for cancer drug development.

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Conflict of the Interest All authors have the no conflict interest.

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Fish Protein Hydrolysates in Indonesia: Their Nutritional Values, Health Benefits, and Potential Applications

16

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Abstract

Fish is an important source of protein with complete and balanced amino acids. In Indonesia, they are available abundantly at a relatively affordable price and found year-round. Thus, they provide human's need of essential amino acids. For the Indonesian people, fish is the main animal protein source. Since Indonesia still has stunting problems, the Government makes efforts, one of which is the provision of high-protein foods. Fish protein hydrolysates (FPHs) are hydrolysis products of fish protein containing peptides and amino acids that are readily absorbed and beneficial for health. The unique functional properties of FPH and its various bioactivities pay much attention for research to develop their applications for functional foods or nutraceutical products. This review paper highlights the nutritional values, health benefits, and potential application of FPH for food, and gives a brief overview of FPH research in Indonesia.

Keywords

Fish protein hydrolysates · Nutritional values · Health benefits · Potential application for food

16.1 Introduction

Protein plays a very important role in human life. It is responsible for various functions including building tissue, cells and muscle, as well as producing hormones and antibodies. The increase of protein demand is related to population growth, socio-economic changes including rising incomes and the increasing elderly population, as

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well as the requirement of nutritious foods especially for the growth of toddlers (Boland 2013; Henchion et al. 2017). The need of protein can be met from both animal and vegetable sources. Currently, the average protein consumption of the Indonesian population is 61.23 g/capita/day. It has fulfilled the adequacy rate of protein consumption which is equal to 57.00 g/capita/day. Among the protein sources, the main animal protein source of Indonesian comes from fish which is estimated at about 48% (Pusdatin Kemenkes RI 2018a). This is due to the availability of abundant fish at a relatively affordable price. However, it has not evenly distributed so that there are still a serious problem of malnutrition occurred in several provinces.

In 2015, there were 19.6 million Indonesian suffered from malnutrition and abnormal growth, although this condition is much lower compared to that of 1990. The prevalence of child stunting in Indonesia reached 37% (ASEAN/UNICEF/WHO 2016) and decreased to 30% in 2017 (Pusdatin Kemenkes RI 2018b). Several factors contribute to unwanted abnormal growth in Indonesia, including nonexclusive breastfeeding for the first 6 months, low household socio-economic status, premature birth, short birth length, and low maternal height and education. The Government has set 5 pillars of national strategies to accelerate stunting prevention and reduction; one of the pillars is Food Security Policy to increase access to nutritious foods and promote food security. This strategy includes: provision of nutritious food, expansion of social aid programs and nutritious food aid for poor families, fulfilling the family needs for food and nutrition, and strengthening regulations regarding food labels and advertisements (Satriawan 2018). Related to this strategy, many research and development on various aspects of food have been carried out to contribute to the prevention and reduction of stunting.

Fish is a source of high-quality protein and generally low fat content. It has an important micronutrient for health and growth. However, the nature of fish is perishable and fishy, and it requires processing technology that produces high protein products with good preference. Fish protein hydrolysates (FPH) are hydrolysis products of fish protein containing peptides and amino acids which are more digestible and absorbable compared to an intact protein. Fish protein hydrolysates had a global market size of over USD 370 million in 2017 and the expected consumption level is at over 100 kilo tons by 2024 (Ahuja and Deb 2019). They can be used in a wider range of products, but at the global market they are found for animal feed, petfood, food, cosmetics, agricultural, and pharmaceutical. The use of FPHs for food is particularly related to their functional properties which are suitable for fortification materials for the supply of high protein foods, such as biscuits, cereal milk, noodles, or other healthy and nutritious foods for kids. So far FPHs that are commercially found in Indonesia are limited for fish sauce which is commonly used as a flavoring agent, and is produced by traditionally fermentation process from by-catch fish. However, many research and development of FPHs have been reported by a number of institutions in line with the increasing need for high nutritious and other healthy foods to reduce malnutrition and other health problem. Currently, researches on FPHs which are purposed for health foods have been mostly conducted using commercial enzymes at a laboratory scale. Limited research was performed at a pilot scale as well as research carried out by using self-prepared enzymes derived from plants or microbes.

16.2 Potency of Raw Material

Fish protein hydrolysate can be produced from whole fish, fish muscle, or fish by-products of fish canning, fish fillets, and other fish processing activities. Also, all types of fish including marine and aquaculture fish as well as by-catch can be prepared for FPHs. However, low economic fish such as by-catch fish are more suitable to be used as raw materials of FPHs which are intended for food ingredients or health foods due to that those raw materials do not compete with the needs of fish for consumption.

Indonesia is a maritime and archipelagic country with a coastline stretches for about 108,000 km, has approximately 17,504 islands and 16,056 of which have been named and registered to the United Nations (Ambari 2018). According to California Environmental Associates (2018), Indonesia is the second-largest fish producer in the world, which supplies seafood for both the international and the domestic markets. Total national fisheries production in 2017 reached 23.19 million tons, consisting of 7.07 million tons from capture fisheries and 16.11 million tons from aquaculture (Pusdatin KKP 2018). The most proportion of the catch is small pelagic fish (e.g., scads, mackerel, sardines), contributing 34% of all fishery production in Indonesia (MMAF and USAID 2018). Meanwhile, the fisheries processing industry is still concentrated in Java, mostly with the MSM (micro-small-medium) scale. The total fish processing units in 2015 were 61,603 units consisting of 718 units of large scale and 60,885 units of MSM scale, with a total production of 1,809,070 tons/year (Kementerian Kelautan dan Perikanan Republik Indonesia 2018). However, the total volume of production is still around 60% of the production capacity or total raw material, which still allowing for being increased. Frozen fish is the main purpose of processing contributes more than 50%, followed by fish canning, drying, and the others. Based on this condition, the use of whole fish from both catches and aquaculture as well as fish processing by-product is possible to develop the production of FPHs in Indonesia.

The type, origin, and condition of raw material will affect the properties and quality of FPHs produced which may determine the purpose of their uses, whether as functional food, ingredient for food fortification, or for feed. Many studies on processing FPHs have been conducted in Indonesia; some of which that have been reported were FPHs from yellowstrip scads (*Caranx leptolepis*) (Nurhayati et al. 2007), sardines (*Sardinella lemuru*) (Handayani et al. 2007), 'kerang mas ngur' (*Atactodea striata*) (Purbasari 2008), green cockle (*Mytilus viridis*) (Nurhayati et al. 2011), and 'lele dumbo' (*Clarias gariepinus*) (Salamah et al. 2012), which were using whole fish as raw materials. Preparation of FPHs produced using by-products of fish processing was reported by Utomo et al. (2014) who used by-product of patin fillet industry, Muzaifa et al. (2012) who studied FPHs from fish by-product, and Kristianawati et al. (2014) who produced fish sauce from viscera of catfish.

16.3 Processing Methods

Basically, hydrolysis of fish protein can be done by chemical or enzymatic methods (Fig. 16.1) through several steps which include: raw material (fish) preparation, homogenization, washing (for fatty fish), hydrolysis (by chemical or enzymatic methods), addition of chemical or heat treatment for stopping the hydrolysis process, separation of FPH, and dehydration.

16.3.1 Preparation of Raw Materials

Starting from the preparation of the raw materials, the fish can be filleted or not depending on the size and types of raw material used. All fishes used, except by-product of fish processing, should be gutted prior to mincing. The minced fishes

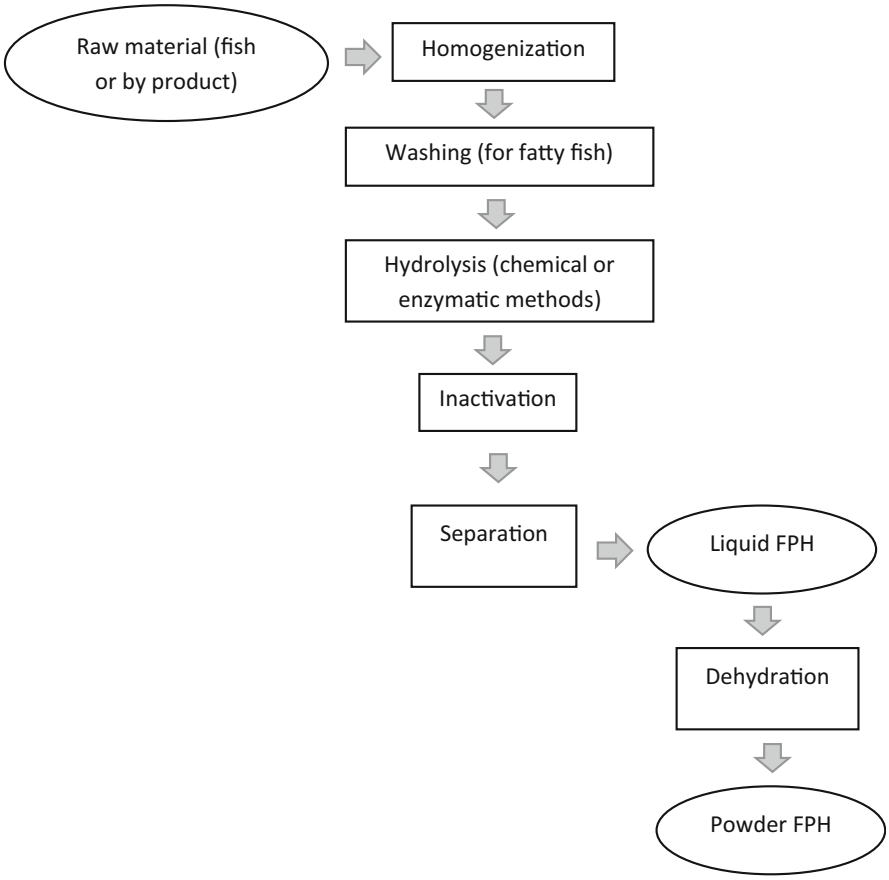

Fig. 16.1 Flow chart of fish protein hydrolysate production

are ready to be hydrolyzed. If high-fat fish is used, minced fish should be washed using cold water to remove or reduce the fat. The presence of fat will interfere with the hydrolysis process and affect the quality of the FPH produced. During preparation, a cold chain system should be applied to keep the fish quality.

16.3.2 Homogenization

Homogenization is intended to make a homogenous fish slurry by mincing the gutted fish or fillets or by-product of fish processing and mixing with water at an equal amount until a homogenous mixture is achieved. The treatment reduces the size of meat producing greater surface area which makes it more accessible to enzymes or chemicals in the hydrolysis process. If fatty fish is used, additional treatments should be introduced to remove the fat, commonly by washing minced fish using cold water. The fish slurry is then left for a while and the floating fat is picked up manually to be discarded. Washing is done 2–3 times to minimize fat content that affects the quality of FPH produced due to the lipid oxidation. A fish protein hydrolysate with high lipid content may darken. After washing, the fat-free slurry is decanted to remove water followed by pressing or centrifugation. Fish meat is ready to be hydrolyzed.

16.3.3 Hydrolysis

Hydrolysis of fish protein into smaller parts (peptides and amino acids) can be done by using chemicals or enzymes to cleave peptide bonds. Chemical hydrolysis is widely used in industry for years ago because it is relatively inexpensive and the process is simpler than enzymatic methods. However, this method tends to be a difficult process to control and produces peptides in various sizes with lower functional properties compared to enzymatic methods. This method may also have a negative impact on the environment, if hard chemicals are used. Moreover, some essential amino acids such as tryptophan and cysteine may be reduced significantly (Jaswal 1990), and racemization of amino acids is taken place causing the reformation of L-form amino acids to D-form amino acids that is unable for human and animal purposes (Wisuthiphaet and Kongruang 2015). Meanwhile, enzymatic hydrolysis which occurred under mild condition produces FPH in better physicochemical, functional, and sensory properties as well as its nutritive value. The use of exogenous enzyme is more manageable than that of endogenous enzyme or autolytic in the hydrolysis process, due to abroad variation in the autolytic process which may be affected by the mixture of enzymes, fish species, and environment conditions.

Chemically hydrolysis of fish protein is commonly performed under acid rather than alkaline condition, usually by using hydrochloric acid at high temperatures. The FPH is then neutralized to pH 5.0–7.0, and dried for solid FPH (Wisuthiphaet and Kongruang 2015).

Enzymatic hydrolysis is employed at an appropriate condition depending on the optimum working condition of the enzyme. Several commercial enzymes available

in the market such as alcalase, neutrase, and papain have been used to hydrolyze fish protein as well as microbial local enzymes or self-prepared enzymes derived from plants such as papain from papaya and protease from biduri (*Calotropis gigantea*) (Muzaifa et al. 2012; Shen et al. 2012; Fawzya et al. 2017; Witono 2007). The type of enzyme, ratio of substrates/enzymes, and reaction period will affect the molecular structures and different functional properties as well as the possibilities of biological activities of FPH produced which could find application in various food formulations, nutraceuticals, or other products. Enzyme is added into the fish slurry, a homogenous mixture of minced fish and water with a ratio of 1:1 or 1:2 (Nurhayati et al. 2013; Muzaifa et al. 2011). However, fish meat could be hydrolyzed without water addition as reported by Himonides et al. (2011) and Rebeca et al. (1991). When a desired %degree of hydrolysis (%DH) is achieved, the hydrolysis is terminated by the addition of chemicals or heat treatment in line with the hydrolysis method. Sometime FPHs have a bitter taste which may affect consumer acceptance and act as an undesirable factor for applications. According to Sujith and Hymavathi (2011) and Aspevik (2016), it may be due to the peptides containing hydrophobic amino acid residues which release during the hydrolysis process.

16.3.4 Separation of FPH

Separation can be done by centrifugation or membranes separation techniques to isolate the liquid FPH from the solids. It needs gradual filtering treatment before using membranes separation, starting from the filter with a large pore size (about 300 mesh) followed by the filter of 600 mesh size, microfiltration and ultrafiltration with pore size of around 0.1 μm. The liquid FPH contains much water up to 90% and the rest is a mixture of peptides and amino acids. Whereas, the insoluble fraction or the sludge may be used as a flavor enhancer or animal feed, because it still contains high protein particularly glutamic acid (Martosuyono et al. 2019, Chasanah et al. 2019). The high water content of liquid FPH makes it unstable and perishable which need more careful handling, storage, transportation, and distribution.

16.3.5 Dehydration

Drying may be applied to reduce the rate of liquid FPH deterioration during storage, although it is difficult and a great challenge. In industrial practice, liquid FPH is spray dried to make a powdered form for further application. But on a laboratory scale, liquid FPH is commonly freeze-dried because it will produce a good quality of powdered FPH with higher protein content. But this is a very expensive drying method. Dried FPH is then packed properly and stored or distributed to the desired destination. Dried FPH is normally stored at 4 °C or lower, in some cases with vacuum packaging to prevent lipid oxidation (He et al. 2013).

16.4 Chemical and Nutritional Properties of Fish Protein Hydrolysate (FPH)

Fish protein hydrolysates have high protein content mainly due to the solubilization of the protein during the hydrolysis process and removal of insoluble materials containing non-protein compounds including fat. Depending on drying methods, the protein content of the FPH scan reaches 93% with complete amino acids composition, thus making them a good nutritional product. Most FPHs are amorphous powders, hygroscopic in nature, containing 81–93% protein, less than 5% fat, 3–8% ash and 1–8% moisture. FPH like this is produced by employing freeze dryer to dehydrate liquid FPHs. Whereas, a lower protein content of FPH may be produced by the use of spray dryer, with the protein content range between 20 and 33% depending on the filler used. Materials commonly used as a filler are maltodextrin, gum, whey protein concentrate, etc., which contain polysaccharides. However, this FPH still has good nutritional value, such as having high digestibility.

Hydrolysates of fish protein are rich in amino acids, whose composition may be varied depending on the type of enzyme used, hydrolysis condition, etc. Fish protein hydrolysate which is superior in lysine and glutamic acid was reported by Chasanah et al. (2019), Salamah et al. (2012), and Amiza et al. (2013). Leucine is another amino acid which is predominant after the two amino acids. A trout roe protein hydrolysates (TRH) produced by hydrolysis using pepsin and alcalase contained high amount of leucine and lysine amino acids (Rajabzadeh et al. 2017). Lysine is an essential amino acid which is very important for growing children, thus required to prevent stunting. Glutamic acid is correlated with umami taste and frequently used as a flavor enhancer. Composition and sequence of amino acids shows various bioactivities, where fragment with a peptide residue length between 2 and 20 amino acids and the presence of hydrophobic amino acids in addition to proline, lysine or arginine group commonly have health benefits due to their bioactivities.

16.5 Health Benefits of FPH

As an important source of dietary protein, fish protein, mainly their bioactive peptides suggest contributing to human health; however, particular researches at the cellular and molecular level are still limited. A number of studies demonstrated that FPHs have various bioactivities which depend on the molecular size of peptides and the type of their amino acid composition. Due to the cleavage of their molecule producing smaller molecules of protein (peptides and amino acids), FPHs can be easily absorbed and used in the body's metabolic system, and therefore they are good nutritional supplements (Nesse et al. 2011). Bioactive peptides (BP) generated from FPH have an important role in the metabolic functions, thus give benefit effect on health. Korczek et al. (2018) stated that the peptides, which are 2–20 amino acids in size, can act as neurotransmitters in controlling biological processes occurring in the body. They may increase the quality of life by affecting the digestive, endocrine, cardiovascular, immune, and nervous system (Sánchez and Vázquez 2017). The

biological activity of BP corresponds with the sequence and composition of amino acids in the protein (Khora 2013; Sánchez and Vázquez 2017; Korczek et al. 2018). Dong et al. (2017) revealed that the antioxidant activities (except for chelating activity) of peptides increased with increasing hydrophobic amino acid content. Similar to the statement, Kitts and Weiler (2003) wrote that many BP contain hydrophobic amino acids in addition to proline, lysine or arginine groups, and have also exhibit to be unaffected by the action of peptidases.

FPHs also gave an effect on the improvement of the immune status of the stunted children, which is thought to be due to the role of di/tri peptides along with essential and nonessential amino acids, micronutrients, and vitamins as a valuable composition of the FPH. Malnourished children may have inadequate intake of nutrition, particularly protein. Their serum compose less amino acids compared to normal-growth children. This condition affects in the suppression of mTORC1 (mammalian target of rapamycin complex 1) to synthesize proteins and lipids (Semba et al. 2016). mTORC1 is a protein complex working as a sensor of nutrient/energy/redox and regulating in the synthesis of protein (Hay and Sonenberg 2004).

Antioxidative peptides can be produced from the FPH by using various processes such as enzymatic hydrolysis, autolytic process using endogenous enzymes, and microbial fermentation. Putalan et al. (2018) found that protein hydrolysates from *Selaroides leptolepis* produced enzymatically by *Bacillus licheniformis* protease showed their antioxidant activity with the highest activity found in peptides at 3–5 kDa fraction. Meanwhile, antioxidant activities of protein hydrolyzates from fermented skipjack roe were found to be higher when it was fermented by *Lactobacillus plantarum* compared to spontaneous fermentation (Aditia et al. 2018). Autolysis with high endogenous proteolytic activity on Pacific hake fish also produced FPH with antioxidative properties (Samaranayaka and Li-Chan 2008). They mentioned that autolyzing fish mince for 1 h at 52 °C and pH 5.50 produced FPH with Trolox equivalent antioxidant capacity (TEAC) in the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical assay of 262 ± 2 μmol/g. However, several factors need to be considered to produce antioxidant peptides, such as fish protein sources, types of enzymes, hydrolysis method and conditions as well as method of hydrolysate separation. Various commercial proteases have been successfully used for the production of antioxidative peptides from fish protein sources (Bougatef et al. 2010; Naqash and Nazeer 2013; Yarnpakdee et al., 2015).

Fish protein hydrolysates may also have antihypertensive effect due to their ability in inhibiting the activity of Angiotensin converting enzyme (ACE). The enzyme catalyzes the transformation of angiotensin I to angiotensin II which potent as a vasoconstrictor causing of blood vessels constriction. As angiotensin II raises blood pressure, therefore inhibition of ACE can decrease blood pressure. Evaluation of FPH as natural ACE inhibitors (ACEI) on the treatment of hypertension has been documented. Chen et al. (2012) found that grass carp protein hydrolysates with MW distributions <3 kDa had the highest ACEI activity with IC_{50} = 0.308 mg/ml. Meanwhile, Putalan et al. (2018) who worked on yellowstripe protein hydrolysate investigated that the highest ACEI activity was found in the fraction of 3–5 kDa peptides with a percentage of ACE inhibitor as 97.15% (with a concentration of

10 mg/mL). Sanchez and Vasquez (2017) explained that inhibitors of ACE show that binding to ACE is strongly affected by the *C*-terminal tripeptide sequence of the substrate. ACE seems to prefer substrates or competitive inhibitors which predominantly have hydrophobic amino acid residues at the three *C*-terminal positions.

Fish protein hydrolysates with antimicrobial activities have recently been isolated from sardine (*Sardinella aurita*) (Jemil et al. 2014), rainbow trout by-products (Wald et al. 2016), Argentine croaker (*Umbrina canosai*) protein isolate (da Rocha et al. 2018), and skipjack roe (Aditia et al. 2018). As other bioactivities of FPH, antimicrobial activities of FPH can be associated with amino acid composition and/or sequence (Najafian and Babji 2012). They mentioned that the protein hydrolysates with antimicrobial activity generally have cationic amino acids in their composition. Da Rocha et al. (2018) reported that Argentine croaker protein isolate hydrolysates inhibit the growth of Gram-positive bacteria (*B. thermosphacta*, *L. innocua*, *L. monocytogenes*, and *S. aureus*) better than Gram-negative bacteria (*A. hydrophila* and *Y. enterecolitica*), but did not show any inhibitory effect on probiotics (*B. bifidum*, *L. acidophilus*, and *L. helviticus).* They also found that increasing degree of hydrolysis affects significantly in rising antimicrobial activities. Investigation on inhibition of protein hydrolysate from sardine against *E. coli* and *S. aureus* reported by Jemil et al. (2014) gave a greater inhibition zone compared to protein hydrolysate from skipjack roe result (Aditia et al. 2018). Antibacterial activity of protein hydrolysate from rainbow trout by-products was detected against fish farming pathogens *Flavobacterium psychrophilum* and *Renibacterium salmoninarum* rather than food contaminants (Wald et al. 2016).

Anti-inflammatory effect of a commercial protein hydrolysate derived from pacific whiting (*Merluccius productus*) through yeast fermentation was shown from evidence that hydrolyzates can induce proliferation and migration in intestinal epithelial cells (Fitzgerald et al. 2005). A novel peptide with inhibitory effects on colitis-induced mice by dextran sulfate sodium from Pacific oyster *Crassostrea gigas* hydrolysates indicated that the hydrolysate has anti-inflammatory activity, and thus potential for application in human disease (Hwang et al. 2012). Another study showed that salmon protein hydrolysates regulate lipid metabolism, gave a high fatty acid anti-inflammatory index and increased plasma carnitine level in high fat-fed mice which will have an impact on reducing obesity (Bjorndal et al. 2013).

Antiproliferative peptides isolated from tuna dark muscle were found to be potential in inhibiting the growth of breast cancer (MCF-7 cells). The peptides contained proline (LeuPro-His-Val-Leu-Thr-Pro-Glu-Ala-Gly-Ala-Thr and Pro-Thr-Ala-Glu-Gly-Gly-Val-Tyr-Met-ValThr) (Hsu et al. 2011). According to Nurdiani et al. (2017), the presence of proline and lysine in the peptide sequence may be important amino acids that act on the antiproliferative activity, as the finding that nutrient mixture (NM) contained proline and lysine had a high toxicity for DU-145 cells (Roomi et al. 2015).

16.6 Application of FPH to Food Products

Application for food is one of the leading FPH application segments of the market after animal feed and pet food. The total market demand of FPH is expected to over USD 525 million by 2024 (Global Market Insight 2019). It is predicted that demand for FPH as a food ingredient will increase due to its advantageous properties. Fish protein hydrolysate as a natural bioactive product has not been well developed in Indonesia; however, in many countries, traditional and commercial production of FPH are currently used as health foods/functional foods/nutraceuticals. As a high protein ingredient, initially the use of FPH in food was primarily intended to increase the protein content of the food. However, findings related to the functional properties of FPH and the benefits of bioactive peptides for health, the purpose of FPH application in food develops for specific purposes. The use of FPHs as functional ingredients in the food application is due to their specific and important characteristics such as water holding capacity, oil absorption capacity, protein solubility, gelling activity, foaming capacity, and emulsification ability (Chalamaiah et al. 2012).

Fortification of protein hydrolysates powder has been tested to various food and drinks such as snacks like cheese stick, crackers, milk cereals, etc., for protein enrichment (Kristinsson and Rasco 2000; Ariyani et al. 2000; Egerton et al. 2018). A commercial FPH generated from salmon in the chocolate drink has been given to malnourished children for trial study and investigated the effect on the immunological parameters (Nesse et al. 2011).

Hydrolysate of the fish protein generated from "kuniran" (local name) or goldband goatfish (*Upeneus moluccensis*) produced enzymatically by a local microbial protease has been used to prepare baby porridges as a complementary food for breast milk. The best formula was then evaluated for digestive nutrition therapy. It was found that adding the FPH into instant baby porridge formula increased significantly the body weight, digestibility, and protein absorption of mice intestine (Chasanah et al. 2018). Baby porridge fortified by FPH produced from *Clarias gariepinus* was reported by Aprillia and Hati (2016).

The use of octopus protein hydrolysate (OPH) prepared enzymatically using crude extract bromelain from pineapple to produce sport nutrition drinks was reported by Riyanto et al. (2016). Nowadays, sport nutrition products have become healthy and convenient lifestyle which are consumed by professional athletes as well as other healthy products consumers. The basic sports drink formulation are water, sugar, and salt, but can be varied by the addition of other components such as proteins and amino acids as well as various of other ingredients. Taurine is an important ingredient in the sport drinks which can increase the stamina of athletes. Application of OPH for the sport drink showed that the best formula was produced from the addition of 4% OPH. The drink in serving size of 600 ml contained taurine 726.06 $\pm$ 0.82 mg, total essential amino acids 4441,75 mg, and total non-essential amino acids 6525,77 mg.

Fish protein hydrolysates from Yellowtail Kingfish have been applied to fried food to study whether it can reduce the oil content of the fried food (He et al., 2015).

It was shown that the FPH can decrease the fat uptake of deep-fried battered fish significantly from about 7–4.5% by replacing 1% (w/w) batter powder with FPH.

Effect of FPH in enhancing water retention in Sous Vide processing of salmon fillets was identified by replacing the most amount of salt added with FPH. The combination of 14% FPH and 0.2% salt achieved almost the same with 2% salt producing the standard level of water retention (Ibarra et al. 2013).

The use of FPH as cryoprotective agents was studied by Cheung et al. (2009). They found that FPH from Pacific hake (*Merluccius productus*) can be used as an alternative to the 1:1 blend of sucrose-sorbitol, a cryoprotectant commonly used for keeping the quality of frozen minced fish.

16.7 Challenges and Opportunities

So far production and application of bioactive peptides are still limited to the research area in a laboratory scale at an early stage. It still needs much more in vivo data for the development of their applications for human health purposes. On the other hand, the production of FPH may be developed at a pilot or commercial scale through a cooperative system between industries and small-medium-scale fish processors. The stages of the process up to the hydrolysis can be done by small-medium-scale processors, while the separation of hydrolysates and drying process that requires more complex of technology, skills and investment are performed by the industry, including packaging, storage and distribution of products, due to the properties of FPH which are very hygroscopic and should be properly handled.

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Caulerpa: Ecology, Nutraceutical and Pharmaceutical Potential

17

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Abstract

The green seaweed *Caulerpa* is one of the green seaweeds which are the most widely distributed seaweed from tropical to subtropical marine waters. Some of *Caulerpa* species are considered as invasive species causing negative impacts to marine ecology. However, the utilization of *Caulerpa* for nutraceutical and pharmaceutical purposes has been studied intensively. *Caulerpa* is well known as nutritious food in Japan, Korea, and Southeast Asia countries. Sulfated polysaccharides and fucose residues of *Caulerpa* are reported to have medicinal activity. PUFA content is about 60.8% of total fatty acids which are dominated by α-linolenic acid. Amino acids dominating in the protein of *Caulerpa* are aspartic and glutamic acid. *Caulerpa* is also found as a good source of minerals, essential trace elements, vitamins, and rich in pigments of chlorophyll-a, chlorophyll-b, β-carotene, and caulerpin. *Caulerpa* is not only highly nutritious but also rich in important compounds as a potential source of therapeutic agents. The majority of secondary metabolites isolated from *Caulerpa* are sesquiterpenoids, acetylenic sesquiterpenoids, xanthophyll, triterpenes, sterols, caulerpin, and caulerpenyne. For pharmaceutical uses, *Caulerpa* is identified to have bio-prospecting compounds demonstrating numerous bioactivities such as anticancer, insecticidal, antibacterial, anti-inflammatory, antidiabetic, and antiplasmodial. Nowadays, *Caulerpa* can be produced extensively by implementing culture methods. The remarkable development of *Caulerpa* leaves many prospects and challenges to make this seaweed become more useful.

Keywords

Caulerpa · Ecology · Nutraceutical · Pharmaceutical · Bioactive

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17.1 Introduction

Caulerpa is one of the green seaweeds which are widely distributed from tropical to subtropical marine waters. Nowadays, some *Caulerpa* species have been utilized as food in Japan, Korea, and Southeast Asia countries. However, some *Caulerpa* species are considered as an invasive species causing negative impacts to marine ecology. On the other hand, the utilization of *Caulerpa* for nutraceutical and pharmaceutical purposes has been studied intensively. The remarkable development of *Caulerpa* leaves many prospects and challenges to make this seaweed more useful. In this chapter, we will comprehensively highlight the ecology, nutraceutical and pharmaceutical potential of *Caulerpa.*

17.2 Biology and Ecology of *Caulerpa*

Caulerpa, one of the genera in the green seaweed, comprises of 97 species and has been recognized universally based on its taxonomical classification (Guiry and Guiry 2019). The genus *Caulerpa* belongs to the *Bryopsidophyceae* (Famà et al. 2002; Van Den Hoek et al. 1995) within the family of *Caulerpaceae* (Schembri et al. 2015). *Caulerpa* is a member of coenocytic siphonous marine green algae (Ohba et al. 1992) which means that each organism consists of one large cell with multiple cell nuclei, not like most organisms composing of many different cells (US Fish and Wildlife Service 2018). *Caulerpa* is the largest and the most different single-celled organism in the world. No cell wall or membrane separates each of the many nuclei and cytoplasm that are close to each other. Even though no cell wall or membrane separates each of the many nuclei and cytoplasm that are close to each other, *Caulerpa* differentiates into the complex structure of leaves, stems, and roots (Guiry and Guiry 2019; Jacobs 1994).

The "roots" of *Caulerpa* are called *rhizoid.* It can grow into the ocean floor or substrate to absorb water and nutrient (US Fish and Wildlife Service 2018). Rhizoid of *Caulerpa* can attach in both unconsolidated and solid substrate (Friedlander et al. 2006; Dawes 1998), and it also can produce bio-adhesive (Levi and Friedlander 2004). The stem-like structures in *Caulerpa* are called stolons. It spread horizontally over the substrate, and it is the place where the rhizoid was anchored. The development level of stolon and rhizoid depends on the substratum. Stolons are interconnected not only with rhizoid but also with the fronds. The fronds are "leaf-like" and photosynthetic structure in *Caulerpa* (US Fish and Wildlife Service 2018). The photosynthetic fronds or assimilators are very diverse morphologically. The variation of morphological shape of fronds is thread-like, blade-like, pinnate, spongy, and vesicular structures (Guiry and Guiry 2019). Another differentiation regarding the type of fronds in *Caulerpa* are feather-like (*Caulerpa ashmeadii, Caulerpa mexicana, Caulerpa sertularoides, Caulerpa scalpelliformis, Caulerpa taxifolia*), grape-like (*Caulerpa racemosa, Caulerpa racemosa var. cylindracea, Caulerpa macrophysa, Caulerpa microphysa*), flat-like (*Caulerpa racemosa var. lamourouxii, Caulerpa brachypus, Caulerpa prolifera*), disc-like (*Caulerpa*

Fig. 17.1 Some *Caulerpa* species collected from Indonesian waters

racemosa var. peltata and *Caulerpa nummularia*), toothed-margins (*Caulerpa cupresoides* and *Caulerpa serrulata*), and fuzzy (*Caulerpa verticilata* and *Caulerpa paspaloides*) (USC Dorsife 2019). There are several types of branching in *Caulerpa*. The most ancient type of branching is radial branching, and the newer type is bilateral. The chloroplast structure supported both types of branching. The thallus of *Caulerpa* consists of a coenocytic filament or siphon (Guiry and Guiry 2019) (Fig. 17.1).

The reproduction of *Caulerpa* species can be performed both sexually and asexually. The most common reproduction process in *Caulerpa* is through the fragmentation process (asexually). In contrast, sexual reproduction in *Caulerpa* seems rare. This kind of reproduction most often happens at warm temperatures (US Fish and Wildlife Service 2018). The differentiation between the fertile plants and non-fertile plants of *Caulerpa* can be detected by looking at its reticulate protoplasmic masses. In contrast to the reticulate protoplasmic masses of infertile plant that is colorful, the non-fertile plant has a homogenous color of reticulate protoplasmic masses (Panayotidis and Žuljević 2001).

The cytoplasms in the rhizome part migrate into fronds, and this condition makes the color of the most rhizome change into white. The protoplasmic network becomes bicolored in light of green and brownish orange 12 h before the gamete release (Panayotidis and Žuljević 2001). There are two types of biflagellate gametes on the same plant. The female gametes contain an orange stigma, and typically its size is larger than the male gametes. The male gametes are smaller and have no stigma in it. The presence of both sex gametes in the same plant is expected to be based on two-color protoplasmic tissue caused by sexual segregation of gametes throughout the thallus and the stigma of redness in female gametes (Panayotidis and Žuljević 2001; Ohba and Enomoto 1987; Goldstein and Morral 1970). The changes in protoplasmic consistency were followed by the formation of numerous slightly white cylindrical papillae, which appeared as outgrowths on the frond axis, branchlets, and rhizomes. When the gametes are released into the water column, these gametes formed a large green cloud around the mother plants. The gametes usually dispersed for 5–10 min. After the gametes are released, all protoplasmic materials streamed out and only the white, ghost thalli persisted (holocarpy). All of the fertile plants released their gametes synchronously approximately 14 min before sunrise: the first gametes cloud appeared 17 min, and the last 12 min before sunrise (Panayotidis and Žuljević 2001). Biflagellate gametes released by the macroscopic thallus fuse to form zygotes which re-establish the macroscopic stage (Ohba et al. 1992; Ohba and Enomoto 1987; Phillips 2009; Price 1972; J. I. H. H and Enomoto 1981).

Besides sexual reproduction, *Caulerpa* can also reproduce by the asexual process. This process is considered to be most important in *Caulerpa.* Asexual reproduction can happen in 2 different ways—first, asexual reproduction by vegetative growth or patch expansion. Second, asexual reproduction by fragmentation, dispersal, and re-establishment of drifting fragments (Ceccherelli and Cinelli 1999; Frada-Orestano et al. 1994). For *Caulerpa*, the most common asexual reproduction occurred is by fragmentation process. It happens when a small piece of the alga breaks off and re-established at another location. This process can happen easily as *Caulerpa* is a single-celled organism (US Fish and Wildlife Service 2018). Several essential sources that lead to the fragmentation process in *Caulerpa* are the wave action in shallow habitats and the human activities that give impact to the fragmentation of *Caulerpa* such as professional and recreational fishing activities in meadows of the alga, impact from the boat propellers and the anchors (Jacobs 1994; Ceccherelli and Cinelli 1999).

Green alga from genus *Caulerpa* can be found in the intertidal and subtidal zones of tropical and subtropical coastal water around the world. It can grow on sandy and rocky reef substrate. *Caulerpa* can colonize to various reef habitats because of its particular stoloniferous systems and its well-developed rhizoid (Belleza and Liao 2007; De Senerpont Domis et al. 2003). Recent research interest has shown that *Caulerpa* has expanded their ranges not only in tropical and subtropical waters but also into the more temperate environment (Famà et al. 2002; Meinesz and Boudouresque 1996; Piazzi et al. 1994; Dalton 2000; Kaiser 2000).

Phenotypic plasticity and morphological variability within members of the genus have resulted in the unstable classification of *Caulerpa* species into numerous

varieties and forms (Calvert 1976). Many taxa form separate units well with relatively little morphological variability. However, some species showed rampant morphological plasticity and unclear taxonomic boundaries (Famà et al. 2002). The upright branches bearing the assimilators have been traditionally used to delineate species within the genus. Environmental factors, such as light intensity and wave action, have been shown to influence the size, shape, and arrangement of assimilators of some species, such that many transitional forms can be recognized within species (Calvert 1976). Variability in the growth forms and the photosynthetic performance of *Caulerpa* species seem to be related to the substrate, light intensity, and water movements (Famà et al. 2002; Collado-vides and Robledo 1999).

17.3 Nutraceutical Products from *Caulerpa*

17.3.1 Nutrient Contents

Macroalgae are rich in nutritional values such as proteins, soluble dietary fibers, vitamins, minerals, phytochemicals, bioactive antioxidants, and polyunsaturated fatty acids (de Gaillande et al. 2017). That nutritional content in macroalgae is mostly depending on the individuals, species, maturity, life cycle, and several environmental factors such as water temperature, salinity, light, and nutrients (Mohamed et al. 2012; Ito and Hori 1989).

17.3.1.1 Polysaccharide Contents

Polysaccharides are water-soluble, biodegradable, and functionally active compound therefore, potential to be applied in biomedical applications. Sources of polysaccharides for such applications are available from agricultural produce, microorganisms, and marine resources (Mayakrishnan et al. 2013). Among marine resources, seaweed comprises several important polysaccharides with different chemical properties among taxonomic classes of seaweeds. Principally, polysaccharides are classified into three groups: (a) structural polysaccharides, (b) intercellular mucilage, and (c) storage polysaccharides (Ito and Hori 1989).

Seaweed polysaccharides are rich in dietary fibers which have a beneficial effect on preventing metabolic syndrome associated with obesity, i.e., type 2 diabetes, and cardiovascular diseases. Dietary fiber consumption increases the feeling of satiety. Due to its bulking capacity, seaweed dietary fibers, especially the soluble and viscous ones, are not only satiating but also reducing appetite and energy intake, slowing gastric emptying as a result modifying the postprandial lipemia, and in turn improving blood glucose levels and dyslipidemia (Jakobsdottir et al. 2014; Bocanegra et al. 2009).

The polysaccharide content of some *Caulerpa* species (%, dry weight) was reported as follows: *C. cupressoides* 47.4%, *C. lentillifera* 11.8–64.0%, *C. racemosa* 3.6–83.2%, *C. racemosa var. clavifera f macrophysa* 52.8%, *C. racemosa var. laetevirens* 50.2%, *C. racemosa var. turbinata* 18.7%, and *C. sertularioides* (nd) 34–39%. Polysaccharides of *Caulerpa* consist of galactose,

sulfate, arabinose, glucose, xylose, and small amounts of mannose, rhamnose, and traces of fucose residues. Polysaccharides derived from seaweed were reported to have medicinal values as antiviral, antitumoral, immunostimulant, immunomodulating, anticoagulative, and antioxidant activities (Ghosh et al. 2004; Maeda et al. 2012). Moreover, marine macroalgae contain sulfated polysaccharides, bioactive compounds which have various biological activities. The sulfated polysaccharides from *Caulerpa* species were reported to exhibit a novel potential source to be explored in biomedical research as antinociceptive and anti-inflammatory activities (Ribeiro et al. 2014), anticoagulant, anti-edematogenic and antithrombotic (Rodrigues et al. 2012), antioxidant activity (Qi et al. 2005), immunostimulatory (Maeda et al. 2012), and antiherpetic (Ghosh et al. 2004).

17.3.1.2 Protein and Amino Acids Profile

Green seaweeds contain moderate amounts of protein ranges from 9 to 26% (w/w). In terms of *Caulerpa* species, the protein contents are ranging from 0.6 to 20.8%, and its quality depends on their essential amino acid content. The protein content is greatly affected by the species, living environments, and seasons (Fleurence 1999). The protein content (%, dry weight) in some edible *Caulerpa* species were reported for *C. cupressoides* (20.8%), *C. lentillifera* (6.6–19.4%), *C. racemosa* (0.6–18.3%), *C. racemosa var. clavifera f macrophysa* (17.4%), *C. racemosa var. laetevirens* (17.3%), *C. racemosa var. turbinata* (12.5%), and *C. sertularioides* (3.1%) (Nagappan and Vairappan 2014; Kumar et al. 2011; Kawaroe et al. 2013; Marquez et al. 2014; Carneiro et al. 2014; Rameshkumar et al. 2012; Santoso et al. 2006; Llah Al-Saif et al. 2014). Additionally, amino acid contents (mg/g, dry weight) of some edible *Caulerpa* are essential amino acids (EAA); threonine (0.51–0.79), valine (0.57–0.87), lysine (0.12–0.82), leucine (0.69–0.99), phenylalanine (0.61–1.99), histidine (0.08–0.20), methionine (0.16–0.18), non-essential amino acids; asparagine (0.83–1.43), serine (0.45–0.76), glutamate (1.13–1.78), glysine (0.51–0.85), arginine (0.49–0.87), alanine (0.49–0.85), tyrosine (0.33–0.48), and proline (0.43–0.57) (Matanjun et al. 2009). The amino acids profile of *Caulerpa* species was dominated by aspartic and glutamic acids content. Otherwise, the contents of histidine, lysine, and methionine are the most limiting amino acids. Therefore, the level of essential amino acids daily intake recommended by World Health Organization could be contributed by *Caulerpa* consumption (Alam Bhuiyan and Qureshi 2016).

Bioactive peptides have been attracted due to their potential in promoting health and reducing disease risks. Bioactive peptides of ribulose-1,5-bisphosphate carboxylase/oxygenase isolated from *Caulerpa cylindracea*, *Caulerpa taxifolia, Caulerpa racemosa var. lamourouxii*, and *Caulerpa racemosa f. occidentalis* exhibited the best potential for ACE and DPP-IV inhibitor, neuroprotective and antioxidative peptides (Agirbasli and Cavas 2017). The bioactive peptide that demonstrated ACE inhibitory and anticancer activity was also isolated from *Caulerpa microphysa* (Lin et al. 2012). Lectin of *Caulerpa cupressoides var lycopodium* was also reported to exhibit anti-inflammatory and antinociceptive activities (Da Conceição Rivanor et al. 2014).

17.3.1.3 Lipids and Fatty Acid Profile

The lipid content of *Caulerpa* species is relatively low (0.1–7.2% of dry matter); however, *Caulerpa* has a high content of polyunsaturated fatty acids (PUFAs), essential nutritional components in humans and animals, lipid composition in marine algae has raised considerable interest (de Gaillande et al. 2017). *Caulerpa* contains four essential fatty acids, i.e., palmitic, α-linolenic, linoleic, and hexadecatrienoic acids; more than 60% of all FAs. The typical *Caulerpa* genus is its high content of hexadecatrienoic acid together with a low concentration of 16:4ω3 and 18:4ω3. Likewise, *Caulerpa* species contain low C18 monounsaturated acids and approximately equal amounts of the ω9 and ω7 isomers, which make them differ from other green algae species (Khotimchenko et al. 1995).

The lipid content (%, dry weight) of some *Caulerpa* species were reported, i.e., *C. cupressoides* (3.8%), *C. lentillifera* (0.8–7.2%), *C. racemosa* (0.1–3.8%), *C. racemosa var. clavifera f macrophysa* (2.2%), *C. racemosa var. laetevirens* (2.1%), *C. racemosa var. turbinata* (2.1%), and *C. sertularioides* (2.3%) (Nagappan and Vairappan 2014). Furthermore, fatty acid compositions (% of total fatty acids) in some *Caulerpa* edible species were reported, i.e., SAFs (13.17–93.06%), MUFAs (5.70–14.10%), PUFAs (9.49–60.80%), PUFAs ω6 (4.0–19.7%), PUFAs ω3 (7.55–38.0%), and ratio ω6/ω3 (0.11–2.85%) (de Gaillande et al. 2017; Nagappan and Vairappan 2014; Kumar et al. 2011; Matanjun et al. 2009; Paul et al. 2014). As recommended by WHO, the daily intake ratio of ω-6 to ω-3 fatty acids should not exceed 10, whereas the ratio ω6/ω3 of *Caulerpa* species is very low (0.11–2.90) (Nagappan and Vairappan 2014).

Among *Caulerpa* species, *C. racemosa* has the highest PUFA content which reached 60.8% of total fatty acids α-linolenic acid (C18:3w3). Some n-3 PUFAs, especially EPA and DHA, play an important role in the prevention of cardiovascular diseases (Mozaffarian et al. 2005; Van Ginneken et al. 2011). PUFAs are major components of brain cells which are crucial for proper development and functioning of the brain and the nervous system (Van Ginneken et al. 2011; Sinclair et al. 2007), increase in lifetime expectancy (Lutz et al. 2008), increase in muscle protein synthesis in elderly and preventing sarcopenic obesity (Van Ginneken et al. 2011).

17.3.1.4 Mineral Content

Due to their life environment, macroalgae have high content of diverse minerals (MacArtain et al. 2007). Mineral content in macroalgae is higher than that in terrestrial plants and animal products (Ito and Hori 1989; Ruperéz 2002). Minerals act as enzymes cofactors in the biochemical body reactions. Therefore, mineral deficiency resulted in severe health impairment (Santoso et al. 2006).

The ash content of *Caulerpa* reached up to 55% of dry matter representing its high minerals content. Moreover, all edible *Caulerpa* contain high amounts of both macroelements (Ca, Mg, Na, P, and K) and microelements (Fe, Zn, Mn, Cu, Se, and Mo) (Matanjun et al. 2009). The macro- and microelements content (g (100 g)$^{-1}$ dry weight) of edible *Caulerpa* species were reported as follows: Na (nd–9.0), K (0.03–3.9), Ca (0.6–5.9), Mg (0.4–4.1), Na/K (nd–100), Fe (<0.01–81.3), Zn (0.002–3.5), Mn (nd–7.9), Cu (<0.01–2.5), Se (nd–12.3), Mo (nd–0.3) (de Gaillande

et al. 2017; Kumar et al. 2011; Matanjun et al. 2009; Paul et al. 2014). Thus, edible *Caulerpa* species are a potential source of minerals. The intake of high Na/K ratios has been associated with hypertension risk. Consumption of seaweed could help to balance the high Na/K ratio diets. Depending on the rinsing process and the methodology of mineral content determinations, the level of Na/K ratios in *Caulerpa* species is highly variable from 0.3 to 2.3 (Matanjun et al. 2009).

Iron content in macroalgae is higher than in many well-known iron terrestrial sources, such as meats and spinach (MacArtain et al. 2007). The iron content of *Caulerpa species* is highly variable. It can reach up to 81.3 mg $(100\ g)^{-1}$ of dry matter for *C. racemosa*, which is more than five times of the recommended daily intake (RDI) (Santoso et al. 2006). Iron plays an important role in cell functions such as in DNA synthesis, oxygen transport, and electron transport (de Gaillande et al. 2017; Mišurcová et al. 2011).

With a high content of magnesium and calcium, the RDI requirement can be attained with less than 15 g of fresh *Caulerpa*. Calcium is not only an essential mineral in bones building, but also in muscle contraction and blood vessel contraction. Whereas, magnesium is essential for synthesizing DNA, proteins, and strengthening bones (Rupérez 2002). Edible *Caulerpa* also contains copper and selenium, the microelements known for having one of the narrowest ranges of beneficent effects for all elements. Depending on the concentration, copper and selenium can act as essential microelements or toxic substances. The high level of these elements shows the high heavy metal contamination of the environment. Thus, for regular consumption, it should be recommended to consume seaweeds that grow in a clean environment (de Gaillande et al. 2017).

17.3.1.5 Vitamin Content

The habitat of macroalgae in the intertidal zone is exposed with oxidative stress from UV radiation, desiccation and extreme temperature fluctuation resulted in the algae to contain many forms of powerful antioxidants including vitamins, carotenoids, phlorotannins, and protective pigments (MacArtain et al. 2007; Lalitha and Dhandapani 2018). The vitamin composition (mg100 g^{-1} wet weight) of edible species of *Caulerpa*, i.e. β-carotene (0.19–8.15), thiamin (nd-0.05), riboflavin (nd-0.06), niacin (1.05–1.09), vitamin C (1.00–13.68), vitamin E (nd-9.40), vitamin A (<3–2160), and vitamin B12 (nd-0.29) (de Gaillande et al. 2017; Matanjun et al. 2009; Paul et al. 2014).

Edible *Caulerpa* has large vitamin C and vitamin E which are up to 46.3 and 62.7% of the RDI, respectively, per 100 g *Caulerpa* consumed (de Gaillande et al. 2017). The vitamin C and E are powerful antioxidants that boost resistance to diseases and oxidative stress. Antioxidant activity of vitamin C protects hydrogen/electron carriers and maintains suitable redox levels for enzyme systems. It also plays in the biosynthesis of hormones and deoxyribonucleic acid. Recently, several research demonstrates the high antioxidant activity in *Caulerpa* genus (Matanjun et al. 2009; Lalitha and Dhandapani 2018; Nguyen et al. 2011; Santoso et al. 2004).

17.3.1.6 Pigment Content

The primary pigments found in green algae are chlorophyll-a and chlorophyll-b, zeaxanthin, lutein together with minor proportion of carotenoids. The pigments content is affected by environmental variables such as light, dissolved oxygen, nitrite, phosphate, and silicate (Sarojini et al. 2015).

Pigment contents in some *Caulerpa* species were reported as follows: *C. racemosa* (Chl-a 5.77 ± 0.45 mg/g, Chl-b 3.22 ± 0.19 mg/g, β-carotene 0.42 ± 0.03 mg/g), *C. lentillifera* (Chl-a 2.58 ± 0.25 mg/g, Chl-b 1.47 ± 0.14 mg/g, βcarotene 0.15 ± 0.01 mg/g), and *C. sertularioides* (Chl-a 14.4 ± 2.9 mg/l, Chl-b 20.2 ± 7.0 mg/l, β-carotene 0.18 ± 0.12 μg/g) (Paul et al. 2014; Sarojini et al. 2015).

17.3.2 Utilization of *Caulerpa*

Asian and Indo Pacific countries were widely consumed edible seaweeds, in the form of fresh, dried, or ingredients in prepared foods (Nagappan and Vairappan 2014; Ratana-arporn and Chirapart 2006). *Caulerpa lentillifera* is one of the edible *Caulerpa* species that favored to be consumed due to its grass-green in color, soft, and succulent texture. *C. lentillifera* usually consumed as fresh vegetable or salad or as a salt-preserved form in Japan, Korea, Philippines, and some other countries in Southeast Asia (Nguyen et al. 2011; Ratana-arporn and Chirapart 2006).

Seaweeds have been utilized in various food products such as cereals, dairy, meat/fish, and fruit/vegetable products to give the additional function of the food. Since the seaweeds are consumed by wider customer, the bakery products can be one of suitable carriers for seaweed utilization as a nutraceutical. Application of *C. racemosa* in biscuit increased the nutrient content such as protein, fiber, mineral and vitamin, and the phenolic content and antioxidant activities (Kumar et al. 2018). When *C. racemosa* was added in jelly candy product, consumers' acceptance was ranging from "neutral" to 'like" level (Tapotubun et al. 2018). Up till now, even that edible *Caulerpa* species are proven to have high nutritional and bioactive contents, but their product developments are still limited.

17.4 Potential Pharmaceutical Properties of *Caulerpa*

Since ancient times, secondary metabolites or natural products have been used as important sources for the treatment of many diseases (Dias et al. 2012). Natural products including those isolated from marine sources have become prospective drug leads (Mishra and Tiwari 2011; Villa and Gerwick 2010). Approximately about 27,000 marine natural products were identified, including eight of them were lead compounds of seven marketed drugs (Altmann 2017). Among marine resources, macroalgae, abundant, and renewable marine resources have been intensively explored for pharmaceutical and nutraceutical applications (Heffernan et al. 2014). It was hypothesized that environmental stress conditions such as saline, light,

temperature, competition, and predation triggered macroalgae to produce bioactive natural products (Barbosa et al. 2014; Christaki et al. 2013; Shalaby 2011).

Among macroalgae, *Caulerpa* spp. Green algae are abundantly found worldwide in the tropical and subtropical marine environment. Various bioactive compounds of the genus have been widely studied and reported for numerous pharmacological properties. Two of the well-known bioactive compounds of *Caulerpa* are the indole alkaloids caulerpin, in which its structure was firstly proposed in 1970 (Liu et al. 2012) and the water-soluble sesquiterpene caulerpenyne (CNY). Both of those natural products were reported to have a wide range of biological activities mainly as antitumor and antibacterial activities (Barbosa et al. 2014; Sfecci et al. 2017). The pharmaceutical and biotechnological application potentials of *Caulerpa* spp. Secondary metabolites will be highlighted here.

17.4.1 Antibacterial

The increasing occurrence of antibiotic resistance is one of the vital global problems in medicine. Hence, discovering new antibacterial compounds is a crucial demand. Up till now, natural products still hold potential as the basis for antibacterial discovery (Jackson et al. 2018) which resulted in an enormous number of antibacterial bioprospecting activities. The studies on the antibacterial potential of marine macroalgae natural products including those from *Caulerpa* spp. were significant.

Caulerpenyne (Cyn), a sesquiterpenoid which firstly isolated from *Caulerpa prolifera* in 1978, is one of the most abundant metabolites of some *Caulerpa* species, i.e., *C. brownie*, *C. taxifolia*, and *C. racemosa* (Sfecci et al. 2017; Sridhar and Vidyavathi 1991; Cevik et al. 2016). Cyn that is acting as a wound closure metabolite of *Caulerpa* (Adolph et al. 2005) was also observed as an antibacterial compound (Hodgson 1984). Another major secondary metabolite from *Caulerpa* is caulerpin (Clp). Clp was not only reported to show some antibacterial activities against several pathogen bacteria, i.e., *E. coli, S. aureus, Salmonella* sp., and *Streptococcus* sp. (Nagappan and Vairappan 2014) but also demonstrated an excellent activity against bacillus *Mycobacterium tuberculosis.* The CLP able to inhibit 70% of tested bacillus *M. tuberculosis*, which indicated that the compound was very potential to be used as a lead compound for the development of antituberculosis drugs (Canché Chay et al. 2014). Nagaraj et al. (Nagaraj and Osborne 2014) isolated 2-(-3-bromo-1-adamantyl) acetic acid methyl ester and Chola-5, 22- dien-3-ol from *Caulerpa racemosa* which exhibited some bactericidal activities against tested pathogens, i.e., *Pseudomonas aeruginosa* and *Escherichia coli.* Studies on antibacterial bioprospecting of *Caulerpa* extracts have been conducted by many researchers in the world. Rizzo et al. (2016) reported that *Caulerpa cylindracea* collected from Torre Guaceto, Italy showed antibacterial activity against *Vibrio species.* Al-Saif et al. (2014) reported the antibacterial activity of *C. occidentalis* and *C. socialis* extracts collected from the Jeddah coast against several pathogenic bacteria. Others studies on antibacterial bioprospecting of *Caulerpa* spp. were also

reported, i.e., *C. prolifera*, *C. ashmeadii*, *C. paspaloides* from Mexico (Freile-Pelegŕn and Morales 2004), *C. racemosa* from Tanzania (Mtolera and Semesi 1996) and Indonesia (Zainuddin et al. 2019; Rusli et al. 2016), and many other reports indicating that natural products of *Caulerpa* spp. showed significant potential as antibacterial.

17.4.2 Anticancer

Up till now, cancer is still the main cause of death in the world. Thus, the search for new potent anticancer drugs to fight the disease is necessary. Historically, natural products have played a dominant role as anticancer drug leads. To date, many researchers in the world are focusing on bioprospecting research of natural products to find anticancer compounds with unique structures and mechanisms of action. In terms of anticancer bioprospecting activities from *Caulerpa* spp., numerous reports were published.

Extract of *C. taxifolia* was reported to induce breast cancer cell cycle arrest that leads to its antiproliferative activity (Mehra et al. 2018). The extract also induced oxidative stress and showed effects on mitochondrial membrane potential on breast cancer (Mehra et al. 2018). The caulerpenyne (Cyn) was also reported as a potential antitumor compound. Barbier et al. (Barbier et al. 2001) observed the antiproliferative activity of Cyn against tumor cell line SK-N-SH without G2/M phase blockage and increases cell death. They also studied that Cyn induces neurites loss and microtubule network compaction at the cell periphery. Furthermore, the cytotoxic activity of Cyn against 8 types of human cancer cell lines, with the best activity against colorectal cancer, was also reported (Fischel et al. 1995). Yang et al. (Yang et al. 2015) reported that racemobutenolids B, 4′,5′-dehydrodiodictyonema A, α-tocospiro A and α-tocopherol quinone compounds isolated from *C. racemosa* showed moderate cytotoxicity against human lung adenocarcinoma cells (A-549) and against human promyelocytic leukemia cells (HL-60).

17.4.3 Neuroprotective Activity

As the prevalence of neurodegenerative diseases, i.e., dementia, Alzheimer's, and Parkinson's are increasing, many researchers have been focusing on natural products bioprospecting with neuroprotective activity.

One enzyme that plays an important role in neurodegenerative disease is lipoxygenase (LOX). LOX, particularly 5-LOX, is upregulated in Alzheimer's disease (Ikonomovic et al. 2008). Cengiz et al. (2011) reported that *C. prolifera* extract showed potent lipoxygenase inhibitory effects. They also reported that the extract of *C. racemosa* also showed LOX inhibition activity with lower level compared to the *C. prolifera* extract due to less caulerpenyne content. Whereas, the pure caulerpenyne inhibited LOX with IC_{50} level of 5.1 μM. Thus, caulerpenyne, which is a major secondary metabolite of *Caulerpa* species, could be potential as a

new therapeutic for Alzheimer's (Barbosa et al. 2014). Liu et al. (2013) isolated 2 novel bisindole alkaloids of Racemosin A and Racemosin B from *C. racemosa*. They reported that Racemosin A, a unique bisindol that possesses seco-indolo[3,2-α] carbazole skeleton, showed a neuroprotective activity which significantly decreased the SH-SY5Y cell damage incomparable level to epigallocatechin as a positive control. Caulerpin that was isolated from *C. racemose* was reported (De Souza et al. 2009) to have antinociceptive activity. De Souza et al. described that in the abdominal constriction assay, caulerpin was able to reduce the acetic acid-induced nociception at 0.0945 μmol which is comparable to dypirone at 0.0426 μmol. Furthermore, caulerpin also showed a favorable result of antinociception (inhibit the sensation of pain) in the in vivo hot plate test (De Souza et al. 2009). They also reported that caulerpin was able to inhibit the inflammatory process through inhibition of inflammation key enzymes, such as cyclooxygenase (COX).

17.4.4 Obesity and Cholesterol-Related Diseases Control

Unhealthy diet of high cholesterol/fat leads to elevated risks of diseases such as hypertension, cardiovascular disease, and diabetes mellitus worldwide. As a result, the need of natural products to control obesity and hyperlipidemia rises.

Among seaweeds natural products, caulerpenyne of *Caulerpa* species showed potential as an anti-obesity agent. Extract of *C. okamurae* significantly inhibited the accumulation of lipid and reduced the expression of adipogenesis master regulator, i.e., PPAR- ϒ, C/EBPα, and SREBP1-c in adipocytes (Sharma et al. 2017). Bitou et al. (1999) studied the ability of Cny to inhibit pancreatic lipase. Their study revealed that Cny inhibited lipase activity of monomeric substrate, i.e., tributyrin and etriolein which indicated that Cny directly interacts with lipase protein. Furthermore, the in vivo study showed that the peak plasma triacylglycerol was reduced and delayed by Cny (Bitou et al. 1999). The antihyperlipidemic activity of *C. racemosa* extract was reported by Ara et al. (2002). They described that *C. racemosa* extract was able to decrease the serum total cholesterol level, triglyceride, and low-density lipoprotein (LDL) cholesterol level in rats. Similar results were also reported by Matanjun et al. (2010), extract of *C. lentillifera* being able to reduce plasma total cholesterol of rats fed with high cholesterol diet.

17.4.5 Antidiabetic Activity

People living with diabetes have been growing worldwide. Bioactive compounds of algae are known for their great potential as antidiabetic. Extract of *C. lentillifera* was able to significantly reduce blood glucose level, plasma insulin, and hepatic glycogen. The extract was also capable to considerably increase key effector molecules for the PI3K/AKT activation pathway (Sharma et al. 2015). Furthermore, the *C. lentillifera* extract also significantly decreased the α-glucosidase and dipeptidyl peptidase-IV enzyme activities, also effectively inhibited cell death and expression

of iNOS in interferon-ϒ and interleukin-1β (Sharma and Rhyu 2014). Both results were suggesting that the *C. lentillifera extract* was potential as an antidiabetic agent. The compound of 4,5-dehydrodiodictyonema A isolated from *C. racemosa* was reported to inhibit protein tyrosinase phosphatase 1B (PTP1B), an important insulin signal negative factor which was a potent therapeutic agent for type 2 diabetes (Qian et al. 2016).

17.4.6 Larvacidal

The bioprospecting of *Caulerpa* extracts as larvacidal were also reported by many studies. Nagaraj et al. (2014) reported the effective larvacidal activity of *C. racemosa* methanolic extract against *Culex tritaeniorhynchus,* a primary vector of Japanese B encephatilis. They also reported that the active compounds in the extract were chola-5, 22-dien-3-Ol, 3 beta and methyl 3-bromo-1-adamantaneacetate. Another larvacidal activity of *C. racemosa* was also reported by Ali et al. (2013). In their study, the extract showed larvacidal activity against fourth instar larvae of *Anopheles stephensi, Aedes aegypti,* and *Culex quinquefasciatus.* Another study reported that acetone extract of *C. scalpelliformis* var. *denticulata* possessed larvacidal activity against late second and early third instar of *Culex pipiens* (Cetin et al. 2010). The *C. scalpelliformis* extract also showed synergism effect with synthetic insecticides as anti-larvacidal (Thangam and Kathiresan 1991).

17.5 Prospect and Challenges of *Caulerpa*-Based Product Development

The green seaweed of *Caulerpa* has a significant potential to be developed in the future years. It can be found almost in every place in the world. The number of human populations in the world will increase rapidly. Consequently, this condition will increase the need for healthy and nutritious food for human consumption. Besides that, the demand for a functional medicine that comes from nature also increases in the years to come. *Caulerpa* can be one of the sources to be the raw materials for human consumption and medicine.

Some countries in the world had already utilized these seaweeds by exporting to other countries. In three Pacific Island countries (Samoa, Fiji, and Tonga), the annual combined crop is 123 tons which valued around US$266,492 (Morris et al. 2014). In Southeast Asia and Western Pacific, some species of *Caulerpa* are increasingly becoming popular as human food (Belleza and Liao 2007; Hatta 2001; Trono 2001). *Caulerpa*, together with other macroalgae, promise to yield economic benefit once it is mari-cultured intensively in the Philippines (Cordero Jr 1990). In Indonesia, the government had started to develop *Caulerpa* in several provinces by developing a pond culture. These data showed that several countries have already realized the potential of *Caulerpa*.

However, several challenges must be faced to develop this seaweed in the future. Issues of environmental concerns have arisen with regards to the invasive species, for example, *Caulerpa taxifolia* C. Agardh, that had invaded and threatened various marine ecosystems in the Mediterranean region (Belleza and Liao 2007; Meinesz et al. 2001). Some species from genus *Caulerpa* had become a problem in several countries because of its invasive behavior. However, this condition can be turned into a benefit by utilizing this seaweed as materials for nutraceutical or pharmaceutical products. As the seaweed can grow rapidly, the adequacy and the continuity of the raw materials can be guaranteed. On the other hand, bringing natural products into real nutraceutical and/or pharmaceutical products is, of course, a problematic challenge, i.e., product development; technology and standardization of the products primarily for the pharmaceutical grade. However, these challenges can be overcome, it is possible that *Caulerpa* will become one of the prominent sources of seaweed in the world to compete with the red and brown seaweed.

17.6 Conclusion

Even though *Caulerpa* is one of the green seaweeds that can be found worldwide, its utilization is still very limited. This kind of seaweed has lots of potentials to be developed as a source for nutraceutical and even pharmaceutical products. *Caulerpa* is prosperous to be utilized as a nutraceutical product due to its excellent nutrient content. It also has potential as a pharmaceutical product due to its bioactive compound content as an anticancer, anti-inflammatory, antibacterial, antidiabetic, and larvacidal. The challenges in the development of *Caulerpa* seaweeds such as the supply of raw material, product development and diversification, and product standardization primarily as pharmaceutical must be handled. If these challenges can be overcome, *Caulerpa* will potentially become an important source of seaweed in the world.

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Part IV

Pharmaceutical Applications of Marine Bioresources

Medicinal Prospects of Marine Flora and Fauna for Drug Discovery

18

Sejal Shah and Sougata Ghosh

Abstract

Marine ecology consists of major forms of life that exist on the earth. Approximately 230,000 marine species are estimated according to the World Register of Marine Species. Marine flora and fauna are tremendously valuable oceanic resources, which contribute to the major part of the oceanic biomass. They are taxonomically diverse, biologically potent, productive, and chemically inimitable in nature. The marine flora contain medicinally useful metabolites in abundance that include polyphenols, alkaloids, and sulfated polysaccharides. It has shown great role in pharmacological properties against cancer, microbial pathogenesis, malaria, viral diseases, inflammation, and diabetes. The phytochemicals possibly activate macrophages, reduce oxidative stress and thus help in immunomodulation. Though marine niche has rich phytochemical diversity, yet it is considered majorly untapped or unexplored. Therefore, a vast number of new biomolecules such as enzymes, polymers, and osmolytes from the marine biotic community are being studied, and there is a growing interest in the industrial production of biomimetics and combinatorial synthesis of naturally occurring marine bioactive principles and their derivatives. Herein, we present a detailed and most comprehensive account of diverse group of bioactive compounds isolated from marine flora and fauna for biomedical applications in this chapter. Further, we have included a detailed account of the mechanism of actions and scientific rationale of these potential drug candidates.

Keywords

Marine ecology · Bioactive principles · Antimicrobial · Anticancer · Patents

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18.1 Introduction

Natural products have always been a rich source for novel molecules explored for drug discovery. According to Dictionary of Natural Products, over 40% of these chemical scaffolds are not synthetic compounds. Numerous compounds are thus being extracted or derived from natural sources which include the flora and fauna from diverse ecological niches (Blockley et al. 2017). Furthermore, although they are complex with well-defined spatial orientations, they serve as excellent starting point for drug discovery. In some countries the traditional medicine has mentioned these valuable resources as complementary and alternative medicine (Ghosh et al. 2012, 2013, 2014). 1184 new compounds were approved between the years 1981 and 2006 as drugs for the treatment of human diseases, the majority of which were derived from natural products and only 30% were of synthetic origin. It is important to note that in 2000 approximately half of the 20 best-selling non-protein drugs were either direct or derivatives of natural products and the acceptance, appreciation, and demand for such products is increasing globally. It is estimated that by 2020 around $115 billion will be spent globally on herbal supplements alone, and in 2014, consumers spent around $6.4 billion on herbal supplements in the United States—a 6.8% increase over 2013 and the 11th consecutive year of increased market growth. The term "natural product" is clearly embraced by consumers and has thus become top preference among other existing chemical ones. One of the most remarkable branches of natural pharmaceutical development is that of the marine natural products (MNPs), known as "blue gold" drug discovery which referred to the broad-spectrum exploration of novel compounds with therapeutic actives from the marine environment. It is scientifically reported that MNPs have greater chemical novelty compared to their terrestrial counterparts, and many of these compounds have advanced to clinical trials and pharmaceutical products. Since 1965, over 20,000 MNPs have been described, which have formed the basis of over 50% of FDA-approved drugs in the period 1981–2002. In 2015 alone 1340 new MNPs have been published in 429 papers; as our oceans cover 71% of the earth's surface, yet remain 95% unknown, the potential for further drug discovery is immense. At the time of writing, more than 30,607 articles focusing on MNPs have been uploaded to MarinLit, a database of MNP literature, many of which relate to MNPs produced by marine invertebrates, most notably marine sponges and corals. Marine invertebrates are significant resource as they account for over 89% of all extant marine animals, comprising over 174,600 species; this diversity is not only found between the animals themselves but also between the symbiotic microorganisms that comprise the entire holobiont. Till date 80% of antibiotics from marine sources come from *Actinomyces* spp., several species of which are associated with marine sponges. However, there can be numerous marine flora and fauna which may harbor a plethora of undiscovered bioactive compounds which is schematically represented in Fig. 18.1. This chapter focuses on the diversity of marine flora and fauna as notable sources of novel bioactive compounds with anticancer, antibacterial, antifungal, antiviral, antimalarial, anti-inflammatory and antidiabetic properties. A brief

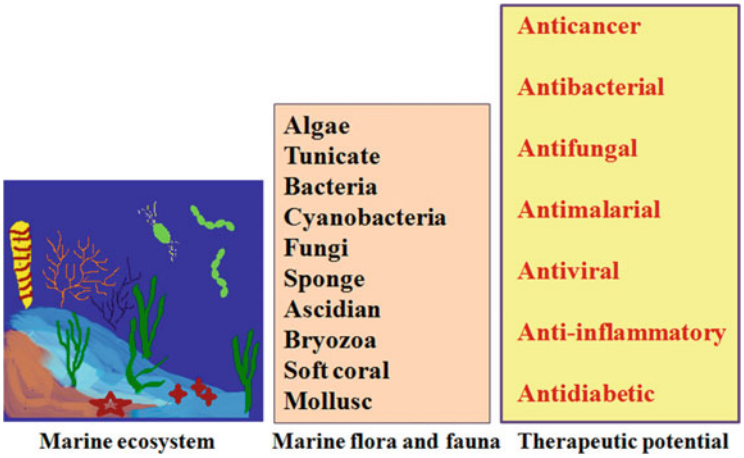

Fig. 18.1 Therapeutically significant compounds from marine flora and fauna

discussion of the mechanism of their therapeutic potential, opportunities, and constraints is also included (Blockley et al. 2017).

18.2 Anticancer Compounds

Cancer is a multifactorial disease, which involves deregulation of several cell signaling pathways. There are a number of signaling pathways which have a role in cell cycle disturbance, cell proliferation, or apoptosis. PI3K/AKT pathway has a major role in tumorigenesis. The genetic abnormalities of *PIK3CA* and *PTEN* genes involved in this pathway have shown great role in a number of cancers. The recent studies in Gujarati population have reported the impact of PI3K/AKT pathway and its relation with development of oral cancer (Shah et al. 2015, 2017, 2018). The products from marine niche have potency to inhibit the tumor growth in in vitro and in vitro murine models as well as for humans. There are several agents, which entered clinical trials for cancer, though very few of them got approval such as cytarabine, Yondelis®, eribulin, and the dolastatin 10 derivative, monomethylauristatin E (MMAE or vedotin) as a warhead for human use (Newman and Cragg 2014). The natural products derived from marine algae are rich in antioxidants. They have also shown health-promoting properties such as antimicrobial, anti-inflammatory, and anticancer due to their higher content of lipids, proteins, omega-3 fatty acids, fibers, and all the necessary vitamins (Lee et al. 2013). Herein an account of antitumor agents is furnished from diverse marine sources (Table 18.1).

Table 18.1 Marine natural products with anticancer activity with underlying mode of action

Sr. No.	Bioactive principle	Organism	Mode of action
1	Polysaccharides	Green algae	Inhibition of the cell proliferation via PI3K/AKT pathway
2	Glycoproteins Fucoidans	Brown algae	Anticancer effect
3	Fucoxanthin	Brown algae	Inhibition of MMP-9 expression and controls the CD44 and CXC chemokine receptor-4 (CXCR4), which are cell surface glycoproteins responsible for invasion
4	Dehydrothyrsiferol	Red algae	Apoptosis induction
5	GA3 polysaccharides	Microalga	Interrupts the process that involves inhibition of topoisomerase I and II
6	Didemnin B	Tunicates	Binding to eEF1A, elongation factor, and inhibition of protein synthesis, mitochondrial activity inhibition
7	Ecteinascidin 743	Tunicates	DNA bending toward major groove, induction of DNA-protein cross-linking, transcription interference
8	Asciddemin	Tunicates	Protein synthesis inhibition, activation of JNK and caspase pathway
9	Aplidine	Tunicate	Cell cycle arrested in G1 phase and blockage at G2 phase, modulation of growth factors, transcription factors, and signal transduction, inhibits the angiogenesis by inhibiting VEGF receptor
10	Benzolactoneenamides-lobatamides A to F	Tunicate	Inhibition of mammalian vacuolar-type (H^+)-ATPases
11	Leptosins C and F	Fungus	Deactivation of AKT pathway, inhibition of DNA topoisomerase I and II inhibition
12	Cycloprodigiosin hydrochloride	Bacteria	Boosting of apoptotic event by (NF-kB) suppression
13	Salinosporamide A	Bacteria	Inhibit the proteasome, a multicatalytic proteolytic complex that is involved in the regulation of cellular protein degradation
14	Laxaphycins A and B	Cyanobacteria	Synergistic role in increased polyploidy by putative alteration in topoisomerase II enzyme activity
15	Aragusterol A	Sponge	Targets the G1 phase of the cell cycle by downregulating CDKs and G1 cyclins involved in G1/S during the cell cycle by blocking the entry of human tumor cells into the S-phase
16	Fascaplysin	Sponge	Inhibition of CDK 4 kinase
17	Melophlins A and B	Sponge	G1 phase cell cycle arrest in RAS-transformed fibroblast
18	Aeroplysinin-1	Sponge	Antiangiogenic activity by inhibition of receptor tyrosine kinase in vitro

(continued)

Table 18.1 (continued)

Sr. No.	Bioactive principle	Organism	Mode of action
19	Agosterol A	Sponge	Multidrug-resistant reversal
20	Halichondrin B	Sponge	Cell cycle disturbance at G2-M phase, and mitotic spindle formation stage
21	Cyclodepsipeptide-jaspamide	Sponge	CD10/neutral endopeptidase expression leading to apoptosis
22	Peloruside A	Sponge	Alters PKC pathway
23	Motuporamines	Sponge	Inhibition of the migration of the cells
24	Mycalamide A and pateamine	Sponge	Apoptosis induction
25	Polyacetylenes	Sponge	Inhibition of topoisomerase I and inhibition of DNA replication
26	Sesterterpenes	Sponge	Induction of enucleation, expression of glycophorin A, and erythroid terminal differentiation in K562 cell line
27	Salicylihalamide	Sponge	Inhibition of mammalian vacuolar-type (H^+)-ATPases
28	Dictyostatin-1	Sponge	Induce tubulin polymerization
29	Dideoxypetrosynol A	Sponge	Enhances Bax expression and caspase activation leading to mitochondrial signaling pathway induction
30	Girolline	Sponge	Inhibits cell cycle at G2/M phase and recruits p53 proteasome
31	Aaptamine	Sponge	Induction of p21 protein expression leading to arrest of cell cycle at G2/M phase
32	Bastadin 6	Sponge	Inhibition of angiogenesis and neovascularization
33	Cortistatins A–D	Sponge	Inhibition of angiogenesis
34	13-Deoxytedanolide	Sponge	Inhibition of protein translation via binding to 60S ribosomal subunit
35	Geodiamolides A, B, H, and I	Sponge	Antitumor activity via disorganization of the actin filaments
36	Geoditins A and B	Sponge	Cytotoxicity by ROS generation leading to apoptosis via caspase-3-mediated signaling pathway
37	Ircinin-1	Sponge	Apoptosis via the Fas/Fas-L pathway
38	Onnamide A and theopederin B	Sponge	Inhibition of protein and activation of p38 kinase and c-Jun N-terminal kinase
39	Stelletin A	Sponge	Activate apoptotic pathway via Fas-L-caspase 3 pathway
40	Strobilinin-felixinin	Sponge	Inhibition of topoisomerase I and pol-alpha primase ends up with DNA synthesis inhibition
41	Variolin B	Sponge	Activation of apoptosis via p53-independent manner by inhibiting CDKs

(continued)

Table 18.1 (continued)

Sr. No.	Bioactive principle	Organism	Mode of action
42	Diazonamide A	Ascidian	Disrupts mitosis and microtubules and inhibits hydrolysis of GTP leading to apoptosis
43	Ningalins	Ascidian	Activation of the tumor-suppressing transforming growth factor-β (TGF- β) signaling cascade
44	Bryostatin-1	*Bryozoa*	Sensitizes cells to radiation-mediated apoptosis
45	Eleutherobin	Soft coral	Enhances anti-mitotic activity
46	Clavulone II	Soft coral	Arresting the cells at G1 phase via apoptotic mechanism
47	Spisulosine	Clam	Promotes disassembly of actin stress fibers
48	Dolastatins	Mollusc	Inhibition of cytokinesis in vitro as well as induction of cytotoxicity
49	Aplyronine A	Sea hare	Binds to the hydrophobic cleft in the actin molecule
50	Fucoxanthinol and halocynthiaxanthin	Sea squirt	Apoptosis induction via BCl-2 protein downregulation

18.2.1 Algal Metabolites

Marine alga is itself edible, and even its products can be used in preclinical studies for drug discovery (Lee et al. 2013). Aqueous extracts of certain red algae such as *Gracilaria corticata* and *Sargassum oligocystum* show cell growth inhibition in human leukemic cell lines. *Gracilaria tenuistipitata* extracts show anti-proliferative activity on Ca9-22 oral cancer cells (Yeh et al. 2012). Ulvan, a polysaccharide derived from alga, exhibits cytotoxicity and cytostaticity. Numbers of drugs are involved to produce oxidative stress and trigger certain miRNAs to express for the carcinogenesis by damaging the DNA (Lee et al. 2012). Polysaccharides from green algae, such as *Capsosiphon fulvescens*, inhibit the cell proliferation via the PI3K/Akt pathway for gastric cancer cells (Kim et al. 2012). Glycoproteins from brown algae such as *Laminaria japonica* and fucoidans from *Sargassum horneri*, *Ecklonia cava*, and *Costaria costata* had anticancer effects on human colon cancer cells (Go et al. 2010; Ermakova et al. 2011). Fucoxanthin derived from the brown algae *Saccharina japonica* has role in suppression of tumor migration and invasion by inhibiting the expression of MMP-9 which is found at higher levels in cancer cells. Moreover, it controls the invasion of B16-F10 melanoma cells as confirmed using transwell invasion assay. Moreover, fucoxanthin controls the CD44 and CXC chemokine receptor-4 (CXCR4), which are cell surface glycoproteins responsible for invasion, migration, and cancer-endothelial cell adhesion. Fucoxanthin is a potent inhibitor for actin fiber formation and interrupts cell migration in wound healing. It has antioxidant, anti-inflammatory, anticancer, antiobese, antidiabetic, antiangiogenic and

antimalarial activities (Chung et al. 2013). Antitumor activity of palmitic acid isolated from the red alga *Amphiroa zonata* has shown lower toxicity and induces apoptosis during in vitro assays with leukemic cell lines. Previous study has shown anti-proliferative activity of caulerpenyne, a sesquiterpenoid isolated from the tropical marine alga *Caulerpa taxifolia*. It inhibited cell growth in human neuroblastoma cell line due to impairment of microtubule assembly. It is a multitargeted agent (Mayer and Gustafson 2004). Dehydrothyrsiferol (DT), a polyether triterpenoid isolated from the red alga *Laurencia viridis* collected from the Canary Islands, induced apoptosis in estrogen receptor-negative breast cancer cells (Mayer and Gustafson 2006).

18.2.2 Tunicate-Derived Metabolites

Several compounds derived from the tunicates such as aplidine, didemnin B, dolastatin 10, and ecteinascidin-743 have a great role in cytotoxicity and neurotoxicity. Didemnin B has a role in mitochondrial activity inhibition. It binds to elongation factor (eEF1A) inhibiting protein synthesis (Marco et al. 2004). Ecteinascidin 743, isolated from the Caribbean tunicate *Ecteinascidia* turbinata, induces DNA-protein cross-linking and DNA bending toward major groove as confirmed by electrophoretic mobility shift (Bonfanti et al. 1999; Zewail-Foote and Hurley 1999). Ecteinascidin-743 inactivates human P-glycoprotein gene (MDR1) promoter. Hence, ecteinascidin-743 may be used as transcription-targeted chemotherapeutic agents and might be helpful for multidrug resistance tumors. Ecteinascidin is promoter-specific, transcription-interfering inhibitor. Its binding causes the widening of the minor groove of the DNA, a bending toward the major groove, which increases protein-DNA interactions which is considered as the underlying mechanism of its proven anticancer activity (Mayer and Lehmann 2001). Asciddemin, an alkaloid, induces apoptosis in human and murine leukemia cell lines by reductive DNA cleavage by ROS. It inhibits protein synthesis in vitro apart from generation of ROS and activation of JNK and caspase-2 eventually leading to apoptosis (Mayer and Gustafson 2006). Aplidine, isolated from *Aplidium albicans*, decreases hematotoxicity in cancer cell lines similar to doxorubicin. Aplidine exhibits G1 arrest/G2 blockage in human leukemic cell lines and modulates growth factors, transcription factors, and signal transduction. It induces apoptosis and fails to activate MKP (MAP kinase pathway). It also affects angiogenesis by inhibiting VEGF receptor 1, glutathione depletion, and COX-2 mRNA, protein, and prostaglandin biosynthesis. Moreover it has a role in mitochondrial apoptosis via Fas/CD95 receptor induction (Mayer and Gustafson 2006). Novel molecules such as benzolactoneenamides-lobatamides A to F and salicylihalamide are extracted from the marine tunicate *Aplidium lobatum* and from the marine sponge *Haliclona* sp., respectively, which inhibit mammalian vacuolar-type (H+)-ATPases that play a key role in angiogenesis, cell proliferation, and apoptosis. This enzyme cascade might be useful as therapeutic target for cancer treatment (Mayer and Gustafson 2004).

18.2.3 Fungi and Bacterial Metabolites

Thiocoraline obtained from the marine fungi shows DNA polymerase alpha inhibition in human colon cell line (Mayer and Lehmann 2001). Molecules derived from different bacteria such as lyngbyabellin A show antitumor activity. A family of antimitotic compounds such as cryptophycin-52 suppresses microtubule dynamics. It induces apoptosis at pico-molar concentrations by binding tightly to a single high-affinity site on tubulin leading to its conformational change. Preclinical animal studies carried out for cryptophycin confirmed its efficiency in combination with doxorubicin, paclitaxel, and 5-fluorouracil. Cryptophycin-52 and cryptophycin-55 in several human colon and ovarian carcinoma xenograft models (Menon et al. 2000; Teicher et al. 2000; Panda et al. 2000). VEGF is known as angiogenic factor which is highly expressed in tumor cells. Indanone derived from the marine cyanobacterium *Lyngbya majuscula* inhibits VEGF expression ($IC_{50} = 25$ mM) in transfected human hepatocellular carcinoma Hep3B cell. Lyngbyabellin A isolated and characterized from the marine cyanobacterium *Lyngbya majuscule* significantly inhibits the cell growth in human nasopharyngeal and colon carcinoma cell lines ($IC_{50} = 0.03$ to 0.5 μg/mL, respectively) and disrupts the cellular microfilament networks in the smooth muscle cells (Mayer and Gustafson 2003). Laxaphycins A and B, which are cyclic depsipeptides derived from the marine cyanobacterium *L. majuscule*, have a synergistic role in increased polyploidy by putative alteration in topoisomerase II enzyme activity. Leptosins C and F, alkaloids isolated from the marine fungus *Leptoshaeria* sp., deactivated AKT pathway and inhibited DNA topoisomerase I and II leading to apoptosis. Among bacteriogenic antitumor compounds, a red-pigmented compound cycloprodigiosin hydrochloride produced by the marine bacterium *Pseudoalteromonas denitrificans* enhances apoptotic event by (NF-kB) suppression. Moreover they have observed synergistic effect of this molecule with epirubicin in vitro as well as in vivo as apoptotic agent (Mayer and Gustafson 2004). Salinosporamide A, a novel marine bacterium-derived alkaloid, inhibits the proteasome, a multicatalytic proteolytic complex that is involved in the regulation of cellular protein degradation. Marine fungus *Myrothecium roridum* produces verrucarin A which targets MAP kinase pathway via p38 and JNK phosphorylation.

18.2.4 Sponge and Other Marine Organism-Derived Metabolites

The steroid aragusterol A, isolated originally from Okinawan marine sponge *Xestospongia* sp., has shown tremendous anticancer activity against the 14 human cancer cell lines in in vitro as well as in vivo murine model. The mechanism behind the apoptosis is by targeting the G1 phase of the cell cycle, downregulating CDKs and G1 cyclins involved in G1/S during the cell cycle, and thus blocking the entry of human tumor cells into the S-phase. The marine polyketide discodermolide shows induction of apoptosis which may rationalize its use as potential promising chemotherapeutic conjugational approach. The alkaloid fascaplysin derived from the sponge *Fascaplysinopsis* sp. inhibits CDK4 kinase in vitro. It binds to the ATP

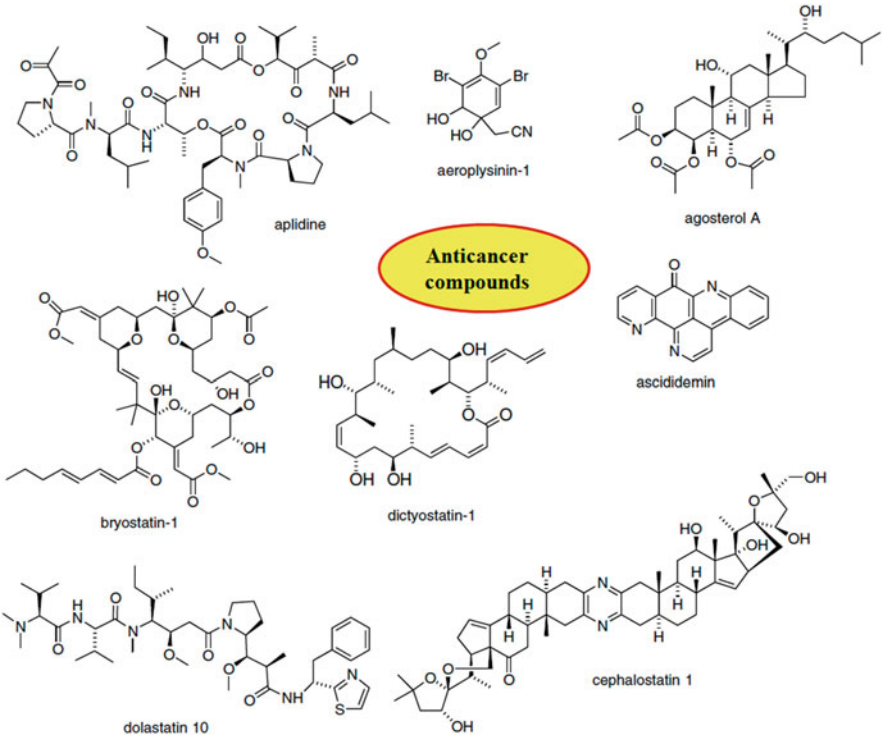

Fig. 18.2 Marine compounds with anticancer activities. (Reproduced with permission from Mayer and Gustafson 2006. Copyright © 2006 Elsevier Ltd.)

binding pocket of CDK4, arresting the cell in G1 phase. The novel compounds melophlins A and B derived from marine sponge *Melophlus sarassinorum* cause G1 phase cell cycle arrest in Ras-transformed fibroblast, showing powerful anticancer activity (Mayer and Gustafson 2003). Various studies were carried out on bryostatin-1 derived from *Bryozoan*, on murine leukemia cell line, human lymphoma cell line, as well as murine in vivo tumor xenograft model for diffuse large lymphoma (Fig. 18.2). The mechanism of action has revealed that it sensitizes cells to radiation-mediated apoptosis (Mayer and Gustafson 2003). The study reported for bryostatin-1 and in combination with interleukin-2 has shown induction of interferon-c gene expression via p38 MAP kinase pathway at transcriptional as well as post-transcriptional levels, in freshly isolated human peripheral blood T cells concluding it as a combinational therapy for cancer prognosis (De Lorenzo et al. 2003). The studies performed in combination of bryostatin and ionomycin in murine cell line have shown activated T cell-mediated tumor regression (Curiel et al. 2001). In contrast, other pharmacological studies have revealed ERK/MAPK signaling was important for bryostatin-1-induced differentiation of the human cell line (Wall et al. 2001). Vrana et al. have reported synergistic induction of apoptosis when treated with bryostatin-1 and lactacystin in human leukemia cells (U937) (Vrana and

Grant 2001). This particular study supports current evidence for the presence of molecular cross-talk between the actions of proteasome inhibitors and the activation of signal transduction pathways. Lin et al. have shown upregulation of X-linked inhibitor of apoptosis protein resulting in impairment of PKC/MAPK pathway in human monocytic leukemia cell line (Lin et al. 2002). One more study performed in the USA has reported that increased level of cytochrome C release ends up with apoptosis in human monocytic leukemic cell lines. Eleutherobin, extracted from the soft coral *Eleutherobia* sp., has shown enhanced antimitotic activity in human breast carcinoma cell line. Spisulosine, isolated from the clam *Spisula polynyma*, which is an alkyl amino alcohol promotes disassembly of actin stress fibers, which is regulated by Rho – the GTP binding protein. Hence, it may act as an antagonist for sphingosine-1 receptor. Altering Rho activity may have a role in its anticancer activity (Mayer and Gustafson 2003). Aeroplysinin-1 (brominated tyrosine derivative), extracted from the marine sponge *Aplysina aerophoba*, shows tremendous antiangiogenic activity by inhibition of receptor tyrosine kinase in vitro, suggesting its potential as antiangiogenic drug in vivo (Fig. 18.2). Agosterol A (polyhydroxylated sterol acetate) derived from the marine sponge *Spongia* sp. shows multidrug resistance reversal by its interaction with P-glycoprotein and multiple drug-resistant associated protein 1 (MRP1). The multidrug-resistant reversal is due to the direct inhibition of MRP1-mediated drug transport through severe reduction of intracellular glutathione levels as revealed by the study performed using human transfected epidermoid carcinoma cell line with MRP1 C DNA. Acetoxyl groups are responsible for reversing P-glycoprotein-mediated drug resistance; the hydroxyl groups of glutathione may be responsible for drug binding to the C-terminal half of MRP1 which end up with the MRP1-mediated drug resistance reversal. Specific amino acids 1223–1295, proximal to C-terminus of the TM helix 17 of MRP1, are found very crucial for glutathione-dependent binding of agosterol A. Aeroplysinin-1, isolated from the marine sponge *Aplysina aerophoba*, shows great potential as antiangiogenic molecule due to its ability to block RTKs (receptor tyrosine kinases). Callystatin A, isolated from the sponge *Callyspongia truncata*, shows rigid and stable binding through strong lipophilic interactions, instead of hydrogen bonding to the targeted amino acid of the receptor molecule (Mayer and Gustafson 2004). Several published articles were reported about a marine cytotoxic microtubule-stabilizing compound, discodermolide, which has a same mechanism of action as the taxanes, best class of chemotherapeutic molecule (Bröker et al. 2002). Halichondrin B and their synthetic ketone analogues show apoptotic activity in vitro as well as in vivo against numerous human cancer cell lines and four human xenografts (breast, colon, melanoma, and ovarian cancer), respectively. Synthetic analogues have a role in cell cycle disturbance at G2-M phase and mitotic spindle formation stage making them as potential anticancer agents. Further preclinical studies have supported the notion of mitotic blockage and apoptosis in human lymphoma and prostate cell lines. One more molecule such as cyclodepsipeptide jaspamide, extracted from *Jaspis johnstoni* and *Hemiastrella minor* sponges, regulates level of CD10/neutral endopeptidase expression ending up with induction of apoptosis in the human promyelocytic leukemia HL-60 cell line which triggers

caspase-independent pathway for apoptosis. Macrolide peloruside A isolated from the marine sponge *Mycale hentscheli* has shown structural similarity to protein kinase C (PKC) binding pharmacophore of bryostatin. Peloruside A, like bryostatin, targets PKC-dependent pathways. Additionally, it is also a novel microtubule-stabilizing agent due to its ability to arrest cell cycle at G2-M phase that might be effective in treating solid tumors. Motuporamine C, extracted from *Xestospongia exigua*, inhibits the cell migration and acts as antiangiogenic factor. Motuporamines D, E, F, G, H, and I also have antiangiogenic properties. Mycalamide A and pateamine isolated from the marine sponge *Mycale* sp. were reported as anticancer agent which has shown antitumor activity via induction of apoptosis during in vitro assays in cell line transformed with RAS and BCR/ABL oncogenes and shown increased susceptibility for apoptosis. Novel molecule polyacetylenes isolated from the marine sponge *Petrosia* sp. have shown inhibition of topoisomerase I and inhibition of DNA replication leading to apoptosis. Certain novel scalarane-type sesterterpenes isolated from the marine sponge *Phyllospongia chondrodes* induce enucleation, expression of glycophorin A, and erythroid terminal differentiation in K562 cell line. Pharmacological studies with squalamine have reported antiangiogenic property with induction of both disorganization of F-actin stress fibers and a reduction of endothelial cadherin (VE-cadherin). The cyclic depsipeptide compound kahalalide F extracted from marine mollusc *Elysia rufescens* is an antitumor agent against prostate tumors. Dolastatins, isolated from marine mollusc *Dolabella auricularia*, enhance actin assembly (Fig. 18.2). Dolastatins 10 and 11 might have a role in the inhibition of cytokinesis inducing cytotoxicity associated with hyperassembly of the cellular F-actin filament network. Dolastatins might have a binding site for actin polymer rather than the other peptides. Dolastatins 10, 11, and 15 bind to amino terminal peptide of β-tubulin and exhibit F-actin stabilization (Mayer and Gustafson 2004). Diazonamide A, isolated from the marine ascidian *Diazona angulata*, disrupts mitosis and microtubules and inhibits hydrolysis of GTP leading to apoptosis. Highly cytotoxic macrolide polyketidedictyostatin-1, originally derived from a marine sponge from the genus *Spongia* sp. isolated from the Republic of Maldives, shows induction of tubulin polymerization. Dideoxypetrosynol A, a polyacetylene from the marine sponge *Petrosia* sp., activates apoptosis by enhancing Bax expression and caspase activation specific to mitochondrial signaling pathway. GA3 polysaccharides, produced by the marine microalga *Gymnodinium* sp., inhibit topoisomerases I and II and have shown in vitro cytotoxicity against 39 human tumor cell lines. Similarly, girolline, isolated from the marine sponge *Peudaxinyssa cantharella*, inhibits cell cycle at G2/M phase and recruits p53 proteasome (Mayer and Gustafson 2006). Among various agents that target the cyclin-dependent kinase inhibitor Cip/Kip p21 protein, aaptamine from *Aaptos suberitoides* induces p21 protein expression and leads to arrest of cell cycle at G2/M phase. Alkyl pyridinium salts isolated from *Reniera sarai* induce apoptosis and simultaneously reduce cell adhesion in NSCLC cells. Aplyronine A, extracted from the sea hare, binds to the hydrophobic cleft in the actin molecule, which is needed to depolymerize actin eventually causing cytotoxicity for HeLa cells. Bastadin 6, an alkaloid extracted from the marine sponge

Ianthella basta, inhibits angiogenesis and neovascularization (in vivo), via apoptotic pathway. Clavulone II, isolated from soft coral *Clavularia viridis*, is antitumor and antiviral in nature. Cortistatins A–D derived from the marine sponge *Corticium simplex* show inhibition of angiogenesis. A macrolide called 13-deoxytedanolide, derived from the marine sponge *Mycale adhaerens*, inhibits protein translation via binding to 60S ribosomal subunit, leading to protein elongation inhibition disrupting protein synthesis. The carotenoids fucoxanthinol and halocynthiaxanthin extracted from the sea squirt *Halocynthia roretzi* trigger apoptosis induction via BCl-2 protein downregulation. Geodiamolides A, B, H, and I that belong to group of cyclic peptides extracted from the marine sponge *Geodia corticostylifera* are known for antitumor activity via disorganization of the actin filaments. Geoditins A and B derived from the marine sponge *Geodia japonica* show cytotoxicity by ROS generation leading to apoptosis via caspase-3-mediated signaling pathway. Ircinin-1, isolated from the marine sponge *Sarcotragus* sp., induces apoptosis during in vitro studies with G1 phase inhibition leading to apoptosis via the Fas/Fas-L pathway. Ningalins, aromatic alkaloids originally isolated from the marine ascidian *Didemnum* sp., show cytotoxicity by activating tumor-suppressing transforming growth factor-β (TGF-β) signaling cascade. Onnamide A and theopederin B, isolated from the marine sponge *Mycale* sp., induce apoptosis via inhibition of protein synthesis and activation of p38 kinase and c-Jun N-terminal kinase. Saponin philinopside A might be used as a potential antiangiogenic factor as isolated from the sea cucumber *Pentacta quadrangularis*. Stelletin A isolated from the marine sponge *Geodia japonica* activates apoptotic pathway via Fas-L-caspase-3 pathway and induces oxidative stress as well. The guanidine alkaloid variolin B, originally derived from the marine sponge *Kirkpatrickia variolosa* from Antarctica, induces p53-independent apoptosis and also inhibits CDKs (Mayer and Gustafson 2008).

18.3 Antibacterial Compounds

Diverse group of marine bacteria, ascidians, bryozoans, sponges, soft corals, and algae are explored for isolation of bioactive principles like loloatins A–D, myticin, and psammaplin A (Fig. 18.3). Marine bacterial isolate MK-PNG-276A from genus *Bacillus* produces loloatins A–D, a family of new cyclic decapeptides which exhibited in vitro antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci, and penicillin-resistant *Streptococcus pneumoniae* (Gerard et al. 1999). Cysteine-rich antibacterial peptide myticin and its isoforms (myticin A of 4.438 Da and myticin B of 4.562 Da) isolated from hemocytes and mussel *Mytilus galloprovincialis* are selectively active against bacterial pathogens like *Micrococcus luteus*, *Bacillus megaterium*, and *Enterococcus viridians* (Mitta et al. 1999). Myticins are comprised of 40 residues with 4 intramolecular disulfide bridges and a cysteine array in the primary structure. Myticin precursors consist of 96 amino acids with a putative signal peptide of 20 amino acids, the antimicrobial peptide sequence, and a 36-residue C-terminal extension. A

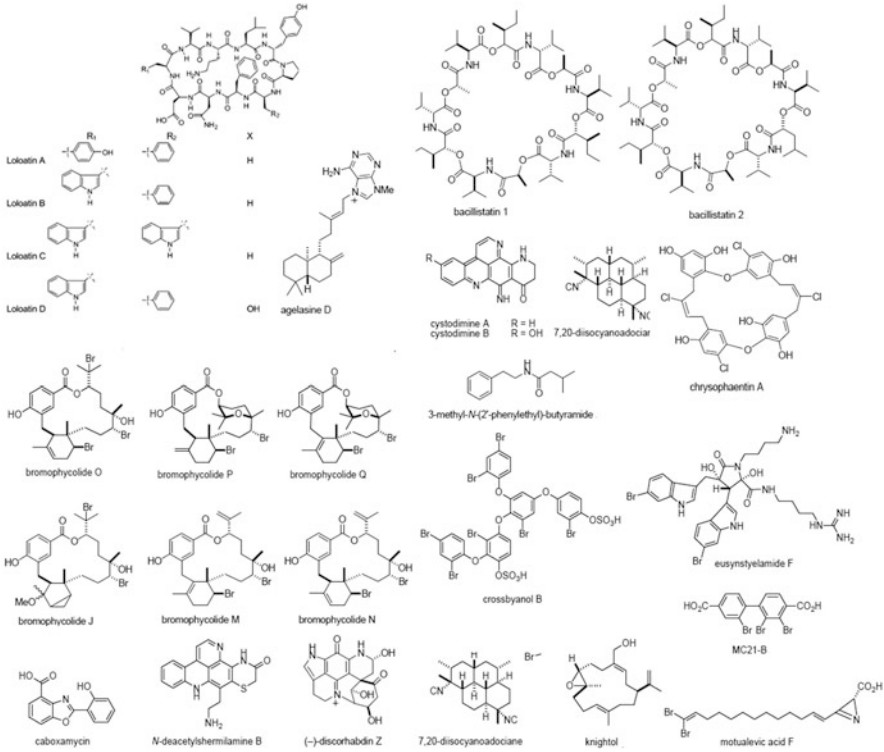

Fig. 18.3 Marine compounds with antibacterial activities. (Reproduced with permission from Mayer and Hamann 2002. Copyright © 2002 Elsevier Science Inc. and Mayer et al. 2013)

bromotyrosine derivative called psammaplin A isolated from marine sponge *Psammaplysilla* sp. demonstrates potent antibacterial activity against MRSA which is comparable to ciprofloxacin, a commercially used quinolone antibiotic. Psammaplin A inhibits DNA synthesis of *S. aureus* SG511 in a dose-dependent manner and effectively inhibits DNA gyrase-mediated supercoiling activity (Mayer and Hamann 2002; Kim et al. 1999). The marine sponge *Oceanapia* sp. contains a novel C14 acetylenic acid which is active against Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and the Gram-positive bacteria *B. subtilis* and *S. aureus* (Matsunaga et al. 2000). Discorhabdin R, a novel antibacterial pyrroloiminoquinone, was isolated from the southern Australian sponge *Negombata* sp. and an Antarctic *Latrunculia* sp. which can partially inhibit *Serratia marcescens* in addition to *S. aureus*, *M. luteus,* and *E. coli* (Mayer and Hamann 2004). Arenosclerins A–C and haliclonacyclamine E, novel tetracyclic alkylpiperidine alkaloids isolated from the marine sponge *Arenosclera brasiliensis*, are effective against antibiotic-resistant *S. aureus* strains. A novel antimicrobial glycolipid caminoside A from the marine sponge *Caminus spaeroconia* can substantially inhibit MRSA and vancomycin-resistant enterococcal strains. The

antimicrobial peptide bogorol A from marine *Bacillus* sp.; novel macrolide antibiotic chalcomycin B from marine *Streptomyces*; unusual peptide, dicynthaurin, from hemocytes of the marivne tunicate *Halocynthia aurantium*; steroidal glycoside, iyengaroside-A, from the marine green alga *Codium iyengarii*; new halogenated G15 acetogenin, lembyne-A, from the marine red alga *Laurencia* sp.; novel halogenated sesquiterpenoids, pannosanol, and pannosane from the red alga *Laurencia pannosa*; chlorinated benzophenone antibiotic, pestalone, isolated from a member of the marine fungus of genus *Pestalotia*; furan carboxylic acid; and an acetyl derivative of Sumiki's acid from the marine fungus *Cladosporium herbarum* are isolated as broad-spectrum antibiotics against bacterial pathogens. Antimicrobial pyrrolidinone derivatives zopfiellamides A and B isolated from the marine fungus *Zopfiella latipes* and the bromotyrosine antibiotic zamastatin derived from the Okinawan sponge, *Pseudoceratina purpurea* are reported to be active against marine biofouling bacteria *Rhodospirillum salexigens* (Mayer and Hamann 2005). A series of kalihinols, diterpenes isolated from the Philippine marine sponge *Acanthella cavernosa*, inhibit bacterial folate biosynthesis. Furanyl-type kalihinols are more selective inhibitors of bacterial folate biosynthesis compared to pyranyl-type kalihinols. The dimeric bromopyrrole alkaloid nagelamide G from the Okinawan marine sponge *Agelas* sp. exhibits antibacterial activity against *M. luteus*, *B. subtilis*, and *E. coli.* The new antimicrobial octapeptide plicatamide from the hemocytes of the marine tunicate *Styela plicata* leads to massive and rapid potassium efflux in the bacterial pathogens indicating cell membrane as its potential target. Manoalide derivatives from a *Luffariella* sp. sponge collected in Palau, β-carboline eudistomin X, isolated from the Micronesian ascidian *Eudistoma* sp. are also antimicrobial in nature. It is important to note that antimicrobial peptide dolabellanin B2 from the sea hare *Dolabella auricularia* shows complete inhibition of growth of *B. subtilis*, *H. influenza*, and *Vibrio vulnificus* at a concentration as low as 2.5–5 μg/mL. Diterpenes pseudopterosins X and Y from the soft coral *Pseudopterogorgia elisabethae*, sphingolipids and glycolipids from soft corals of the Andaman Islands (Indian Ocean), bromophenols like bis(2,3-dibromo-4,5-dihydroxybenzyl) ether isolated from the marine red alga *Rhodomela confervoides*, nitrogen heterocyclic compound cribrostatin 6 isolated from the dark-blue marine *Cribochalina* sp. sponge, purpuramine L from the Indian marine sponge *Psammaplysilla purpurea*, nitrogenous sesquiterpene germacrane isolated from an *Axinyssa* n. sp. sponge, bicyclic guanidine alkaloid from the marine sponge *Ptilocaulis spiculifer*, diterpenes membranolides C and D derived from an Antarctic cactus sponge, small 21-residue peptides arenicin-1 and arenicin-2 from the coelomocytes of the marine lugworm *Arenicola marina*, and highly basic and hydrophobic peptide perinerin, from the marine clamworm *Perinereis aibuhitensis*, are considered as well-known antibacterial agents of marine origin (Mayer et al. 2007). Bisdiarylbutene macrocycle chrysophaentin A from the chrysophyte alga *Chrysophaeum taylorii* significantly inhibits MRSA and vancomycin-resistant *Enterococcus faecium* by inhibiting GTPase activity of protein FtsZ which is essential for bacterial cell division. Some of the bioactive principles isolated from marine bacteria can inhibit quorum sensing, thereby inhibiting cell-to-cell signaling and associated infections.

Phenethylamide metabolites like 3-methyl-*N*-(2′-phenylethyl)-butyramide isolated from a marine Gram-positive *Halobacillus salinus* strain interfere with quorum sensing regulation. Small cyclopropane-containing fatty acid, lyngbyoic acid, isolated from the marine cyanobacterium *Lyngbya* cf. *majuscula* affect both quorum sensing pathways (acylhomoserine lactone receptor LAsR as well as gene expression in *Pseudomonas aeruginosa*. Pyrroloiminoquinone alkaloids of the discorhabdin class isolated from the Korean marine sponge *Sceptrella* sp. are also antibacterial in nature. Novel alkaloid (−)-discorhabdin Z, with a unique hemiaminal group, inhibits sortase A which is a bacterial transpeptidase that covalently attaches proteins to the bacterial cell wall. Similarly, a new alkaloid agelasine D isolated from the marine sponge *Agelas nakamurai* collected in Bali, Indonesia, the maleimide mixture aqabamycin E from marine *Vibrio* sp. growing on the surface of the Red Sea soft coral *Sinularia polydactyla*, cyclodepsipeptides bacillistatins 1 and 2 purified from cultures of the Chilean bacterium *Bacillus silvestris* obtained from a crab, new diterpene-benzoate macrolides bromophycolides J–Q isolated from the Fijian red alga *Callophycus serratus*, benzoxazole antibiotic caboxamycin produced by the deep-sea *Streptomyces* sp. NTK 937 isolated in the Canary Basin, a brominated polyphenyl ether crossbyanol B isolated from the Hawai'ian marine cyanobacterium *Leptolyngbya crossbyana*, pyridoacridine alkaloids characterized from the Mediterranean ascidian *Cystodytes dellechiajei*, Fijian *Cymbastela hooperi* diterpene isonitrile, a novel alkaloid eusynstyelamide F isolated from an Arctic bryozoan *Tegella* cf. *spitzbergensis*, and a novel cembranoid diterpene, knightol, found in the Colombian gorgonian octocoral *Eunicea knighti* are found to have efficient antibacterial properties. A novel antibiotic MC21-B produced by the marine bacterium *Pseudoalteromonas phenolica* O-BC-30T; long-chain 2*H*-azirine 2-carboxylic acid, motualevic acid F, described from a Fijian marine sponge *Siliquariaspongia* sp.; the thiopeptide TP-1161 isolated from a Norwegian marine sediment derived Gram-positive *Nocardiopsis* sp. Bacterium; two novel α-pyrone macrolides neurymenolides A and B extracted from the Fijian red alga *Neurymenia fraxinifolia*; two polybrominated metabolites synthesized by Hawai'ian marine bacterium *Pseudoalteromonas* sp.; pseudopterosin U found in the Caribbean octocoral *Pseudopterogorgia elisabethae*; an alkaloid called 5-bromo-8-methoxy-1-methyl-β-carboline isolated from the New Zealand marine bryozoan *Pterocella vesiculosa*; and a new rifamycin antibiotic, salinisporamycin, purified from a culture of the Micronesian marine actinomycete *Salinispora arenicola* YM23-082are among the novel antibacterial marine compounds (Mayer et al. 2013). Other marine products with antibacterial properties include a novel bioactive alkaloid, synoxazolidinone A, discovered in the sub-Arctic Norwegian ascidian *Synoicum pulmonaria*; a novel casbane diterpenoid 10-hydroxydepressin from the Hainan soft coral *Sinularia depressa*; a novel guai-2-en-10α-methyl methanoate from the marine alga *Ulva fasciata*; a novel gymnochrome F from the deep-water crinoid *Holopus rangii*; fatty acids ieodomycins from a marine *Bacillus* sp.; sulfated sesterterpene alkaloid 19-oxofasciospongine A from a marine sponge *Fasciospongia* sp.; phenalenone derivative from the marine fungus *Coniothyrium cereal*; 4,4′-oxybis [3-phenylpropionic acid] from the marine bacterium *Bacillus licheniformis*;

sargafuran from the marine brown alga *Sargassum macrocarpum;* tetracyclic brominated diterpene from the red alga *Sphaerococcus coronopifolius*; and bromotyrosine alkaloid tyrokeradine B from a Verongida marine sponge (Mayer et al. 2013). Novel marine antimicrobial peptides are also being explored for their potential antibacterial properties. Dimeric peptides centrocins 1 and 2 from the Norwegian green sea urchin *Strongylocentrotus droebachiensis*; halocyntin and papillosin isolated from hemocytes of the Mediterranean ascidian *Halocynthia papillosa* and; hyastatin, a glycine-rich multi-domain peptide from hemocytes of the Norwegian spider crab *Hyas araneus* are reported as highly effective marine antimicrobial peptides (Mayer et al. 2013).

18.4 Antifungal Compounds

Several antifungal agents with superior activity are reported from diverse groups of marine flora and fauna which are included in Fig. 18.4. Novel marine natural product called lyngbyabellin B, an antifungal depsipeptide, isolated from the marine cyanobacterium *Lyngbya majuscula*, is active against *Candida albicans*. Similarly, the novel imidazole alkaloid naamine D is extracted from the Red Sea sponge *Leucetta* cf. *chagosensis* simultaneously with naamidine A, B, D, and G. Among these alkaloids naamine D is effective against *Cryptococcus neoformans*. Naamine D competitively inhibits murine macrophage-inducible nitric oxide synthase (Mayer

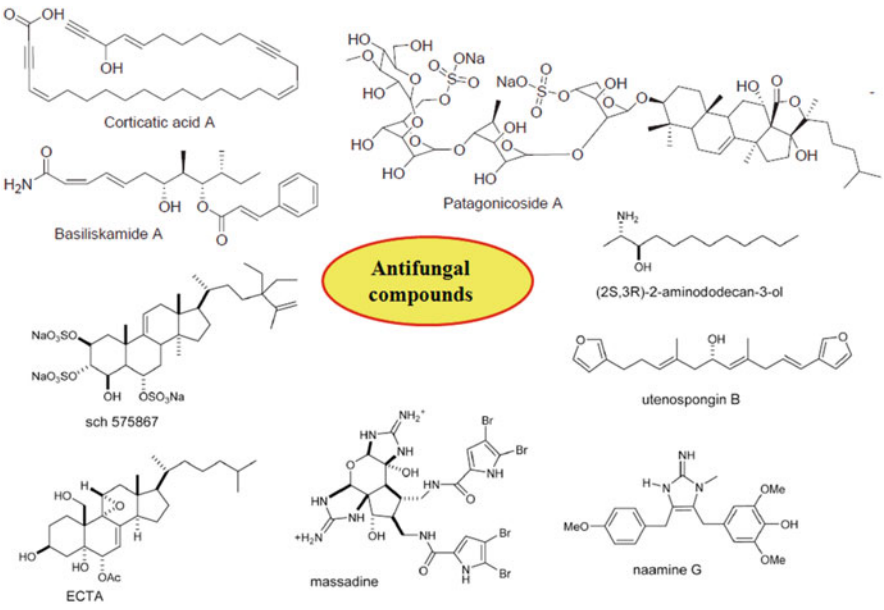

Fig. 18.4 Antifungal compounds from marine flora and fauna. (Reproduced with permission from Mayer and Hamann 2005. Copyright © 2005 Elsevier Inc. and Mayer et al. 2007. Copyright © 2007 Elsevier Inc.)

and Hamann 2004). Marine bacterium *Bacillus laterosporus* produces the novel polyketides basiliskamides A and B that shows antifungal activity against *Candida albicans* and *Aspergillus fumigatus*, which is as potent as amphotericin B. It is important to note that basiliskamide A is fourfold less cytotoxic to normal human fibroblasts compared to amphotericin B. Polyacetylenic acids like corticatic acids D and E isolated from the marine sponge *Petrosia corticata* inhibit geranylgeranyltransferase type I (GGTase I), an enzyme that plays a critical role in fungal cell wall biosynthesis. Swinhoeiamide A, a calyculin derivative isolated from the marine sponge *Theonella swinhoei*, shows strong antifungal activity toward *C. albicans* and *A. fumigates*. Disulfated triterpene glycoside patagonicoside A, from the sea cucumber *Psolus patagonicus* is active against the pathogenic fungus *C. cucumerinum*. Similarly, the antifungal metabolite 3,3 V-oxybis[5-methyl-phenol] isolated from marine fungus *Keissleriella* sp. inhibits human pathogens, particularly *C. albicans*, *Trichophyton rubrum*, and *A. niger*. Polyester 15G256b from *Hypoxylon oceanicum* and decalactone xestodecalactone B from *Penicillium* cf. *montanense* isolated from the host marine sponge, *Xestospongia exigua* are active against *C. albicans* (Mayer and Hamann 2005). Sterol isolated from the marine sponge *Dysidea arenaria* inhibits MDR1-type efflux pump in multidrug-resistant *C. albicans*. An alkaloid called massadine from the marine sponge *Stylissa aff. massa* inhibits fungal GGTase (IC50 = 3.9 μM). The imidazole alkaloid naamine G from the Indonesian marine sponge *Leucetta chagosensis* inhibits phytopathogenic fungus *Cladosporium herbarum*. Untenospongin B from the Moroccan marine sponge *Hippospongia communis* is a potent antifungal agent against *Candida tropicalis* and *Fusarium oxysporum*. Polyketide (2*S*, 3*R*)-2-aminododecan-3-ol (39), isolated from the Brazilian ascidian *Clavelina oblonga*, is active against *C. albicans.* Activities of many of the antifungal agents of marine origin are comparable or more potent than amphotericin B, nystatin, and ketoconazole. Thus there is a growing need to investigate the in vivo antifungal activity in addition to finding out the mechanism of action and developing strategies for selective/targeted delivery to the site of infection (Mayer et al. 2007).

18.5 Antimalarial Compounds

A polyketide, ascosalipyrrolidinone A, isolated from obligate marine fungus *Ascochyta salicorniae* inhibits both *Plasmodium falciparum* strain K1, a strain resistant to chloroquine, and strain NF 54, a strain susceptible to standard antimalarials. Homofascaplysin A and fascaplysin, sesterterpenes isolated from the Fijian marine sponge *Hyrtios* cf. *erecta*, are highly effective against *P. falciparum* but show reduced cytotoxicity against muscle myoblast cells and mouse peritoneal macrophages rationalizing the fact that they can be considered as candidate antimalarial drugs. Manzamine A, a β-carboline alkaloid, inhibits the growth of *Plasmodium berghei* which is a rodent malaria parasite. Manzamine A can be administered by either oral or intraperitoneal route (Mayer and Hamann 2004). Aigialomycin D, isolated from the marine mangrove fungus *Aigialus parvus* BCC 5311, inhibits

multidrug-resistant *Plasmodium falciparum* (K1 strain). Figure 18.5 shows the ophiobolane sesterterpene halorosellinic acid from the marine fungus *Halorosellinia oceanica* BCC 5149, trypyrrole bacterial pigment heptyl prodigiosin from proteobacteria isolated from a marine tunicate, and ent-8-hydroxymanzamine A, manzamine F, and neo-kauluamine isolated from Indo-Pacific sponge which are reported as potent marine antimalarial compounds (Mayer and Hamann 2005). *P. falciparum* is moderately inhibited by bielschowskysin, an oxygenated hexacyclic diterpene isolated from the Caribbean gorgonian octocoral *Pseudopterogorgia kallos*, as well as by briarellins K hydroperoxide and D/L hydroperoxide, isolated from the gorgonian *Briareum polyanthes.* Chloroquine-resistant *P. falciparum* W2 is inhibited by cembradiene, a diterpenoid isolated from the Caribbean gorgonian octocoral *Eunicea* sp. Likewise, dolastatin 10, a peptide microtubule inhibitor isolated from the sea hare *Dolabella auricularia* which is a potent anticancer drug also, shows antimalarial activity by selectively affecting the schizont stage of intraerythrocytic development, which has the highest concentration of tubulin. Manzamine A and trioxacarcins A and D exhibit antimalarial activity which is comparable to artemisinin (Mayer et al. 2007).

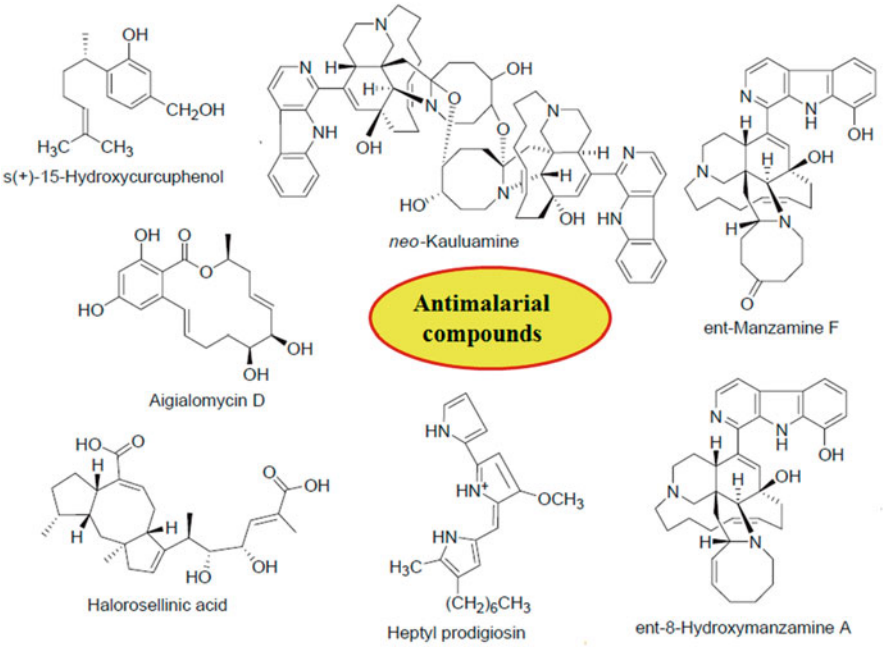

Fig. 18.5 Antimalarial compounds from marine flora and fauna. (Reproduced with permission from Mayer and Hamann 2005. Copyright © 2005 Elsevier Inc.)

18.6 Antiviral Compounds

Cyclodidemniserinol trisulfate isolated from Palauan ascidian *Didemnum guttatum* inhibits HIV-1 integrase and topoisomerase enzyme of the *Molluscum contagiosum* virus. Antiviral bromoindole alkaloid, dragmacidin F, isolated from the Mediterranean sponge *Halicortex* sp. inhibits herpes simplex virus (HSV)-1 infections and syncytia formation by HIV-2. Lobohedleolide, (7Z)-lobohedleolide, and 17-dimethylaminolobohedleolide isolated from Philippine soft coral *Lobophytum* sp. exhibit HIV-inhibitory activity. Anti-HIV bromotyrosine-derived lipids, namely, the mololipids, are reported in a Hawaiian sponge of the order Verongida (Mayer and Hamann 2004). Clathsterol is a novel active sulfated sterol from the Red Sea sponge *Clathria* sp., which is a potent HIV-1 reverse transcriptase inhibitor. Similarly, the cyclic depsipeptide microspinosamide isolated from the marine sponge *Sidonops microspinosa*; polyacetylenetriol, isolated from the marine sponge *Petrosia* sp.; and the sulfated-flavone glycosides thalassiolins A–C, isolated from the Caribbean sea grass *Thalassia testudinum*, inhibit HIV infections (Fig. 18.6). Sulfated calyceramides A–C are metabolites from the marine sponge *Discodermia* calyx which are novel influenza virus neuraminidase inhibitors (Mayer and Hamann 2005). Many metabolites from marine organisms like polycyclic guanidine alkaloid called crambescidin 826 isolated from the marine sponge *Monanchora* sp.; a C_{22} furanoterpene designated dehydrofurodendin from a Madagascan *Lendenfeldia* sponge; depsiundecapeptide neamphamide A isolated from the Papua New Guinea marine sponge *Neamphius huxleyi*; diterpenes, Da-1 and AcDa-1, isolated from the marine alga *Dictyota menstrual*; and bis-quinolizidine alkaloids petrosins isolated from the Indian marine sponge *Petrosia similis* are found to be potent inhibitors of HIV infections (Mayer et al. 2007).

18.7 Anti-inflammatory Compounds

Anti-inflammatory activity of marine metabolites like carvernolide, contignasterol, cyclolinteinone, and oxenamide A is well reported. Carvernolide, a novel C21 terpene lactone isolated from the sponge *Fasciospongia cavernosa*, significantly inhibits tumor necrosis factor-α, nitric oxide, and prostaglandin E2. Inhibition of human synovial phospholipase A2, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) gene expression is considered as the underlying mechanism of action. Contignasterol, a highly oxygenated sterol isolated from the sponge *Petrosia contignata*, inhibits allergen-induced bronchoconstriction. Similarly, cyclolinteinone, a sesterterpene from the sponge *Cacospongia linteiformis*, regulates iNOS synthase and COX-2 enzyme by inhibiting nuclear transcription factor-κ B. Among oxepinamides and fumiquinazolines, alkaloids isolated from marine fungus *Acremonium* sp., only oxepinamide A exhibited good in vivo anti-inflammatory activity (Mayer and Hamann 2004). Halipeptins A and B isolated from the marine sponge *Haliclona* sp. are reported as anti-inflammatory agents. Marine cyclopeptide hymenamide C from the marine sponge *Axinella carteri*, and

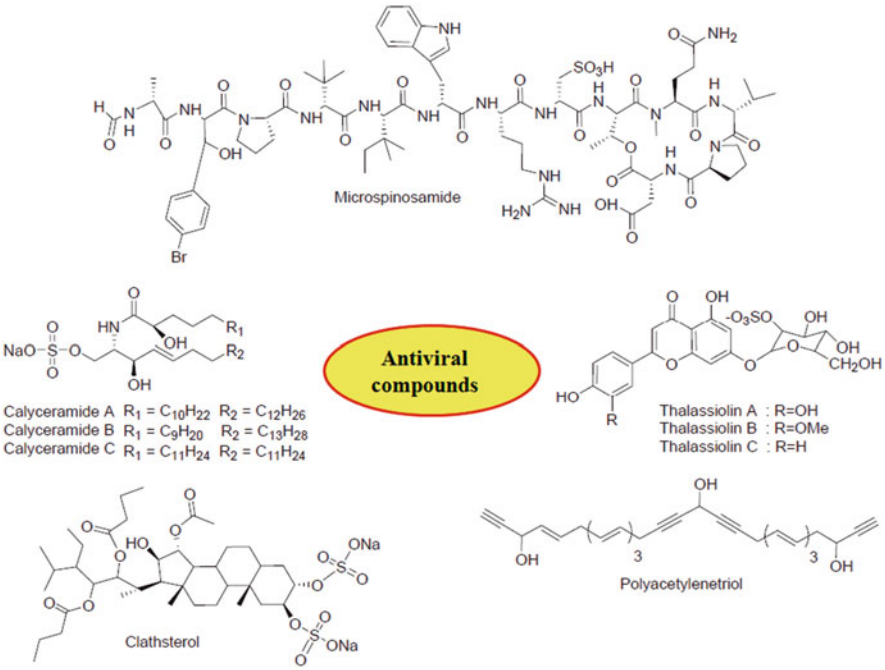

Fig. 18.6 Antiviral compounds from marine flora and fauna. (Reproduced with permission from Mayer and Hamann 2005. Copyright © 2005 Elsevier Inc.)

sesterterpene petrosaspongiolide M from the marine sponge *Petrosaspongia nigra* show remarkable anti-inflammatory potential (Fig. 18.7). Scytonemin, a yellow pigment isolated from marine cyanobacteria, exhibits anti-inflammatory and anti-proliferative activities by inhibiting polo-like kinase 1 and protein kinase C h1 (Mayer and Hamann 2005). Among other anti-inflammatory marine compounds, astaxanthin, bolinaquinone, cacospongionolide B, clathriol B, conicamin, cycloamphilectene 2, elisabethadione, plakohypaphorine, pourewic acid A, methylpourewate B, cadlinolide C, petrocortyne A, petrosaspongiolides M–R, pseudopterosin N, pseudopterosin R, and seco-pseudopterosin E are considered as notable. The mechanism of action mostly involves inhibition of nitric oxide, prostaglandin E2, and TNF-α generation. At the molecular level, cacospongionolide B inhibits nuclear factor-κ-DNA binding activity and enhances IκB-α expression (Mayer et al. 2007).

18.8 Antidiabetic Compounds

Very few antidiabetic compounds are reported from marine sources which are furnished herewith. Purification, characterization, and biological activity of insulins from the European spotted dogfish, *Scyliorhinus canicula*, and the hammerhead

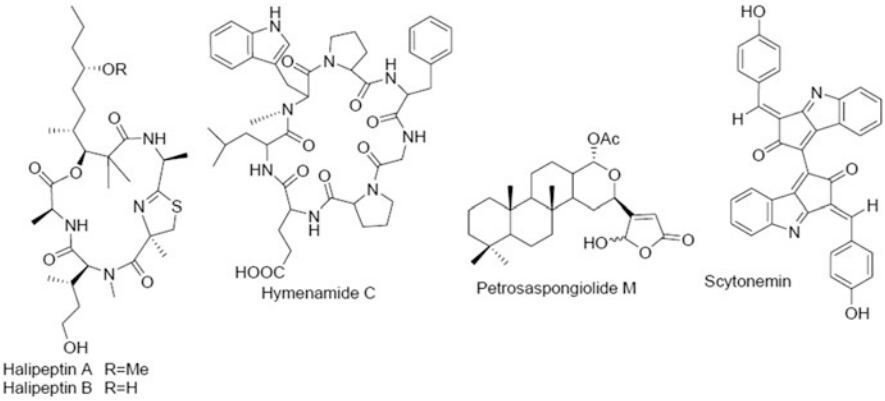

Fig. 18.7 Anti-inflammatory compounds from marine flora and fauna. (Reproduced with permission from Mayer and Hamann 2005. Copyright © 2005 Elsevier Inc.)

shark, *Sphyrna lewini*, showed that the elasmobranch insulins are markedly different from human insulin. Administration of dogfish insulin by bolus arterial injection leads to significant reduction of blood glucose only after 12 h which persists for 48 h indicating metabolic action identical to mammalian insulin (Mayer and Hamann 2005). Diphlorethohydroxycarmalol [DPHC] isolated from the marine brown alga *Ishige okamurae* controls postprandial hyperglycemia in diabetic mice by potent inhibition of both α-glucosidase and α-amylase which are key enzymes for carbohydrate metabolism (Fig. 18.8). Sesquiterpene dysidine from the Hainan marine sponge *Dysidea villosa* activates insulin pathway by inhibition of human protein phosphatase 1B which is considered as a potent drug target for treatment of type II diabetes and obesity. It also regulates glucose uptake and glucose transporter 4 translocation in vitro (Mayer et al. 2013).

18.9 Future Prospects

Several studies on marine pharmacology and toxicology have reported novel bioactive principles from marine flora and fauna which include indoles and carbazole alkaloids, proteins, immunomodulatory glycolipids, anticoagulant sulfated glycosaminoglycans, sea anemone-derived pore-forming proteins, cytolytic peptide, protein toxins, okadaic acid, and many more. Thus the potential of marine natural product is a major source of new therapeutic entities in the pharmacopeia, and major thrusts are for exploration of candidate drugs. Due to the emergence of multidrug resistance in every sphere of medical treatment, there is a continuous need to search for novel and effective bioactive compounds. In view of the background, the need for sources of diverse and pharmacologically active leads grows ever larger, and marine environment provides a treasure of untapped natural products with immense therapeutic potential. Dynamic cooperation and collaboration between academic

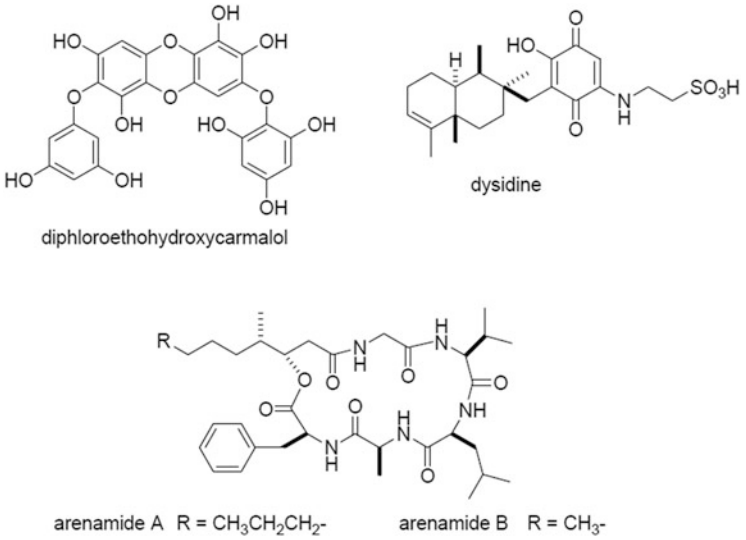

Fig. 18.8 Antidiabetic compounds from marine flora and fauna. (Reproduced with permission from Mayer et al. 2013)

scientists and those in smaller biotechnology companies can prove to be instrumental to the early preclinical development. Exploration of mechanism of action based on studies will provide crucially important preclinical data to rationalize the involvement of larger pharmaceutical companies to support the late preclinical and clinical trials. Similarly, it is very important to identify molecular targets for these biologically active metabolites of marine origin and develop chemical synthesis routes in order to minimize overexploitation of marine flora and fauna. Metagenomic studies may provide a strong base for identifying the potential source as well as determination of therapeutic index of the isolated compounds.

18.10 Conclusion

The present chapter on bioactive natural products from marine fauna and flora highlights the anticancer marine pharmacology research which mainly focuses on the fact of preclinical and experimental studies in the domain of molecular and cellular pharmacology of marine cytotoxic agents, which includes mainly bryostatin-1, cryptophycins, dolastatins, and ecteinascidin-743. Moreover, the chapter has discussed the mechanism of antitumor activity and elaborated the mode of action of the each compound isolated from the marine species. Similarly, various metabolites derived from marine sources are included in this chapter which are potentially significant antibacterial, antifungal, antimalarial, antiviral, anti-inflammatory, and antidiabetic agents. Multinational and multidisciplinary efforts between marine biologists, natural product chemists, and pharmacologists may come

together to fill up the lacuna toward characterization and identification of the molecules, formulation development, and successful clinical trials for final drug development. This untapped source of marine metabolites certainly has the potential to revolutionize the global healthcare and drug discovery.

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Tapping the Potential of Marine Resources in the Arena of Cosmetics

19

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Abstract

The demand for new alternatives is very high in the cosmetic world and hence it provides a new dimension of opportunities. Marine resources play a pivotal role in the field of cosmetic industry. The macronutrients (proteins, amino acids, carbohydrates, and lipids) and micronutrients (copper, zinc, iron, etc.) are richly present in marine organisms. These ingredients serve properties in photoprotection, anti-aging along with antioxidant potential. Yet more exploration is called for optimization of production and extraction of the active ingredients from marine resources. Also, more research is claimed to ensure the effectiveness and safety of new components for cosmetic applications.

Keywords

Marine resources · Cosmetics · Anti-aging · Antioxidant

19.1 Introduction

The products that are used on the body with the intention of beautifying and improving appearance are called cosmetics. The cosmetic products are mixtures of synthetic or natural chemical compounds. Till date, marine resources are reported as active ingredients for pharmaceuticals and cosmeceuticals applications (Wijesinghe and Jeon 2011). There has been an increase in the popularity of cosmetics from natural sources as compared to synthetic cosmetics (Bom et al. 2019). Hence, the investigations of new marine resources along with its biotechnological potentials are

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a promising area for cosmeceutical studies (Medina-Franco 2019; Ye et al. 2019). In the marine environment, seaweeds, micro- and macroalgae are renowned for bioactive compounds having the ability to serve as cosmeceutical products (Chandini et al. 2008; Kladi et al. 2004). These compounds are present in the form of secondary metabolites, vitamins, protein, oils which act as a rich source of functional ingredients (Kashman and Rudi 2004; Plaza et al. 2008; Guillerme et al. 2017). Skin undergoes the aging process being in direct contact with the environment (Gao et al. 2008). Cosmetics and their ingredients are applied to the skin, showing complex interaction phenomena, sometimes leading to adverse or toxic effects (Nohynek et al. 2010; Antignac et al. 2011). The research of the marine habitat domain showed the significance of natural compounds with less or no adverse effect in contrast to synthetic materials (Fisher et al. 2002; Antignac et al. 2011; Wijesinghe and Jeon 2011). Table 19.1 summarizes marine sources and their skin whitening properties.

19.2 Factors Responsible for Skin Problems

19.2.1 Aging

The aging are of two types, one is natural aging and another is photoaging also known as intrinsic and extrinsic aging, occurs due to passage of time and sun exposure respectively (Wlaschek et al. 2001; Uitto 1986). The natural and photoaging causes the laxity and fine wrinkling and damage to the connective tissue and elastin-containing material (Lavker 1995; Fisher et al. 1997). As the age increases, properties of skin such as richness in collagen content along with its tightly packing are lost and it becomes fragmented and unsystematic (Fisher et al. 2008; Quan et al. 2010). As far as natural aging is concerned, no one in this world can escape this process (Fisher et al. 2008; Quan et al. 2010; Quan and Fisher 2015). It is impossible to control the adverse effect of aging but we can search novel active compounds from marine resources with its maximum effect on preventing aging of the skin making it look healthy and young.

19.2.2 UV Radiation Smash up to Skin

The UV-A range (315–400 nm) is divided into two categories UV-A-1 (340–400 nm) and UV-A-2 (320–340 nm). These radiations lead to human skin aging, wrinkles and pigmentation, and actinic keratosis. The longer time exposure of these radiations can lead to skin cancer (Wang et al. 2011; Brunt and Burgess 2018). UV-B radiation belongs to the range of 280–315 nm and reaches the epidermis to interact directly with cellular targets like DNA and induces the intracellular reactive oxygen species leading to sunburn and skin cancer (Zhong et al. 2015). It also causes inflammation and redness, increase in the temperature, and bruises on the skin (de la Coba et al. 2009; Pallela et al. 2010). UV-C has a very

Table 19.1 List of some substances from marine resources and their skin whitening properties

Sr No.	Source	Compound and its Action	Reference
1.	*Sanguisorba officinalis* and *S. japonicus*	Extract showed the inhibition of tyrosinase activity	Yoon et al. (2010)
			Lee et al. (2010)
			Husni et al. (2011)
			Kim et al. (2016)
2.	Korean Sea cucumbers	Glycoprotein fraction of boiled liquid extract showed the inhibition of tyrosinase activity and the suppression of melanin synthesis	Suh et al. (2014)
3.	*E. cava* and *S. silquastrum*	Fucoxanthin compound reduced melanin synthesis and tyrosinase activity	Maeda et al. (2005)
			Shang et al. (2011)
			Cha et al. (2011)
4.	*E. cava*	Phlorotannins reported for the inhibitory effect on tyrosinase activity as well as melanin synthesis	Yoon et al. (2009)
5.	*E. cava*	7-phloroeckol acted as an inhibitor of melanin formation	Yoon et al. (2009)
6.	*E. cava*	Diphlorethohydroxycarmalol showed the inhibition of tyrosinase activity and melanin synthesis	Yoon et al. (2009)
7.	Marine algae	Tyrosinase inhibitory effect	Liang et al. (2012)
8.	*Laminaria japonica*	Fucoxanthin showed the suppression of tyrosinase activity and mRNA expression linked to melanogenesis	Thomas and Kim et al. (2013)
9.	Brown algae	Phloroglucinol derivatives indicated tyrosinase inhibitory activity by chelating copper	Babitha and Kim (2011)
10.	*E. stolonifera*	Extract displayed the tyrosinase inhibition activity	Kang et al. (2004)
11.	*Fucus vesiculosus*	Sulfated polysaccharide showed the suppression of melanin synthesis	Song et al. (2015)

short range of wavelength 100–280 nm and is capable of penetrating in ozonosphere. It has no hazards to the skin (Matsumura and Ananthaswamy 2004; Gomez et al. 2009; Wang et al. 2015).

19.2.3 Skin Pigmentation

Melanin is a natural pigment mainly responsible for the color of skin, eyes, and hair and is found in most organisms. Melanocyte cells are located in the bottom layer of the epidermis and the middle layer of the skin and eye, respectively (Agar and Young 2005). Melanocyte cells produce the melanin through process of melanogenesis. It absorbs light which causes an increase in melanogenesis as a response to DNA photodamage (Lee and Noh 2013). Further, it has the ability to protect the skin cells from damage (Tsatmali et al. 2002). Melanin is produced from the tyrosine (amino acid) by the action of tyrosinase (enzyme) which has the ability to catalyze eumelanin and phenomelanin synthesis. The eumelanin and phenomelanin developed by the catalysis of tyrosine to 3,4- dihydroxy-L-phenylalanine (L-DOPA) which further gets converted to dopaquinone leading to pigmentation (Gao et al. 2008; Solano et al. 2006; Parvez et al. 2006). Thus, pigmentation of the skin, eyes, and hair has been induced by tyrosinase. Thus tyrosinase inhibitory activity is essential for depigmentation and whiting of skin (Likhitwitayawuid 2008; Liang et al. 2012). Till date, the koijc acid and arbutin showed good tyrosinase inhibition activity but with adverse effects (Cheng et al. 2007; García-Gavín et al. 2010). Still, it is needed to explore new active molecules for this purpose (Fais et al. 2009; Sima et al. 2011).

19.2.4 Acne Vulgaris

The common and long-time problem of skin which is observed in teenagers and youth is acne vulgaris, which is recognized as acne. It leads to permanent marks, spots along with open pores of skin showing the unpleasant effect on physiological growth (Leyden 1995). The multidimensional factors such as hair follicle keratinization and sebum production, the infection of *Staphylococcus epidermidis, S. aureus,* and Gram-positive anaerobic *Pseudomonas aeruginosa*, are reported for causing the acne (Farrar and Ingham 2004; Yamaguchi et al. 2009). Till date clindamycin and erythromycin are used as traditional medicines for acne but have also been reported for skin allergies. So it has opened the new area for the identification of novel compounds with no adverse effect from marine recourses (Lee et al. 2009; Pérez et al. 2016).

19.3 Tapping the Potential of Marine Resources to Explore the Benefits in Cosmetics

19.3.1 Scope for Topical Anti-aging

Skin aging is a slow, intricate progression of intrinsic and extrinsic aging (Yaar 2006). Skin aging causes several changes like thinning, dryness, laxity, fragility, enlarged pores, fine lines, and wrinkles (Wang et al. 2015).

The extract from *Codium tomentosum*, green algae, is a good source of glucuronic acid, which regulates water distribution within the skin, and protects the skin from the damaging effects of a dry environment. The extract from *Laminaria saccharina* contains proteins, vitamins, minerals, and carbohydrates, which regulates sebaceous gland activity, and has anti-inflammatory and healing properties (Fitton et al. 2007).

Studies on sulfated polysaccharide from *Porphyridium cruentum* has been found to improve the amount of cornified envelope maturation in stratum corneum and reinforces the dermal-epidermal junction (DEJ). Therefore, it seems to be a good choice for improving the skin characteristics of dry or aged facial skin and can prolong the effect of moisturizers when applied topically (Ghibaudo et al. 2014).

Microalgae extracts are used in the formulation of skin care products (e.g., anti-aging cream, regenerating skin care products, antioxidant and anti-irritant products, and emollient products). Dermochlorella DG, XCELL-30, Alguronic Acid, and Alguard are few to name. Dermochlorella DG is a *Chlorella* sp. extract comprising oligopeptides that increases firmness and skin tone. XCELL-30 developed from microalgae acts on cellular turnover in the basal layer of the epidermis, preserving the youthfulness of the skin. Alguronic Acid is a mix of polysaccharides produced by microalgae with significant anti-aging properties (Jaspars et al. 2016).

Macroalgae and microalgae extracts have been quite promising in this direction. A noteworthy study has shown the antibacterial activity of extracts of macroalgae *Himanthalia elongate* and *Synechocystis* spp. against *Escherichia coli* and *Staphylococcus aureus* (Plaza et al. 2010). Bacteria like *Pseudomonas aeruginosa* or *Klebsiella pneumonia* were inhibited by extracts from microalgae named *Isochrysis galbana*, *Chlorella marina*, *Nannochloropsis oculata*, *Dunaliella salina*, and *Pavlova lutheri*. The leaves of halophyte *Crithmum maritimum* have a polyacetylene Falcarindiol. It has been reported that it robustly hampers the growth of bacteria such as *Micrococcus luteus* and *Bacillus cereus* (Srinivasakumar and Rajashekhar 2009).

Some cosmeticians have even invested in their own microalgal production system (e.g., LVMH, Paris, France and Danial Jouvance, Carnac, France). Some commercialized products include a liposome-based product containing a photolyase from blue-green algae, *Anacystis nidulans*, manufactured from the US company (AGI Dermatics). The product (Phycosaccharides®) prepared from the extract of *Laminaria digitata* is a skin penetrant (Mungo 2005). La Mer and Sisley produce internationally known anti-aging skin care products prepared from the Kelp.

Anti-aging products with a mixture of PSs derived from *Pseudoalteromonas* sp., *Pseudoalteromonas antarctica,* and *Halomonas eurihalina* have been formulated. This mixture increases collagen I synthesis thereby improving skin structure (Martins et al. 2014). Oligosaccharides present in *laminarin* have been found to stimulate, regenerate, and rejuvenate human fibroblasts and human epidermis keratinocytes (Yvin et al. 1999).

Hyaluronic acid is a component of the extracellular matrix of the skin that is commonly used as an anti-aging substance (Price et al. 2007). Extract from *Macrocystis pyrifera* not only induce hyaluronic acid synthesis but also stimulate syndecan-4 synthesis, another important protein of the extracellular matrix (Couteau and Coiffard 2016). Progerin, along with telomeres, triggers cellular senescence in

human fibroblasts (Robert et al. 2009; Reddy and Comai 2012; Nikolakis et al. 2013). *Alaria esculenta*, an edible seaweed, decreases the amount of progerin in aged fibroblasts, although this inhibitory effect was not observed in younger cells (Verdy et al. 2011).

Pentapharm (Basel, Switzerland) has launched new products of the extracts from *Nannochloropsis oculata* with excellent skin-tightening effects. Blue Retinol™, algae extract from *D. salina*, stimulates skin cells growth and proliferation. Marestil® prepared from marine algae extracts is a strong moisturizing, elasticizing, and toning complex. (Kim et al. 2008).

Matrix metalloproteinases (MMPs) are Zn^{2+} extracellular endopeptidases enzymes that degrade collagen resulting in wrinkle formation. Peptides isolated from seahorses (SHP-1) enhance collagen release by inhibition of collagenases 1, 3, and 13 (Ryu et al. 2010a, b). The various seaweed species have been reported with MMP inhibitory capabilities (Sanjeewa et al. 2016). Eckol and dieckol (phenolic compounds) obtained from *E. stolonifera* have shown strong inhibitions of MMP-1 expression (Joe et al. 2006).

19.3.2 Room for Skin Photoprotection

Photoprotection is an essential prophylactic and therapeutic element (Wang et al. 2013a, b). External aging arises from exposure to UV radiation and related irritation, characterized by deterioration of the dermal extracellular matrix and keratinocyte dysplasia in the epidermis, causing wrinkles, laxity, coarseness, and mottled pigmentation (Kim et al. 2013).

Macroalgae are known to produce photoprotective compounds to combat photoaging (Pallela et al. 2010). Such compounds can absorb UV-A and UV-B, inhibit the formation of MMPs, and can scavenge the reactive oxygen species. Some of these compounds include shinorine, porphyra-334, palythene, eckstolonol, eckol, mycosporine-glycine, mycosporine methylamine-serine, sargachromenol, fucoxanthin, tetraprenyltoluquinol chromane meroterpenoid, scytonemin, and sargaquinoic acid (Daniel et al. 2004; Urikura et al. 2011; Kim et al. 2013; Ryu et al. 2014; Balboa et al. 2015).

Fucoidans are well known for their radical scavenging, antioxidant, anticoagulant, anti-inflammatory, antiviral, antithrombotic, and anticancer activities (Kim et al. 2014). Incorporation of fucoidans as ingredients into cosmetic preparations has proven to provide skin improvement or cosmeticizing action by preventing and alleviating skin aging conditions such as freckles, wrinkles, and blotches (Wijesinghe and Jeon 2011).

Fucoidan derived from brown algae has recently been shown to inhibit UVB-induced MMP-1 expression in vitro by the suppression of extracellular signal-regulated kinase (ERK) (Thomas and Kim 2013). Highly concentrated fucoidan extract (89% fucoidan) from *Undaria pinnatifida* is available commercially as Maritech ReverseTM. It is known to protect skin against UV irradiation, prevent wrinkles, and also act as a soothing agent. The extract has been found to be

non-sensitizing and non-allergenic to skin. Moreover, it is Halal Kosher certified (Fitton et al. 2015).

Mycosporine-like amino acids (MAAs) are among the most efficient UV-A-absorbing compounds found in cyanobacteria, algae, corals, and many marine invertebrates, and also demonstrate good anti-oxidant activity (Wada et al. 2015; Řezanka et al. 2004). MAAs have the ability to absorb 310–362 nm light (UV radiation) and scattered these energies in the form of heat radiation (Gröniger et al. 2000). The red algae *Porphyra umbilicalis* is a rich source of mycosporine-like amino acids (MMAs) which are potent UV light absorbers, and therefore work as sunscreen (Shick and Dunlap 2002). These studies give noteworthy clue that MAAs can be made use in sunscreens and other cosmetic products.

19.3.3 Wide Horizon in the Field of Antioxidants, Preservatives, and Essential Oils

19.3.3.1 Antioxidants

The human skin which is exposed to UV radiation can be sheltered from oxidative harm by antioxidants. UV-induced reactive oxygen species disturb membrane lipids, proteins, and DNA creating profound damage on human skin. Against these dents generated by ROS, antioxidants have a protective effect. Remarkably, lipid peroxidation by ROS is involved in reducing the youthful form of skin (Kim 2014). Also, in cosmetic formulations, antioxidant compounds assist in preventing oxidation of ingredients.

Therefore, synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tertbutyl hydroquinone (TBHQ) are used by the cosmetic industry to tackle ROS-induced oxidation. But, it has been found that these synthetic antioxidants possess probable health dangers (Park et al. 2001; Hettiarachchy et al. 1996). Natural antioxidants thus embody a harmless substitute for the cosmetics industry (Al-Amoudi et al. 2009).

Carotenoids are organic pigments that are made up of 8 isoprene units with 40 carbon atoms. They have been categorized into two groups: first and second xanthophylls containing oxygen and carotenes respectively, which are merely hydrocarbons (Menon and Lele 2015). Plants, bacteria, and some fungi are the ones that chiefly produce them from fats. There are some reports that displayed the properties of antioxidant and anti-inflammatory attributes of carotenoids. For this reason, carotenoids have been found to be responsible for skin photoprotection by stalling ROS toxicity induced by UV. Hence many sunscreens formulation encompass carotenoids (Morabito et al. 2011).

Marine organisms are bacteria, yeast, and fungi that act as a source of carotenoids (Vílchez et al. 2011; Mata-Gómez et al. 2014). Bacteria such as *Paracoccus* and *Agrobacterium* and yeast genera like *Rhodotorula, Phaffia, Xanthophyllomyces* produce *astaxanthin* (Corinaldesi et al. 2017). β-carotene is also profoundly present in algae (Takaichi 2011). In the late 1980s, the commercial-scale production of β-carotene has been optimized from *Dunaliella*. High salinity and intense light are

the best triggers for the best β-carotene production (Borowitzka 1999). The protists of marine origin *Ulkenia* sp. and *Thraustochytriidae* sp. AS4-A1 are also reported to produce astaxanthin (Quilodrán et al. 2010). Saproxanthin and myxol are rare carotenoids that were isolated from marine bacteria (family Flavobacteriaceae) which illustrated high antioxidant potential (Shindo et al. 2007).

Phenolic compounds with potent antioxidant activities are present in marine halophytes (Lopes et al. 2016; Surget et al. 2015). There is a direct correlation between the antioxidant potential of a plant extract and its phenolic content. Reports have cited that a prominent amount of phenolic compounds have lead to high antioxidant activity in numerous halophytes (Surget et al. 2015; Aniya et al. 2002; Falleh et al. 2012). The halophyte *Crithmum maritimum* L. possesses compelling antioxidant activity and has been found to contain phenolic compound chlorogenic acid (CGA) (Meot-Duros and Magne 2009).

The potential benefits of fucoidans from brown algae *Chnoospora minima* and *Sargassum polycystum* have been reported by Fernando et al. (2018). The fucoidans demonstrated potential antioxidant activities (2,2-diphenyl-1-picrylhydrazyl (DPPH) and alkyl radical-scavenging activities) and UV-protective effects. In the study by Kelman et al. (2012), they found the significant antioxidant activity of Hawaiian algae checked by employing the FRAP (ferric reducing antioxidant power) assays. Brown algae such as *Fucus vesiculosus* and *Turbinaria conoides* are a source of polysaccharides like laminaran, fucoidan, and alginate, also encompass antioxidative properties (Je et al. 2009).

19.3.3.2 Preservatives

In cosmetics products, microbial contamination leads to spoilage of the product. If the contamination turns out to be pathogenic, it corresponds to a deleterious health hazard. To avoid alteration and microbial contamination, preservatives are added to cosmetic products. The parabens (preservatives with antimicrobial properties) also showed disagreement about their safety (Routledge et al. 1998; Darbre et al. 2004). Thus, it is required to explore novel and safe antimicrobial preservatives.

Marine organisms possess a rich pool of antimicrobial peptides. These are at present considered for cosmetic products such as moisturizing creams and shampoos. HAHp2-3-I fraction extracted from the pepsin hydrolysate of *Setipinna taty* (half-fin anchovy) showed good antibacterial activity. Besides these, from sea cucumber *Holothuria scabra*, xanthophyll, β-cryptoxanthin, and β-carotene were isolated which strongly inhibited *Staphylococcus aureus* (Sarhadizadeh et al. 2014).

19.3.3.3 Essential Oil

The characteristic lemony fragrance has been found in essential oil from halophyte *Crithmum maritimum* L. (Coiffard et al. 1993). Essential oils in the algae extract offer antiseptic and anti-inflammatory roles. The essential oil extracted from edible seaweed, *Laminaria japonica* L., has been shown to display antioxidant and antibacterial activities (Patra et al. 2015).

19.3.3.4 Skin Whitening Properties

There are several marine resources-derived ingredients that have been reported for skin whitening properties. Some of the skin whitening substances obtained from the various sources are depicted in Table 19.1.

19.4 Concluding Remarks

The current cosmetic market is a booming industry. Hence there is a constant need to investigate new cosmetic molecules. In the last decades, dermatological researchers have opened a new umbrella for cosmeceutical products with synergetic benefits in the field of cosmetic and pharmaceutical industry (Choi 2006). Nowadays, traditional cosmetics such as creams, lotions, and balms are shifted to cosmeceuticals products with naturally dynamic ingredients asserting to have medical and drug benefits (Chen et al. 2005; Schürch et al. 2008; Wijesinghe and Jeon 2011). Marine sources can be an exciting alternative to search for novel compounds. Numerous molecules from marine sources like carotenoids and polyphenolic compounds have proved to have good cosmetic benefits. They exhibit excellent antioxidant and anti-aging properties. The review has listed various compounds derived from different marine sources like algae, bacteria, and protists. These can address the demand for new cosmetically active molecules. Still, the marine niche is underexplored as far as cosmetic innovation is concerned and hence it would be a thrilling arena to search for more novel molecules.

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Marine Pharmacognosy: An Overview of Marine-Derived Pharmaceuticals

20

Kavya Bisaria, Surbhi Sinha, Ashutosh Srivastava, and Rachana Singh

Abstract

Medical science has come a long way in improving the life span and its quality. However, we still face challenges regarding a range of incurable diseases, developing resistance to current prognosis, as well as in the lack of new sources for drug development. Consequently, pharmaceutical industries are constantly in the search for new and advanced resources to produce safe and functional drugs to combat these challenges. Marine biosphere is one of the largest and intricate biospheres as water covers more than 70% of the earth inhabiting a number of flora and fauna. In the hostile marine environment, as part of their adaptation mechanisms, organisms produce specific secondary metabolites to survive in the ocean. These metabolites have very high biological activity and are explored for their potential therapeutic application. To date, seven marine-derived drugs have been approved as "first-in-class drugs" and several are in the clinical and pre-clinical pipeline. These drugs are successfully used in the market for the treatment of cancer, viral diseases, chronic pain, and to lower blood triglyceride levels. This chapter presents an overview of biodiversity in marine life, drug development, marine bioactive agents, and challenges faced in drug discovery, elucidating that the ocean is a largely untapped source of new-era pharmaceuticals.

Keywords

Marine drugs · Anti-microbial agents · Sponge · Bioactive molecules from marine

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20.1 Introduction

With the advancement in medical science, life expectancy and quality of life have greatly increased in the past few decades. However, there are life-threatening disorders and diseases that need attention. Mortality, especially at a young age, is a key indicator of population health. Avoiding premature mortality due to any health-related cause is the main aim of every health system (WHO 1946). Diseases such as cancers, diabetes, cardiovascular disorders, etc., do not have a 100% cure yet. The current treatment involves heavy medication, invasive surgery, and close follow-up, which often causes multiple side-effects. Sometimes, the disease might relapse or become resistant to the present dose, resulting in a poor quality of life. Therefore, with the present challenges and the additional advent of multiple drug resistant (MDR) and extreme drug resistant (XDR) bacterial strains, the need to search for potential novel drug sources has been emphasized.

Oceans cover more than 70% of the planet's surface, which makes them the largest habitat on earth. Extreme environmental conditions in the oceans give rise to the chemical diversity. Thus, recently, they are being explored for the derivation of new drugs. Approximately 30,000 compounds of marine source have been identified and, since 2008, more than 1000 compounds are being discovered every year. These compounds are generally characterized by their chemistry, species source, complexity, and diversity (Kiuru et al. 2014).

Marine pharmacognosy is a branch of pharmacy that deals with drugs derived from marine flora and fauna. The oldest marine product known is the dye Tyrian puple, which was extracted from marine mollusks in 1600 BC by Phoenicians. Agar, carrageenan, vitamin A and D from fish cod liver oil, or polyunsaturated fatty acids like docosahexaenoic acid and eicosapentaenoic acid are well-known products of the marine source. In the 1940s, the cephalosporins class of antibiotics was developed by isolation of cephalosporin C, produced by the fungus *Acremonium crysogenum* (Konig 1992) isolated from Mediterranean Sea water. However, the real marine drug development started with the discovery of bioactive nucleosides spongothymidine and spongouridine from the Caribbean sponge *Tethya crypta* in the 1950s (Bergmann and Feeney 1951; Bergmann and Burke 1955; Sagar et al. 2010; Anjum et al. 2016; Newman and Cragg 2014).

The present chapter gives an understanding of biodiversity in marine life, marine drug development, classification of marine agents on the basis of their pharmaceutical potential along with the drugs in various phases of clinical trial, and approved drugs with special mention to sponges and challenges in drug discovery.

20.2 Biodiversity in Marine Life

The marine environment is one of the richest as well as extreme biospheres on earth. Living conditions in the ocean differ drastically as compared to that on the earth's surface. Temperature ranges from −1.5 °C in the ice sea to 350 °C in deep hydrothermal vents, and pressure can vary from 1 atm to 1000 atm, from complete

darkness to extensive photic zones, and from nutrient-rich to nutrient-sparse regions. According to the World Register of Marine Species (2019), there are currently 247,009 known species. This number does not include microorganisms, whose diversity could count over a billion (Sogin et al. 2006). The International Census of Marine Life (CoML), a 10-year project that aimed at catalyzing discoveries on the abundance, diversity, and distribution of marine life, found approximately 5000 new species. Novel environments like hydrothermal vents and ice biota are continuously showing new species discoveries.

The discovery of unique hydrothermal vent fauna reported some 40 years ago (Cavanaugh 1983) gave a new perspective of a fundamentally different ecosystem in the fact that it is driven by chemicals such as hydrogen sulfide and methane, which are emitted from immensely hot sea water from the sea bed, rather than by sunlight as that on the earth surface (Cavanaugh 1983). Its complicated biogeography (Van Dover et al. 2002), a high number of endemic species and taxonomic distinctness, may yield a potentially higher number of species, with biologically active compounds, uncommon in the terrestrial species. Anoxic basins such as that in the Mediterranean Sea lack most of the living life forms but DNA analysis points out the presence of living Protista.

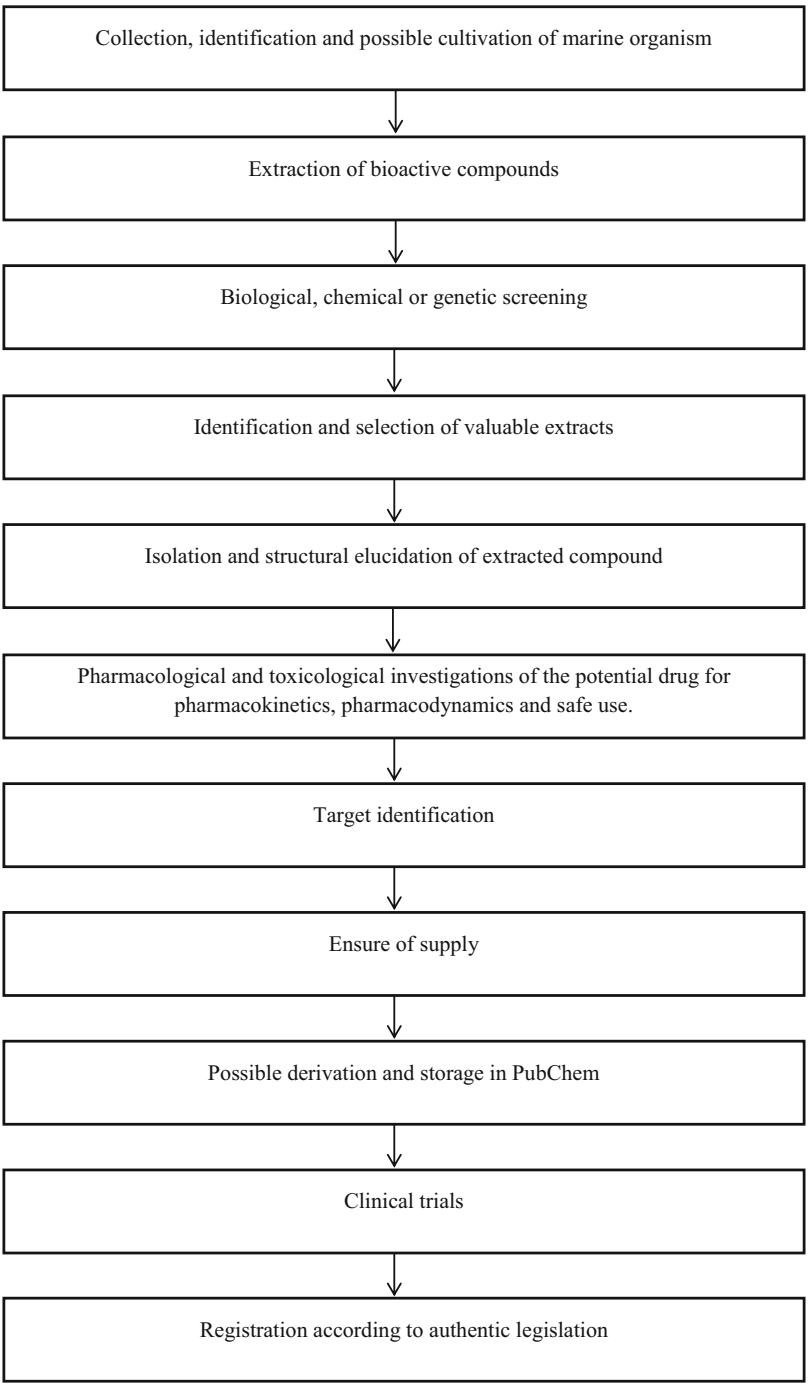
Even though the ice biota offers less diversity, with only a few species per major taxonomic group (Gradinger et al. 2010), they are found in extreme living conditions (temperature less than −10 °C and salinity more than 100 ppm).

Deep-water coral patches were first reported in the 1800s (Snelgrove and Smith 2003) but were not identified as distinct habitats until the twentieth century. They have been recognized as a definite habitat for a variety of microorganisms and invertebrates.

These findings suggest that the vastness of oceans, the stark contradictory living conditions as compared to that on earth as well as in the different regions in the ocean, has led the metabolically active life forms in such environment to have physiology we may have never known. Thus, to combat such an environment, these marine flora and fauna produce secondary metabolites that can be used for potential drug development.

20.3 Marine Bioprospecting and Drug Development

Bioprospecting refers to the systemic process of discovery and commercialization of medicinal drugs obtained from natural flora and fauna. It also targets marine organisms (Olson et al. 2007) and their bioactive products (Leal et al. 2012) for the development of drugs. Different steps involved in the development of a drug from these bioactive molecules are summarized below (Kiuru et al. 2014; Martins et al. 2014).


Collection, identification and possible cultivation of marine organism
Extraction of bioactive compounds
Biological, chemical or genetic screening
Identification and selection of valuable extracts
Isolation and structural elucidation of extracted compound
Pharmacological and toxicological investigations of the potential drug for pharmacokinetics, pharmacodynamics and safe use.
Target identification
Ensure of supply
Possible derivation and storage in PubChem
Clinical trials
Registration according to authentic legislation

20.4 Marine Drugs

20.4.1 Antimicrobial Agents and Antibiotics

Antimicrobial peptides (AMPs) act as host defensive peptides and are therefore regarded as an essential part of innate immunity. AMPs can protect the host from a broad spectrum of pathogenic microorganisms and hence they are an attractive target for pharmaceutical use. The marine AMPs are structurally different from their terrestrial counterparts (Charlet et al. 1996). They are advantageous in the fact that they are diverse, have a low bio-deposition rate in the host body tissues, and have a spectrum of antibiotic activities and high specificity for targets. A few such antimicrobial agents are described in Table 20.1.

20.4.2 Anti-cancer Agents

Marine-derived bioactive molecules have been found to be successful in anti-cancer therapy. Four out of seven approved marine-derived drugs are being used in various types of cancer treatment. A number of drugs are in the clinical phase and more are being tested in laboratories. Some of the anti-cancer agents are mentioned in Table 20.2.

20.4.3 Anti-viral Agents

Advances in biological science have greatly reduced the diseases caused by viruses. However, a substantial number of patients fail therapy due to recombinant viruses, drug resistance, and cell toxicity (Tantillo et al. 1994, Morfin and Thouvenot 2003, Gilbert and Boivin 2005). It is hence necessary to explore alternative anti-viral products. Table 20.3 summarizes some of the anti-viral agents derived from marine source.

20.4.4 Analgesic and Anti-inflammatory Agents

Millions of people worldwide suffer from chronic pain. Standard treatment options like morphine (opioid) has severe disadvantages like dependency, tolerance, the possibility of abuse, and side effects. Inflammation is a complex biological response against infection and injury-inflicted tissue damage. It is characterized by increased blood flow, vasodilation, vascular permeability, and cellular extravasation. Chronic inflammation has been reported to be linked to cancer, autoimmune disorders, asthma, etc. (Serhan and Savill 2005; Coussens and Werb 2002). Therefore, novel drugs are an urgent demand. The marine anti-inflammatory and analgesic agents and drugs from the last decade are listed in Table 20.4.

Table 20.1 Antimicrobial marine agents

Compound name	Chemistry	Source species	Action spectrum	Status	References
Indolepyrazines A and B	Alkaloid	*Acinetobacter* sp. ZZ1275	MRSA, *C. albicans, E. coli*	In vitro	Anjum et al. (2019)
L-amino acid oxidase (LAAO)	Amino acid	*Pseudoalteromonas luteoviolacea*	Broad spectrum	In vitro	Andreo-Vidal et al. (2018)
Chitosan oligosaccharide-streptomycin conjugate (CO-strep)	Polysaccharide conjugate	Chitin (from shrimp and crustaceans) treated with alkali	Microbial biofilms	In vitro	Li et al. (2019)
Ethanol extract	Polyphenol	*Gracilaria gracilis*	*Bacillus subtilis*	In vitro	Capillo et al. (2018)
Skin mucus	NA	*Dasyatis pastinaca*	*Klebsiella pneumoniae, E. coli, Pseudomonas aeruginosa*	In vitro	Fuochi et al. (2017)
Ageloxime B	Terpenoid	Sponge	*Staphylococcus aureus*	Pre-clinical	Yang et al. (2012)
Crysophaentins	Shikimate	Marine alga	FtsZ inhibitor	Pre-clinical	Keffer et al. (2013)
Hyrtimomine D	Alkaloid	Sponge	*Staphylococcus aureus*	Pre-clinical	Tanaka et al. (2013)
Cephalosporin C	Antibiotic	*Acremonium chrysogenum*	Broad spectrum	Approved	Konig (1992)
Anthracimycin	Polyketide	Actinomycetes	*Bacillus anthracis*	In vivo	Jang et al. (2013)

Table 20.2 Cytotoxic marine agents

Compound name	Chemistry	Source species	Action spectrum	Phase	References
Gukulenin A	Tetraterpenoid	*Phorbas gukhulensis*	Ovarian cancer cell lines (A2780, SKOV3, OVCAR-3, and TOV-21G)	In vitro	Ahn et al. (2019)
λ-carrageenan oligosaccharides	Polysaccharide	Red seaweed	Heparanase inhibitor	In vitro	Groult et al. (2019)
Cytarabine (Ara-C)	Modified nucleotide	*Cryptotethia crypta*	Acute myelocytic leukemia and non-Hogkin's lymphoma	Approved	Lindequistet al. (2019)
5-O-Acetyl-Renieramycin T	Tetrahydroisoquinoline marine alkaloids	*Xestospongia* sp.	Lung cancer stem cells	In vitro	Chantarawong et al. (2019)
Tetracenomycin X	Polyketide	*Streptomyces* and *Nocardia* sp.	Lung cancer cells- cyclin D	In vitro	Qiao et al. (2019)
Fascaplysin	Bis-indole alkaloid	*Fascaplysinopsis Bergquist* sp.	Cyclin-dependent kinase 4 (CDK4)-lung Cancer	In vitro	Rath et al. (2018)
Brentuximab vedotin (SGN-35) attached to dolastatin-10	Pentapeptide. Antibody-drug conjugate	*Dolabella auricularia*	Hogkin's lymphoma	Approved	Cheung et al. (2015)
Trabectedin	Alkaloid	*Ecteinascidia turbinata*	Soft tissue sarcoma	Approved	D'incalci et al. (2014)
Plitidepsin	Depsipetide	*Aplidium albicans*	Induces cell cycle arrest or apoptosis	Phase II clinical trial	Munoz-Alonso et al. (2009)
Gemcitabine	Nucleoside (derivative of cytarabine)	Sponge	Ribonucleotide reductase inhibitor	Phase III clinical trial	Krege et al. (2014)
Glembatumumab vedotin	Antibody drug conjugate	Mollusc	Breast cancer and melanoma	Phase II clinical trial	Bendell et al. (2014)

(continued)

Table 20.2 (continued)

Compound name	Chemistry	Source species	Action spectrum	Phase	References
Elisidepsin	Depsipetide	Mollusc	Antineoplastic agent, modifying lipids from cell membrane	Phase II clinical trial	Molina-Guijarro et al. (2015)
PM-10450 (Zalypsis®)	Alkaloid	Sponge	Transcription inhibitor	Phase I/II	Petek and Jones (2014)
Bryostatin-1	Polyketide	Bryozoa	Non-Hogkin's lymphoma, leukemia	Phase II	Varterasian et al. (2000)
Pinatuzumab vedotin	Antibody drug conjugate	Mollusc	Non-Hogkin's lymphoma, leukemia	Phase I	Forero-Torres et al. (2016)
PM-060184	Polyketide	Sponge	Microtubule interfering agent	Phase I	Newman and Cragg (2014)

Table 20.3 Marine anti-viral agents

Compound name	Chemistry	Source species	Action spectrum	Status	References
Halistanol sulfates	Sulfate sterols	*Petromica citrina*	HSV-1	In vitro	da Rosa et al. (2013)
Stachybogrisephenone B	Xanthone	*Stachybotrys*	EV71	In vitro	Qin et al. (2015)
Phlorotannins	Phloroglucinol	*Ecklonia cava*	Porcia epidemic diarrhea virus	In vitro	Cho et al. (2019)
11a-dehydroxyisoterreulactone A	Lactone	*Aspergillus terreus* SCSGAF0162	HSV-1	In vitro	Nong et al. (2014)
NK- lysins	Peptide	*Scophthalmus maximus*	Spring viremia of carp virus	In vitro	Falco et al. (2019)
Echinochrome A	Naphthoquinoid	Sea urchin	HSV-1 and TBEV	In vitro	Fedoreyev et al. (2018)
Oroidin	Alkaloid	*Stylissa carteri*	HIV-1	In vitro	O'Rourke et al. (2016)
Cladosin C	Polyketide	*Cladosporium sphaerospermum*	Influenza A H1N1 virus	In vitro	Wu et al. (2014)
Sulfoquinovosyldiacylglycerol (SQDG)	Glycolipids	*Osmundaria obtusiloba*	HSV-1 and HSV-2	In vivo	De Souza et al. (2012)

HSV-1 herpes simplex virus-1, *TBEV* tick borne encephalitis virus, *HIV* human immunodeficiency virus

Table 20.4 Marine agents with analgesics and anti-inflammatory effect

Compound name	Chemistry	Source species	Action spectrum	Status	Reference
Excavatolide B	Diterpene	*Briareum excavtum*	Gene expression of iNOS and COX-2	Lab	Lin et al. (2015)
ω-Conotoxin (Ziconotide)	Peptide	*Conus magus*	Blocking N-type calcium channels on the primary nociceptive nerves of the spinal cord	Approved	Malve (2016)
β- thymosin	Polypeptide	*Crassostrea gigas*	LPS induced RAW264.7 macrophage cells	Lab	Hwang et al. (2019)
Neorogioltriol	Diterpenes	*Laurencia glandulifera*	Inflammatory bowel disease- suppresses M1 and promotes M2-like macrophage responses	Lab	Daskalaki et al. (2019)
Eckol,dieckol and 8,8′-bieckol	Phlorotannins	*Ecklonia cava*	Alzheimer disease- amyloid-β peptide induced damage on PC12 cells	In vitro	Lee et al. (2019)
Tetrodotoxin	Aminoperhydroquinazoline	Puffer fish	Pain from chemotherapy	Clinical phase III	Hagen et al. (2017)
Flexibilide	Diterpene	*Sinularia flexibilis*	Antinociceptive pain	Animal model- chronic constriction injury in rats	Chen et al. (2014)
Heterofucan (F2.0v)	Sulfated polysaccharide	*Dictyota menstrualis*	Antinociceptive and anti-inflammatory	In vitro	Albuquerque et al. (2013)
Kunitz type HCRG-1 and HCRG-2	Polypeptide	*Heteractis crispa*	Inflammatory proteases, modulation of cytokine expression	In vitro	Gladkikh et al. (2015)

20.4.5 Anti-thrombin and Anti-coagulant Agents

Thrombin is a serine protease enzyme that participates in a series of reactions that ultimately leads to activation of clotting factors. Anti-thrombin and anti-coagulants are agents that prevent clotting of blood.

Heart and blood-related ailments such as ischemic heart disease, deep vein thrombosis, atherosclerosis, and stroke are a major cause of mortality. According to the World Health Organization, approximately one-fourth of the total deaths worldwide are due to these complications (WHO 2018). Such episodes are managed by giving patients anti-thrombin and anticoagulant medicines. However, they are often accompanied by moderate to severe side-effects (Mannucci and Franchini 2011). Heparin is one such drug currently in use; its side-effects include thrombocytopenia (Ahmed et al. 2007) and hemorrhage (Clark et al. 1991). Therefore, novel drugs with minimal side effects are urgently needed. Few such agents from marine source are listed in Table 20.5.

20.4.6 Anti-diabetic Agents

Diabetes is a chronic metabolic disease characterized by high blood sugar level, which arises due to age or poor lifestyle. It is manageable up to an extent with strict dietary and physical changes. However, if uncontrolled, it can be life-threatening. It can progress to blurred vision, cardiovascular disease, and organ damage (Andreoulakis et al. 2012). Diabetes is of two types: type I, which is caused due to a lowered production of insulin by the pancreas, and type II, which is a result of insulin resistance. Protein tyrosine phosphatase 1B (PTPB 1) catalyzes the dephosphorylation of phosphotyrosine residues and controls the phosphorylation level of proteins involved in insulin signaling, thereby downregulating its production. PTPB 1 agents have poor cell permeability and bioavailability (Lund et al. 2004). Hence, the development of more efficient PTPB 1 inhibitors and new anti-diabetic pharmaceutical agents is greatly significant.

Few of the anti-diabetic agents isolated from marine life are listed in Table 20.6.

20.5 Marine Sponges: Major Role as a Potential Therapeutic

Marine life with its seemingly infinite biodiversity is a promising source of bioactive compounds. Among the great biodiversity of ocean and sea, marine sponges have been one of the key resources for natural, bioactive compounds with potential therapeutic activity. This is owed to the fact that sponges produce a wide variety of secondary metabolites with unique structural characteristics. Sponges are sessile, immobile, and filter-feeders belonging to the phylum *Porifera* and lack body symmetry (Hadas et al. 2009). The majority of the sponges are soft and since they are incapable of movement, they become an easy target of predators like fish, turtles, and invertebrates. Therefore, as a survival tactic, sponges produce a variety of

Table 20.5 Marine anti-coagulant and anti-thrombotic agents

Compound name	Chemistry	Source species	Action spectrum	Status	References
F-I and F-II (extracts)	Sulfated galactans rich extracts	*Udotea flabellum*	Inhibits thrombin, similar effects as that of heparin	In lab	Marques et al. (2019)
3-linked 2-sufated α-galactan	Sulfated galactans	*Echinometra lucunter*	Inhibitory effects in the in vitro coagulation assay, in vivo thrombosis assay, and in vitro platelet aggregation	In vivo, in vitro	Marques et al. (2019)
Sulfated rhamnan	Polysaccharides	*Monostroma angicava*	High fibrinolytic and thrombolytic activities	In vivo, in vitro	Liu et al. (2018)
Abalone extract	Sulfated polysaccharide	*Haliotis rubra*	In vitro prothrombin time, activated partial thromboplastin time were prolonged.	In vitro, in vivo animal model	Suleria et al. (2017)
Dichotomanol, pachydictyol A, isopachydictyol A	Diterpenes	*Dictyota menstrualis*	NA	In silico	Pereira et al. (2017)
2-sulfated galactan	Sulfated galactan	*Echinometra lucunter*	Platelet aggregation	In vitro and in vivo	Vasconcelos et al. (2018)
Fucoidan	Sulfated fucose rich carbohydrates	*Undaria pinnatifida*	Hemostasis	Clinical study	Irhimeh et al. (2009)

Table 20.6 Marine anti-diabetic agents

Compound name	Chemistry	Source species	Action spectrum	Status	References
Polysaccharide chromium (III) derivatives	Rhamnam type sulfated polysaccharide derivative	*Enteromorpha prolifera*	Improved glucose metabolism	In vivo	Cui et al. (2019)
Aquastatin A	NA	*Cosmospora sp.*	PTP1B inhibition	In vivo	Debbab et al. (2010)
Astaxanthin	Carotenoid pigment	*Chlorella Zofingiensis*	Inhibits lipid peroxidation and scavenging reactive oxygen species	In vivo	Sun et al. (2011)
Docohexaenoic and Eicosapentaenoic acids	Polyunsaturated fatty acids	*Isochrysis galbana*, *Nannochloropsis oculatat*	Clinical values and intestinal inflammation in rats	In vivo	Nuño et al. (2013)
CYC27	Synthetic derivative of marine bromophenol BDB	*Rhodomela confervoides*	Decreased blood glucose levels, reduced total serum cholesterol and triglyceride levels	Animal model – BKS db mice	Luo et al. (2019)
Collagen peptides	Peptide	Fish hydrosylates	Improved glucose and lipid metabolism	Clinical study	Zhu et al. (2010)
Sargahydroquinoic acid, sargachromenol and sargaquinoic acid	Plastoquinones	*Sargassum serratifolium*	PTP1B and α-glucosidase inhibitory activity	In vivo	Ali et al. (2017)
HPN	Bromophenole analogue	*Rhodomela confervoides*	PTP1B inhibition	Animal model – C57BL/KsJ-*db*/*db* mice	Shi et al. (2013)

HPN- 3,4-dibromo-5-2-bromo-3,4-dihydroxy-6 (isopropoxymethyl) benzyl benzene-1,2-diol

chemical compounds, including terpenes, sterols, cyclic peptides, fatty acids, alkaloids, peroxides, amino acid derivatives, and unusual nucleosides, to deter predators from preying upon them (Thomas et al. 2010). They also secrete defensive materials to keep small plants and animals from settling upon them (Hertiani et al. 2010).

These bioactive nucleosides were the main agents in the formation of anti-viral drug ara-A and the first marine-derived anti-cancer drug, ara-C (Proksch et al. 2002). It is now used to treat leukemia and lymphoma. Many sponge-derived substances are now in pre-clinical or clinical phases of anti-inflammatory and anti-cancer drug testing (Martins et al. 2014).

The marine sponge also exhibits low-intensity anti-bacterial properties against marine bacteria and at greater intensity against terrestrial bacteria (Xue et al. 2004). It is reported that approximately 800 antibiotic substances have been extracted from marine sponges (Torres et al. 2002). Manoalide, a sesterterpenoid isolated from *Luffariella variabilis*, was found to be an antibiotic (de Silva and Scheuer 1980). However, no commercial anti-bacterial product has been obtained from them yet.

Anti-viral properties have also been studied in the marine sponge. Different sponges producing HIV-inhibiting compounds have been reported in several papers (Ford et al. 1999; Qureshi and Faulkner 1999; Yasuhara-Bell and Lu 2010; Sagar et al. 2010). Avarol, one such example, has been reported in vivo and in animal studies to inhibit HIV progression from infected cells by 50% and 80% at the concentration of 0.3 μM and 0.9 μM (Müller et al. 1987). Moreover, the derivatives of avarol are a stronger inhibitor of HIV reverse transcriptase enzyme.

Immuno-compromised people are at a greater risk of getting a fungal infection, and sometimes it becomes a direct cause of death for cancer patients (Sandven 2000). Currently, used fungicides are not as diverse as anti-microbial drugs and are restricted in use due to their biological toxicity. Jaspamide, a macrocyclic depsipeptide, isolated from *Jaspis sp.* has a selective in vitro anti-fungal activity against *Candida albicans*. Purine derivatives agelasines and agelasimines, isolated from *Agelas sp.* is reported to have anti-fungal property against *Candida krusei* (Vik et al. 2007). 24-methoxypetrosaspongia C, a sesterterpene isolated from *Hyrtios erectus* and Plakortide P, a polyketide isolated from *Plakortis angulospiculatus*, has anti-tumor properties (Elhady et al. 2016) (Kossuga et al. 2008).

Immunosuppressants are drugs that are given to patients in case of organ transplant, to avoid rejection of donor organ or graft by the immune system. Simplexide, isolated from Plakortis simplex, acts as an immunosuppresant by inhibiting T-cell proliferation (Costantino et al, 1999). *Dysidea sp.* have great contribution in the portion of biomolecules (Mayer et al. 2011). Three polyoxygenated sterols derived from *Dysidea sp.* from North Australia block the binding of interleukin 8 with its receptor (de Almeida Leone et al. 2000).

20.6 Approved Drugs Derived from Marine Bioactive Molecules

The past 30 years have been vital in the extraction of the bioactive compounds from marine source. Out of these several agents, seven have reached the market as clinical drugs (four anti-cancer, one anti-viral, one analgesic, and one for hypertriglyceridemia) (Mayer et al. 2010). Ziconotide, a peptide discovered in Tropical cone snail, was the first ever marine-derived drug in 2004. It was used for the treatment of pain. Trabectedin in 2007 was the first marine-anti-cancer drug. In 1986, a polyether metabolite halichondrin B was isolated from the sponge *Halichondria okadai* (Hirata and Uemura 1986). This agent showed toxicity toward cancer cells and after subsequent studies leading to structural modification finally gave rise to Eribulin. Eisai pharmaceuticals marketed this agent under the brand name Halaven and are now used to treat drug refractory breast cancer (Menis and Twelves 2011).

20.7 Challenges and Future Prospects

Procuring drugs from marine sources have some indisputable challenges. Firstly, the variation in the environmental conditions could lead to the production of different metabolites every time. Therefore, to extract a particular metabolite becomes tricky. Many of the natural compounds extracted are of their microbial associate. These microbial populations usually cannot be grown on a pure culture since they are symbiotically associated with their marine host. Even if they are cultivated in vitro, only a part of their biosynthetic genes transcribe. Exploiting the full metabolic potential of these microorganisms is a challenge (Martins et al. 2014). One of the reasons for the lack of marine drugs in clinical studies is the paucity of "continuous supply." Several hundred grams of the compound are required for pre-clinical studies and several kilograms required for clinical studies.

In the coming future, bioactive compounds from marine organisms can be chemically modified with different bioisosteric units to develop "drug-like molecules." A sustainable supply of micro-organisms can be aided by techniques in mariculture and aquaculture. Recently, the concept of genome mining has been introduced where researchers can understand biosynthetic, evolutionary, and defensive strategies employed by the ocean inhabitants. Collaborative efforts from all the fields of science like organic chemistry, medicinal chemistry, bioinformatics, and pharmacology can also facilitate the commercialization of marine natural products as therapeutics.

20.8 Conclusion

Even though a variety of new compounds have been extracted and isolated from marine organisms with strong biological activity, very few have been marketed as pharmaceutical products. Thus, the pharmaceutical potential of marine life is yet to

be realized for the possible development of various adjuvants. Marine compounds and their derivatives can have a path-breaking improvement in the current scope of medical science, and therefore more research is required for the development of novel drugs, agrochemicals, and research biochemicals. As the drugs in the pre-clinical and clinical pipeline are considered, several of which will almost certainly reach the market, the ocean will probably be a major source for the pharmacy of the future.

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Compatible Solute Ectoines: Fancy Marine Product for Pharmaceuticals and Cosmeceuticals

21

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Abstract

Oceans being immense source of natural products of biological origin still host enormous biodiversity and product diversity which are to be explored. Marine waters, soils and sediments, saltern crystallizer ponds, and alkaline soda lakes are of the major interest among the researchers for the study of industrially important biological products like "compatible solutes". Halophilic microbes belonging to bacteria, archaea and actinomycetes are reported for the accumulation of compatible solutes like ectoine and its hydroxy derivative. Studies related to the synthesis of these compounds revealed the molecular foundations and their physiological correlations with the expressions of the responsible genes. Genetic engineering-aided research has provided the deep insight into the probable role of these compounds in the maintenance of osmotic balance as well as in the osmo- and thermoprotection and stabilization of cellular components like DNA, enzymes and other proteins; applaud them as chemical chaperons. Such crucial functions make ectoine an indispensable compound for some of the biotechnology, medicine and skincare industries. Very tiny amount of product recovery is one of the agitating problems in the large-scale production processes, making the purified product a pretty penny.

Keywords

Compatible Solutes · Halophilic Microbes · Ectoine · Osmoprotection

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21.1 Introduction to Compatible Solutes

Since hundreds of years salt solutions are being used in day-to-day life of human being for various applications like preservation, because of the inhibitory effect of high salt concentrations on microbial growth. While on the other side a small quantity of salt is desirable for the better growth of microorganisms, as in the microbial cultivation media.(Melmer 2011; Ingram and Kitchell 1967) An important parameter that affects all the microorganisms is osmolarity due to the salinity of the growth medium. The salt concentration ranges which can be tolerated by microorganisms are different in case of each different organism, because increase or decrease of salts in the surrounding makes a change to water activity across the cell membrane. As per the limits of tolerance, microorganisms can be distinguished into salt-tolerant and salt-intolerant ones.(Czech et al. 2018)

The organisms surviving strictly in high concentrations of salt in their environment (hypersaline environments) are named as halophiles ("salt lovers"), which further are partitioned as slight, moderate and extreme halophiles as they can live with 2–5% NaCl [0.34–0.85 M], 5–20% NaCl [0.85–3.4 M] and 20–30% NaCl [3.4–5.1 M].(Ollivier et al. 1994) On the other side, defining halotolerant organism is a bit difficult task as the tolerance for salts in the environment can be of a wide range. Even the quantities of organic molecules or inorganic ions gathered inside cytoplasm can aid into the development of "halotolerance".(Melmer 2011) Generally, microbes do not possess active transport channels for water in their cell membrane. Hence the water activity across the cell membrane can only be maintained by "osmosis". As the organic molecules like protein, nucleic acids, sugars and other metabolites can together affect the osmotic potential of the cell, influencing the water influx together with development and maintenance of turgor pressure on the cell wall. Extreme conditions like high concentration of salts in the surrounding medium or environment can affect the survival of non-halophilic and halotolerant microorganisms dramatically.(Czech et al. 2018) To the surprise, halophiles and halotolerant ones can survive not only the high salt concentrations but also higher or lower temperatures. Hence it can be considered that halophiles and halotolerant microbes are very diversified and need different isolation methods and nutritional requirements and seek more attention to grow at higher cell densities for the purpose of detection and analysis of the unique compounds or molecules produced by the cells.(Melmer 2011)

In case of the majority of the microorganisms, growing at high salt concentrations maintains osmotic balance across the cell membranes by one of the two strategies to maintain the gradient of water across the membrane. The first mechanism (salt-in strategy) uses potassium K^+ and chloride Cl^- ions to achieve osmotic equilibrium. In detail, this method includes replacement of the Na^+ ion by K^+ ion in the cytoplasm which is an energetically favourable process. But as it involves the accumulation of positively charged ions in cytoplasm, the molecules like proteins need to be adapted to get solubilized. This leads to modification of protein compositions and the majority of proteins of such a cell shows more negatively charged amino acids on their surface, making them little bit acidic in nature. Further, this strategy is less

adaptive and can put the organism in threat while suddenly exposed to lower salt concentration. The mechanism is widely used by the archaea.(Czech et al. 2018; Kunte et al. 2014)

The second mechanism is organic-osmolyte strategy or salt-out strategy, used by eubacteria. This strategy takes advantage of the accumulation of non-ionic low molecular weight organic compounds to counterbalance the osmotic stress imposed by the environment. These low molecular weight organic compounds are called compatible solutes. At the initial stage, this strategy too intakes the K^+ ions to immediately adjust with the changing environment but after a period of time, as the sufficient quantity of compatible solute will be generated, it will release the K^+ ions. The major benefit of the strategy is that the organisms which are adopting it do not need to have additional adaptations at their protein or enzyme level against extreme conditions.(Czech et al. 2018; Kunte et al. 2014) For example, compatible solutes protect proteins against freezing, drying and high temperature.(Lippert and Galinski 1992)

Non-salt-tolerant bacteria like *Escherichia coli* are not able to synthesize compatible solutes in excess amounts but still can resist certain extent of salt concentration gradient by taking up compatible solutes.(Schubert et al. 2007)

21.2 Various Compatible Solutes

The organic compounds or molecules used as compatible solutes include sugar derivatives (trehalose, sucrose), amino acid derivatives (betaine and ectoines), polyols and derivatives. Presence of compatible solutes has been reported in all the three domains of life. These compounds can either being synthesized or being uptake from the environment by the organisms.(Kunte et al. 2014) These solutes can be divided into non-charged or neutral, negatively charged and zwitterionic ones for easy understanding. And very often the negatively charged compounds can be found in archaeal life forms while bacteria and eukaryotes accumulate non-charged and zwitterionic compounds.(Roberts 2005) A comprehensive list of compatible solutes and the microorganisms synthesizing them is provided by Roberts(Roberts 2005) as well as by Pastor et al.(Pastor et al. 2010) respectively.

Amongst all, the ectoine is a solute which can be found most widely; that is throughout various halophile and halotolerants belong to α-proteobacteria and γ-proteobacteria and actinobacteridae. Limited synthesis has also been observed in β-, δ-, ε-proteobacteria, firmicutes and plantomycetes.(Pastor et al. 2010)

A number of scientists have studied the organisms which are producing ectoine and derivatives. The early work of Galinski and co-workers has been seen to provide the foundation in the study of ectoines and their production. Ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidinecarboxylic acid) being heterocyclic amino acid was discovered in the extremely halophile *Ectothiorhodospira halochloris* and characterized by ^{13}C-NMR spectroscopy, mass spectrometry as well as infrared spectroscopy.(Galinski et al. 1985) *Ectothiorhodospira halochloris,* when exposed

to 5 M NaCl concentration, showed the accumulation of glycine-betaine has also been observed for simultaneous production of ectoine.(Raymond and Sistrom 1969)

Hydroxyectoine, 5-hydroxy tetrahydro pyrimidine derivative, was reported in *Streptomyces parvulus* by incorporating ^{13}C- and ^{15}N-labelled L-glutamate for the cultivation of the organism. It was further noticed that providing D-fructose in the growth medium, which got converted to glutamate intracellularly, aided in the accumulation of ectoine. And both hydroxyectoine and ecoine took part in actinomycin D synthesis proving both the solutes as probable nitrogen sources for the cells. In addition to that, it was observed that an increase in intracellular concentration of the ectoine first helped in the accumulation of hydroxyectoine(Inbar and Lapidot 1988); the mechanism which has been observed often in other ectoine-accumulating organisms. Moreover hydroxyectoine, though being almost similar to ectoine, has been observed to play a role in thermoprotection as well in addition to the osmoregulatory and cryoprotective functions. In their studies on the derivation of the hydroxyectoine and ectoine from streptomyces strain and its effect on *E. coli*, Malin and Lapidot observed the accumulation of hydroxyectoine in *E. coli* via *ProP* and *ProU* gene products for maintenance and restoration of cell growth. (Malin and Lapidot 1996)

21.3 Genetic Basis of Ectoine Synthesis by Microbes: Operon *ectABC*

Synthesis of ectoine by microorganisms and its regulation have been studied since years. Most studied species till date is *Halomonas elongata*¸ which is used for the industrial-scale production of the ectoine and hydroxyectoine. To make use of a microbial species for industrial scale, it is mandatory to know its genome construction for process optimization. Hence researchers started looking for the genes responsible for ectoine production.

The pathway of ectoine synthesis was first proposed in *Ectorhodospira halocholris* and *Halomonas elongata*. Findings of Peters et al. (Peters et al. 1990) suggest that the pathway starts with phosphorylation of L-aspartate. The pathway can be diverted in either way to the synthesis of amino acid L-lysine, threonine, methionine and homoserine or to ectoine synthesis by first two steps, mediated by aspartokinase and L-aspartate-beta-semialdehyde dehydrogenase. In addition to these two enzymes, L-diaminobutyric acid transaminase, L-diaminobutyric acid acetyl transferase and ectoine synthase have been reported to be present in both of the organisms. Moreover, pyridoxal 5′-phosphate and K^+ are essential factors for the transaminase activity, while rest of all the enzymes can also be activated NaCl/KCl, indicating that intracellular concentration of cations may regulate the ectoine synthesis.(Roberts 2005; Ono et al. 1999)

Further studies were done using a moderate halophilic Gram-positive bacterium *Marinococcus halophilus* to draw ideas about genes producing enzymes involved in the pathway of ectoine synthesis. According to the report of Louis and Galinski, they cloned the genes from *Marinococcus halophilus*, responsible for the functional

expression of the ectoine biosynthesis, in *E. coli*. On the basis of sequencing of 4.4 kb fragment and deletion mutation studies, they were able to conclude that four ORFs denoted as *ectA*, *ectB*, *ectC* and *orfA* are the major ORFs for ectoine synthesis in the organism under study. And significance of *ectA*, *ectB* and *ectC* genes was provided by sequence comparison studies against the known protein sequences and the challenges in the form of increasing salinity of the medium, provided in physiological experiments. Deletion studies have aided to prove the ability of the genes to synthesize one or more intermediates in the pathway. It has been demonstrated that *ectA*, *ectB* and *ectC* code for L-diamiobutyric acid acetyltransferase, L-diaminobutyric acid transaminase and L-ectoine synthase; more than that a region upstream to the *ectA* gene has been noted important for the regulated synthesis of the ectoine.(Louis and Galinski 1997)

More investigations on the genetic information led to the detection of stress responding promoter region in *Marinococcus* genome. This promoter region is situated upstream of the *ectABC*, denoted as *ectUp* and has a sequence length of 480 bp. *Marinococcus halophilus* genes were cloned in $pBRGFP_{UV}$ plasmid. It was noticed from the experiments that only osmotic stress led to higher fluorescence detection; not the heat or cold stress, indicating that transcription and translation of the gene can only be controlled by water stress.(Bestvater and Galinski 2002) Comparison of the data published by Louis and Galinski(Louis and Galinski 1997) and Bestvater and Galinski(Bestvater and Galinski 2002) suggests that orfA and the *ectUp* promoter have to be the same regions.

More studies on the genome analysis for ectoine biosynthesis pathway in various microorganisms have provided the details about *ectD* gene which has been recorded present in organisms belong to the proteobacteria, firmicutes and actinobacteria. *Chromohalobacter salexigens*, a member of γ-proteobacteria, have been studied quite in deep for the synthesis and regulation of ectoine and hydroxyectoine by various groups of scientists. *ectD* gene has been characterized from *C. salexigens* and reported to produce ectoine hydroxylase, an enzyme necessary for hydroxyectoine formation from the ectoine either synthesized by organism itself or already present in the medium. ^{13}C NMR study of the cells of *C. salexigens* revealed the observations that cells grown in minimal medium at 45 °C temperature accumulated hydroxyectoine in addition to ectoine as one of the compatible solutes. Accumulation of ectoine has been up- and down-regulated with increase in salinity/osmotic stress and temperature respectively, while that of hydroxyectoine has been upregulated with respect to increase in salinity and temperature; providing evidence that hydroxyectoine is necessary for thermoprotection of *C. salexigens* and transcription of gene *ectD* is controlled by temperature.(García-Estepa et al. 2006)

Deeper studies on *C. salexigens* provided the evidence of transcriptional regulation of *ectABC*. S1 nuclease assay of total RNA extracted from the organism provided idea of four putative initiation sites at 44, 96, 134 and 149 bp upstream to the *ectA* start codon; indicating the presence of four putative promoters of *ectA*, namely *ectAp1*, *ectAp2*, *ectAp3*, and *ectAp4*. One more putative promoter site was found 25 bp upstream to *ectB* coding region, named as *ectBp*. Products of putative promoter *ectAp1*, *ectAp2* and *ectAp4* were found in higher concentrations at higher

salinity, proving them as osmoregulated promoters. Presence of glycine-betaine in the medium showed suppressed transcription of *ectAp1*, *ectAp2* and *ectAp3*, indicating negative regulation of *ect* genes by glycine-betaine. It has been observed that consensus sequence of *ectAp1* and *ectAp2* resembled σ^{70}, *ectAp3* resembled σ^{S} and *ectBp* resembled σ^{32} (a heat stress inducible factor), which are dependent promoters of *E.coli* (Isabel Calderón et al. 2004).

One more gene responsible for the regulation of ectoine production was identified by Mustkhimov et al.(Mustakhimov et al. 2009; Mustakhimov et al. 2010; Mustakhimov et al. 2012), named *ectR*. This novel gene *ectR* was identified in haloalkaliphilic methylotrophic bacteria *methylophaga alcalica*[20] and a halotolerant methanotroph *Methylomicrobium alcaliphilum*[21]. Since the compatible solute accumulation is directly related to osmotic stress, it is obvious to look for a regulatory mechanism which can control the expression of the genes responsible for the synthesis of enzymes of ectoine production pathways. Studying *M. alcalica* nucleotide sequence revealed that biosynthesis of ectoine in the organism is due to *ectABC-ask* operon; *ask* gene producing aspartokinase enzyme. In addition to that, 268 bp upstream to *ectA* is the location of an ORF made up of 567 bp (arranged in reverse direction than *ectA*), which encodes for 188 amino acid residues containing protein EctR which belongs to MarR transcription regulatory protein family. Proteins of MarR family mainly regulate physiological functions like bacterial stress response, synthesis of virulence factors and catabolism of aromatic compounds. Lower identity between primary protein sequences and higher identity between secondary structures of protein is a character of this family of proteins. (Mustakhimov et al. 2009) Further studies on *M. alcaliphilum* gave the idea that the *ectR* gene product binds to the two promoter regions of the *ectA* gene namely *PectA1* and *PectA2* and controls *ectABC-ask* operon negatively (null mutation of *ectR* resulting in overexpression of *ectABC-ask* operon). Binding site of EctR is situated on promoter 1 of the *ectA* gene and is made up of pseudopalindromic sequence made up of 8 bp half sites separated by 2 bp; TATTTAGT-GT-ACTATATA. Findings suggest that the binding site of EctR is situated between the transcription and translation initiator sites of the *ectR* gene, pointing towards the thought of self-regulation of gene.(Mustakhimov et al. 2010) Phylogenetic analysis of *ectR* from *Methylophaga thalassica* revealed the presence of homologous genes in the genome of marine bacteria like *Alcanivorax borkumensis*, *Reinekea sp.*, *Oceanospirillum sp.*, the soil bacterium *Nitrobacter sp.*, and some others. In most of the others the ORF is situated immediately upstream to *ectA* in opposite direction. The *ectR* gene contains three promoter elements *ectRp1*, *ectRp2* and *ectRp3* amongst them ectRp1 is a potential promoter element as it shows sequence homology to σ^{70}-dependent promoters of the other bacteria.(Mustakhimov et al. 2012)

21.4 Transporters of Ectoine and Other Compatible Solutes

Compatible solutes, as per the definition, can make the cells compatible against adverse environmental conditions by preserving cells physiology; and their respective concentrations are continuously changing as per the measure of adversity. Hence it is very obvious to think about the mechanisms which can mediate the uptake and accumulation or release of these organic molecules(Oren 1999); the channel proteins or transporters. Various studies have been made to reveal the idea about the osmoporters.(Jebbar et al. 2005) For most of the non-halophilic organisms these transporters are of broad or narrow specificity types.(Jebbar et al. 2005) For *E. coli*, it's been noted that ProP and ProU systems are involved in ectoine uptake in case of a slight increase in osmotic stress. There are 3 systems involved in proline transport in *E. coli*, denoted as PutP, ProP and ProU, to use proline as carbon or nitrogen source for the cell. Amongst them ProP and ProU are involved in proline and glycine-betaine accumulation. ProP is a constitutive low-affinity transport system, while ProU is a high-affinity binding protein which includes three protein products ProX, ProV and ProW, functions as periplasmic glycine-betaine binding protein, and two integral membrane protein components of the system respectively. Further studies of the mutant *E. coli* strain, having mutation in *proP* and *proU* genes showed no uptake and accumulation of ectoine against the wild type strains of *E. coli* having active *proP* and *proU* genes; accumulating ectoine provided in media in case of osmotic stressed condition.(Jebbar et al. 1992)

Similarly, the transporter system for ectoines has been studied in *Halomonas elongata* mutants defective in ectoine synthesis. 7200 insertion mutants were prepared out of which one mutant showed difficulties in the accumulation of ectoines; which on genetic analysis revealed that the insertion in one ORF of 1281 bp named *teaC* made this happen. Upstream regions of *teaC* were also sequenced and two found ORFs were *teaB* (603 bp) and *teaA* (1023 bp). On deletion of these two ORFs, the mutants showed good amount of leaking of the ectoines out of cells, providing evidence that all the three genes are necessary for ectoine accumulation by *H. elongata* cells.(Grammann et al. 2002; Tetsch and Kunte 2002) Further investigations on protein sequence comparison studies led to the conclusion that all the three proteins TeaA, TeaB and TeaC are identical to an ATP-independent periplasmic transporter family TRAP-T. TRAP-T systems are made up of three components mainly a large and a small transmembrane proteins as well as a periplasmic protein which is a key component to substrate-binding function of the system.(Grammann et al. 2002) These transporters were found to be osmoregulated during the study.

Studying the transporter systems for ectoine in *Marinococcus halophilus,* it has come to the knowledge that the bacterium contains two different proteins responsible for uptake of ectoines. Plasmid containing genes of *M. halophilus* has been transferred to *E. coli* strain MKH13 (deletion mutation of *proP*, *proU* and *putP*) lacking the uptake systems for ectoines. Transferred bacteria were studied for sequence analysis revealing the two ORFs *ectM* (1578 bp) and *betM* (1482 bp). EctM, a putative hydrophobic protein consisting of 525-residues weighing 58.48 kDa; while

BetM is a putative hydrophobic protein made up of 493-residues weighing 53.9 kDa. Both the proteins belong to betaine-carnitine-choline transporter (BCCT) family. EctM has been found 35% identical to EctP and BetP transporters from *C. glutamicum*; EctP being a transporter for ectoines, glycine-betaine and proline. Furthermore, both of these proteins were tested for substrate affinity on the growth mediums containing 765 mM NaCl and presence or absence of compatible solutes like ectoine, hydroxyectoine, glycine-betaine, proline and carnitine; revealing that EctM supports the uptake of ectoines only (ectoine and hydroxyectoine), while BetM supports uptake of glycine-betaine and ectoine, not hydroxyectoine and the others. Hence EctM can be considered as a narrow substrate-specific transporter. (Vermeulen and Kunte 2004)

After getting the idea of the osmoprotective effect of ectoine, Jebbar and colleagues tried to study the effect of ectoine in *Rhizobium meliloti*. During their studies, they have found ectoine as effective as glycine-betaine. Furthermore, it was observed that insensitive strains to glycine-betaine can also grow on ectoine supplement.(Talibart et al. 1994) Proteomic studies also revealed that ectoine can induce about 10 different proteins in *R. meliloti*. Among the ectoine-induced polypeptides, four were the putative ABC transporter proteins, which are an amino-acid-binding protein (Smb20428), a periplasmic component of a putative amino acid ABC transporter system which also comprises three other components, an ATPase (Smb20427) and two permeases (Smb20429 and Smb20430). Genes responsible for these proteins were named *ehuB*, *ehuA*, *ehuC* and *ehuD* respectively (named by function Ectoine Hydroxyectoine Uptake), which are located on the megaplasmid pSymB amongst the three replicons of *R. meliloti* known to be a chromosome (3.7 Mb) and two megaplasmids pSymA (1.4 Mb) and pSymB (1.7 Mb). Downstream to the ABC transporter gene were the genes for ectoine utization (*eut* genes). *eutA*, *eutB*, *eutC*, *eutD* and *eutE* encode a hypothetical arylmalonate decarboxylase, a putative threonine dehydratase, a putative cyclodeaminase, a putative hydrolase peptidase, and a hypothetical protein that might be involved in ectoine catabolism, respectively. Upstream of the *ehu* transporter genes, there is a hypothetical transcriptional regulator gene (*smb20426*) of the GntR family, which is oriented in the same direction as the *ehuABCD* genes. Moreover, from the BLAST analysis it came to note that the *S. meliloti* EhuA, EhuB, EhuC and EhuD components of the Ehu transporter exhibit high homologies to components of putative amino acid uptake systems, and EutABCDE exhibits high homologies to proteins with unknown functions and to putative amino acid catabolic enzymes. Study of *ehuA* and *eutA* mutants of *R. meliloti* added to the knowledge that at least one more transporter system and catabolic pathway do exist in the organism for ectoines.(Jebbar et al. 2005) As well as, ectoine being an inducer of various protein and their functions in the organism led to a conclusion that ectoine can be considered as a mediator osmolyte molecule which triggers endogenous osmolytes (genuine osmolytes) like glycine-betaine.(Talibart et al. 1994)

In brief we can say that the biosynthesis and regulation of ectoine have been studied in several different bacteria belonging to Gram-negative or Gram-positive groups. Biosynthesis of ectoine in *H. elongata* has been studied in deepest insight.

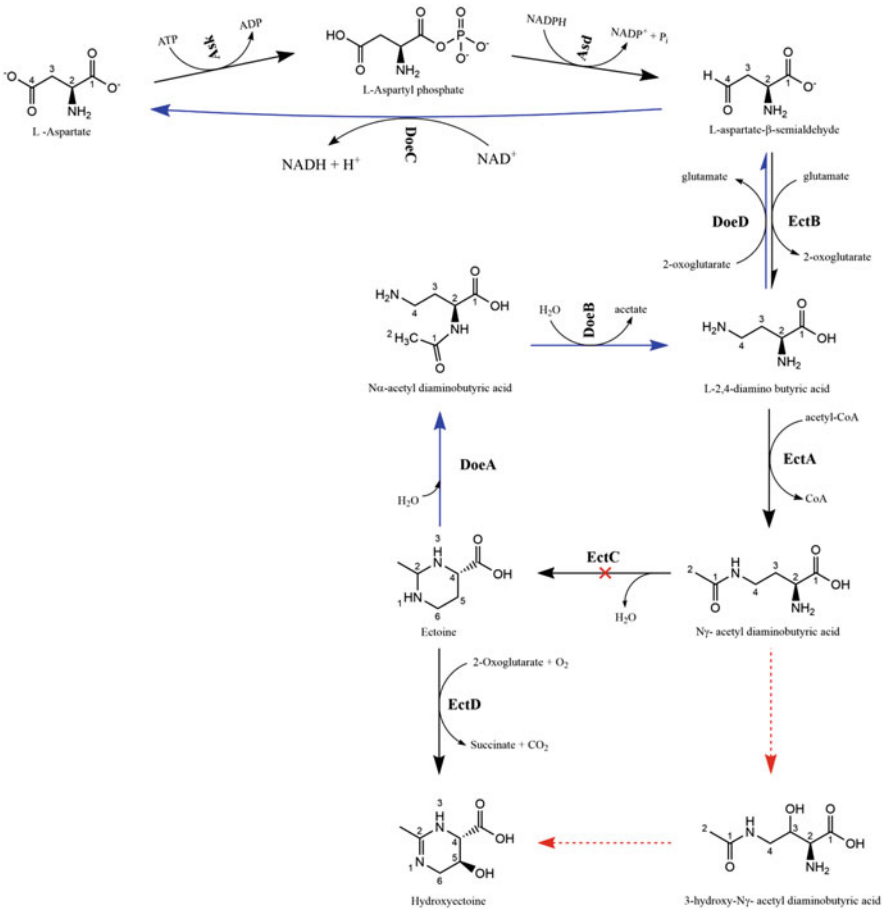

Fig. 21.1 Ectoine metabolism pathway. (a) Pathway of biosynthesis/anabolism of ectoine; reactions shown by black arrows.(Peters et al. 1990; Louis and Galinski 1997; García-Estepa et al. 2006; Cánovas et al. 1999; Cánovas et al. 1997; N Khmelenina et al. 2010) (b) Pathway of catabolism/breakdown of ectoine, serving as source of carbon; reactions shown by blue coloured arrows.(Kunte et al. 2014; Schwibbert et al. 2011) (c) a possible divergent pathway for synthesis of hydroxyectoine from NADA in case of null mutation of ectC gene; reactions shown by red dotted arrows(García-Estepa et al. 2006)

Aspartate can be considered as precursor amino acid. A molecule converted from aspartate can make entry into ectoine biosynthesis; which is aspartate semialdehyde, an intermediate in amino acid metabolism.(Roberts 2005) As shown in Fig. 21.1 the aldehyde gets converted to L- 2,4-diaminobutyric acid, which is then acetylated to from Nγ-acetyldiaminobutyric acid (NADA). The final step is a cyclization reaction of this solute to generate ectoine. The genes for biosynthesis of compatible solute ectoine were identified after the isolation of salt-sensitive mutants of *H. elongata* and further cloning of genes.(Roberts 2005; Cánovas et al. 1999) Ectoine synthesis is

carried out by the products of three genes: *ectABC*. In most of the halophoilic bacteria it is common arrangement of genes in *ectABC* form. But recent studies on *Spiribacter salinus* draw attention on different arrangement of the genes, that is, *ectAC* and *ectB*. And genomes of many phylogenetically similar organisms have the similar non-canonical arrangement of *ectA*, *ectB* and *ectC* genes,(León et al. 2018) as another recent study on *Nocardiopsis gilva* reveals that arrangement of *ectABCD* genes can be of two different kinds, either genes can be found in *ectABC* together in one cluster and *ectD* in other cluster located distally or all the four *ectABCD* arranged together in a single cluster. In *Nocardiopsis sp.* the transporter systems are of two types *ehuABCD* and *proXWV*, both expressing at higher salinities.(Han et al. 2018)

In *H. elongata*, *C. salexigens* and other halophiles ectoine synthesis is regulated by internal cation concentration and at transcriptional level by osmoregulated promoter regions which are similar to σ factor-controlled mechanism.(Roberts 2005) As discussed previously, the level of ectoine concentration is also modulated by the presence or absence of glycine-betaine, so there must be additional post-transcriptional control. The effect of glycine-betaine on *ectABC* expression and ectoine accumulation was of negative regulation type; as shown in *Marinococcus halophilus* [(16)], as betaine accumulation suppressed the ectoine synthesis. Hydroxyectoine, a derivative of ectoine has also been studied, and it has been observed that hydroxyectoine is synthesized from ectoine. However, *H. elongata* demonstrates an alternative pathway, observed in mutants defective in EctC. These mutants that cannot synthesize ectoine can still convert NADA (N-γ-acetyldiaminobutyrate) into hydroxyectoine as shown by the data of Canovas et al.(Cánovas et al. 1999) NADA can be hydroxylated to 3-hydroxyl-N-γ-acetyldiaminobutyrate, which is then converted to hydroxyectoine by putative hydroxyectoine synthase, the enzyme which is not detected from the cell extracts. This study also stated that the NADA can act as osmoprotectant itself. Due to mutation in *ectC*, the organism accumulates NADA which can act as enzyme stabilizer in high osmolarity as well as heat stress protector of the cell during heat stress up to 50 °C. Thus, NADA can act as a compatible solute if ectoine synthesis is blocked,(Cánovas et al. 1999) may be a precaution measure developed by bacteria.

21.5 Catabolism of Ectoine

For compatible solutes, being small organic molecules and synthesized by cells itself, there must be some mechanism to utilize those solutes for cell growth or sustainability. With the knowledge of the ectoine being utilized as carbon or nitrogen source by *H. elongata*, and findings of Jebbar and colleagues, that *S. meliloti* containing EutABCDE proteins which probably can function as degrader of ectoine and other molecules, a study for analysis of whole genome of *H. elongata* has been carried out. Homology analysis reveals that homologues for each *eutBCDE* genes were found on chromosome of *H. elongata*. Homologues of *eutBC* and *eutDE* were found on two different gene clusters, which are separated by three ORFs. Null mutational study of *eutDE* ORFs and two ORFs from the *eutBC* and *eutDE* cluster

separating ORFs have revealed that, due to absence of these gene products, mutants either were unable to utilize ectoine as carbon source or their growth in the presence of ectoine in medium was suppressed. As per the function of these ORFs, they were named *doeABCD* (degradation of ectoine). Individual protein products of these genes were DoeA (ectoine hydrolase), DoeB (Nα-acetyl-L-2,4-diaminobutyricacid deacetylase), DoeC (aspartate-semialdehyde dehydrogenase) and DoeD (diamino butyric acid transaminase). Deletion mutational study of *eutBC* homologue ORFs revealed that the growth of mutants was not affected either by the presence of ectoine or in the utilization of the same, suggesting no involvement of these two genes in ectoine degradation.(Schwibbert et al. 2011)

21.6 Production Strategies

Ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid) and its hydroxy derivative, which are widespread compatible solutes in bacteria(Kunte et al. 2014), have been proven to provide extra protective benefits compared to the other solutes, and stabilizes even whole cells against UV radiation, inflammation caused by nanoparticles or cytotoxins.(Kunte et al. 2014; Lippert and Galinski 1992; Buenger and Driller 2004; Graf et al. 2008; Kanapathipillai et al. 2005; Peuschel et al. 2012; Yao et al. 2013) These properties of ectoines made it a choice for industries of health care. During the nineties ectoine was synthesized chemically, an economically non-favourable method. The increasing demand in markets forced to produce ectoines by biosynthetic methods, which is achieved by two different methods of choice, either by "bacterial milking" procedure(Sauer and Galinski 1998) or development of leaky mutants.(Grammann et al. 2002) Ectoine is synthesized on a large scale by the company bitop (Witten, Germany) in a process utilizing *H. elongata* as producer strain.(Kunte et al. 2014) A few more researches have provided records of production of ectoine on a considerable scale which are discussed below.

As per previous discussions *H. elongata* is the strain which is studied most rapidly. As per the record of Sauer and Galinski, *H. elongata* was the organism of choice because of its distinctive properties like broader salt tolerance and its rapid response to osmotic downshock, which helps releasing large amount of ectoine in the environment and rapid intracellular resynthesis of ectoine against osmotic upshock. During their study they have designed a fed batch fermentation procedure. The procedure was optimized to achieve dry cell mass of 48 gm per litre of medium. To attain this the glucose concentration as carbon source was carefully monitored, not to exceed 700 mM, as it has been observed that the carbon source glucose was the only limiting factor in the production medium. Similarly, the temperature was also maintained slightly higher than the growth temperature of the organisms, which increased the production slightly. Ammonium chloride was used as a nitrogen source. After attaining the sufficient cell density, the bacterial cells were exposed to osmotic downshock by shifting them from 15% to 3% NaCl containing medium. As per their observation, within an hour majority of ectoine has been released in the low salinity medium. The product was recovered in pure form by a cross flow

filtration and subsequent elution by NaOH on cation exchange resins. For separation of ectoine and hydroxyectoine, methanol solubility difference of both the compounds were utilized and separated products were crystallized. In this method they were able to reuse the cell mass as their next inoculum nine times subsequently and the ectoine productivity was achieved to 155 mg per cycle.(Kunte et al. 2014; Pastor et al. 2010; Sauer and Galinski 1998)

By using *Halomonas salina* strain DSM 5928^T a study has been conducted in which the intracellular ectoine production by cells have been attained by combining actively growing cell in a batch culture and the resting cells after completion of the batch process. In first step cells were grown in optimal growth conditions, to obtain maximum biomass. The phase 2 was production phase, in which the phosphate limited environment employs cells to produce and to release the ectoine. Major influencing factors for phase 1 were monosodium glutamate (80 gm L^{-1} initial) and NaCl concentration (0.5 M initial), while these concentrations for phase 2 were 200 gm L^{-1} and 0.5 M initials. Total production of ectoine in one batch of 10 litres was 14.86 gm which was considerably good amount recovered.(Y-j et al. 2011)

In order to increase ectoine production by *Halomonas sp.* H02 strain, a placket-burman design and a response surface methodology experiment designs were prepared to optimize the medium components in the fermentation process. PB experiment results showed that three main components from the medium were influencing the ectoine production; these components being $C_5H_8NNaO_4$ (monosodium glutamate), NaCl concentration and initial pH. The optimum combination of three influencing factors was found to be 41 g/L of monosodium glutamate, 87.2 g/L of NaCl and initial pH 5.9, and the predicted amount of ectoine was 1835.8 mg/L.(Li et al. 2017)

Due to thermal protective effect of hydroxyectoine, industrial scale production of hydroxyectoine is much more of interest to the researchers. Hydroxyectoine production using Gram-positive bacterium *Marinococcus sp.* strain M52 has been extensively studied. A high cell density exponential fed batch fermentation technique was occupied earlier. For the production of hydroxyectoine, as seen previously that ectoine accumulation can get affected by the presence of glycine-betaine, complex medium supplemented with 10% NaCl concentration, containing glucose as carbon source and glycine-betaine free peptone source was designed. Total of 56 gm dry cell mass was achieved per litre of medium, and hydroxyectoine contributed 13.5% of total dry cell mass. As the strain M52 can tolerate broader osmolarity changes, extraction of product was attained by osmotic downshock by diluting the media with desalted and demineralized water, and pure product was recovered by the methanol extraction method.(Frings et al. 1995) A deeper study of the same strain revealed that the accumulation of hydroxyectoine can be increased by providing dissolved oxygen higher than 10%. In addition to that a microfilteration bioprocess was utilized to get higher biomass and final yield, attaining final product recovery of 3.6 gm hydroxyectoine per litre of medium. To improvise the recovery of the product hydroxyectoine, as it cannot be liberated as easily from *Marinococcus* M52 strain as *Halomonas sp.*, hydroxyectoine release was induced by osmotic downshock coupled with high temperature (55 °C) for better release of hydroxyectoine with

simultaneous release of ectoine in four consecutive recovery cycle process.(Schiraldi et al. 2006)

Recently genetically modified *E. coli* K-12 strain BW25113 was used for ectoine production. Introduction of *ectABC* from *Halomonas elongata* to *E. coli* under arabinose inducible promoter has shown significant production of ectoine while aspartate and glycerol were used as direct substrate. Optimal fermentation condition (100 mM sodium phosphate buffer (pH 7.0), 100 mM sodium aspartate, 100 mM KCl and 100 mM glycerol), when provided to high-density culture (20 OD/ml), the reported concentration of extracellular ecotine was the highest till date (25.1 gm L^{-1}) (He et al. 2015).

21.7 Applications of Ectoine and Hydroxyectoine in Pharmaceuticals and Cosmeceuticals

Ectoine and hydroxyectoine show good osmoprotective properties as well as they are noted as great UV protective agents; they are in high demand in pharmaceutical and cosmeceutical utilities. Hence researchers started exploring the more beneficial properties and uses of ectoine.

21.7.1 Skin Care Agents

Though ectoine is a major product of halophilic microorganisms and till a few years back, their properties were thought to be useful for organisms from lower domains; some recent studies established the idea of applying ectoine for human welfare. Report of Graf et al.(Graf et al. 2008) from Merck KGaA has shown the use of ectoine containing oil-water emulsion for the purpose of protection of skin cell membrane against damage caused by surfactants like SDS. They have reported the strengthening of barrier function of the epidermal cells. Molecular dynamic simulation study revealed that ectoine can bind to water molecule for longer time spans and application of high concentration of ectoine leads to binding and retention of more water molecules at a time. The results of these studies have proven ectoine as an important moisturizing agent.

Moreover, it has been observed that ectoine can reverse the UVA-induced second messenger release, transcription factor AP-2 activation and mitochondrial DNA mutations, which reveals the idea that ectoine can possibly reduce the effect of UVA-induced skin ageing.(Buenger and Driller 2004) A study conducted by Yao et al.(Yao et al. 2013) for testing the effect of ectoine on melanoma cell lines depicted the inhibitory effect of ectoine on tryrosinase activity. Overall melanogenesis was also reduced by the application of ectoine on mouse and human melanoma cell line, B16-F0 and A2058, which leads to inference that ectoine can be a good whitening agent as well as cell viability was observed till 500 μM concentration of ectoine, which further add that cytotoxicity of ectoine is very poor; aiding in the establishment of ectoine as a safer whitening agent for human use.

21.7.2 Ectoine as Enzyme Stabilizer

Report of Canovas and co-workers has generated the idea that NADA, and intermediate of the ectoine synthesis pathway, extracted from *H. elongata* can act as a thermostabilizer for rabbit muscle LDH.(Cánovas et al. 1999) This can be considered as one of the evidences for the enzyme-stabilizing and thermoprotective ability of NADA. Working ahead with this ideology research reports have been generated showing the effect of various compatible solutes like ectoine, hydroxyectoine, trehalose, maltose, sucrose and glycine-betaine. They were tested for the preservation of the enzymes LDH (lactate dehydrogenase) and PFK (phosphofructokinase); as the enzymes under study are highly susceptible to freezing and thawing. A four cycle fast freezing and slow thawing experiments were performed in the presence of each compatible solutes; the results indicated best preservation of LDH in the presence of hydroxyectoine and that of PFK in the presence of ectoine. Rest of the compatible solutes in both the cases showed approximately 80 – 90% of recovered activity of enzymes after four cycles of freeze-thaw.(Lippert and Galinski 1992)

Moreover, the effect of ectoine and hydroxyectoine on zymogens was also studied. Activation of trypsinogen and chymotrypsinogen respectively by enteropeptidase and trypsin was checked in the presence of ectoine, hydroxyectoine and betaine. Observation showed ectoine as promising zymogen protective agent, reducing the enteropeptidase activity to 4% and formation of chymotrypsin was reduced to 23%. In addition to that the effect of ectoine on individual peptidase activity was also studied, results of which depicted that 49% of trypsin activity and 55% of chymotrypsin activity were preserved while incubated with 800 mM of ectoine against 20 mM Tris–HCl, 150 mM NaCl, pH 8.4 and 6% DMSO. These results provided the insight in chaperon like activity of ectoine.(Kolp et al. 2006)

21.7.3 Ectoine as Anti-Inflammatory Agent

Pathogenic effect of xenobiotic air pollutants on lung epithelial cells may lead to inflammatory reactions. The responsible factor is the activation of epidermal growth factor receptor (EGFR) which can initiate signalling events leading to such unwanted reaction. Carbon nanoparticles were exposed to rat for the study of the in vivo effect of xenobiotic compound. Accumulation of ceramide was observed subsequent to exposure. Ceramide being intermediate of sphingolipid metabolism has been identified as the causative agent of pulmonary disease. Ceramide-mediated phosphorylation of EGFR induces the signalling cascade of the inflammation reaction. Compatible solute ectoine has been recorded as a preventive agent of this phosphorylation reaction, leading to suppression of activation of signal cascade. (Peuschel et al. 2012)

21.7.4 Ectoine as Inhibitor of Amyloid Formation

As discussed in previous sessions, ectoine and hydroxyectoine can act as protective agent and chaperon like agent for proteins. Stretching ahead this fact, they have been applied to amyloidogenic proteins, which undergo amyloid formation (an alternative folding process) during the stressed condition.(Arora et al. 2004) Such amyloids are causative agents for disease like Alzheimer's disease. Responsible agent for this condition is β-amyloid peptide. Aggregation of this peptide makes it a neurotoxic agent. Effect of ectoine and hydroxyectoine has been checked on Aβ42 amyloid formation. Thioflavin T-induced fluorescence revealed that Aβ42 aggregation process follows a sigmoidal kinetics. It has been observed in the study that for control reaction the lag phase time was around 12 hours while incubated reaction with 100 mM of ectoine lengthened the lag phase to about 24 hours, which indicates that the presence of ectoine possibly can delay the Alzheimer's disease.(Kanapathipillai et al. 2005)

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In Silico Identification of Drug Targets and Drug-Like Molecules against *Vibrio splendidus* LGP32

22

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Abstract

On a productive mode, this research specifies a genomics approach. It talks about the retracements of pathogens that act as an aid to treat infections through genomics. Two kinds of genomics approach that were used in this process are comparative and subtractive genomics. The principle used here for identification is in silico, which defines "a good drug target is a gene essential for the bacterial survival, yet cannot be found in a host." To state, the development of genetic and genomic approaches progress to another basement of enhancing drug designs. Drugs specific to the novel drug targets are required to design against antibiotic sensitive pathogen *Vibrio splendidus* LGP32 that causes vibriosis in Oyster. On a measured study, the in silico–based approach is capable of creating screening proteins that on combination add on to attain potential drug targets. Inevitability forms the only result of these targets.

Under deep research, we herewith describe the study that was aimed to identify drug-like molecules that can block 3-oxoacyl-(acyl carrier protein) synthase 1 protein of *Vibrio splendidus* LGP32. This protein carrier imbibes the quality of producing new drugs made available from chemical molecules to block the protein tar. The microorganism *Vibrio splendidus* LGP32 is fast gaining resistance to the existing drugs. New types of credible and effective drugs should be

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established that help devastates infections. Thus, this research can be the best place of switching standard theories for current therapies available.

Keywords

Drug design · Bioinformatics · Docking · In silico screening

22.1 Introduction

The use of genomics holds several purposes and very significant one is the comparative genomics whose data can be used as an antibiotic to retrieve medicinal values for treating the deadly pathogens (Shahbaaz et al. 2016). Analyzation of its genes and its products can be used as a potential drug to understand the very use of it. Similarly, on the other hand, novel drug targets have been identified successfully for various pathogens by using this approach (Georrge and Umrania 2012; John et al. 2012; Georrge and Umrania 2011; Vaishnav et al. 2015; John and Kotadiya 2015; Trivedi and Georrge 2016; Georrge 2016; George and Georrge 2019; Abouelwafa and Georrge 2017; George et al. 2017).

Combinatorial chemistry, which is high throughput screening and also includes in silico virtual screening, has many features and some are into absorption, distribution, metabolism, and excretion–toxicity screening, and de novo and structure-based drug designs serve to expedite as well as economise the modern-day drug discovery process (Baig et al. 2016).

On mere observation, there are various species of *Vibrio* genus that are affected by deadly pathogens even in aquatic life. The most affected mammals are finfish, shellfish, and mammals. To an extent, *Vibrio*–host interaction appears to play a critical role in the survival and dissemination of these bacteria in the marine environment. *V. splendidus* LGP32 is also known as *V. splendidus* strain Mel32 (Balbi et al. 2013). This stain is caused by significant mortalities in oysters, such as *Crassostrea gigas*, during the summer. To this, a question arises: what is a genome? Many researchers have stated that genome sequence outpours accurate information about the proteins involved in virulence and pathogenesis. The *Vibrio splendidus* consists of two circular chromosomes of 3299 Mb in chromosome 1 and 1675 Mb chromosome 2 with an average percent G + C content of 44.03 and 43.64, respectively (Federhen et al. 2014; Le Roux et al. 2009). To add on, it is this genome sequence of LGP32-revealed homologs of genes that has been associated with virulence in other organisms (S-i and Shinoda 2000; Villicana et al. 2019; Zhang and Austin 2005; Coulthurst 2019). The research done here focuses on questioning what is the vital function of this homolog and what is its reaction to the organisms of oysters. Related observation and experiments have proven that strains associated with the "summer mortalities" syndrome in oysters have ended the multifaceted farming of oysters in contemporary life. On vibrant testing, many sectors and departments relating to biology, etiology, and extended natural forums like environmental studies, agent infections, blocks of physiology, and genetic hosts have stood

an example for such testing and research (Clerissi et al. 2020; King et al. 2019). From general testing on different samples of aquatic life have developed to another step of producing variable virulence for aquatic animals when tested independently. On a broader scale, populations of oyster experiments have been commonly found in moribund animals, and that these agonisms settled between strains are *V. splendidus* vibriosis that takes its form to be an interaction of microbe (Bruto et al. 2018; Gay et al. 2004).

22.2 Materials and Methods

22.2.1 Identification of Drug Targets

The research switches over to the process of identification in drugs and its target. As discussed earlier, the drug *Vibrio splendidus* LGP32 was sequenced by the Institut Pasteur, Paris. The reference proteins of *V. splendidus* LGP32 were retrieved from the NCBI website (www.ncbi.nlm. nih.gov), and this was subjected to tblastn against expressed sequence tag (EST) and nucleotides of *Ostreidae*, with an E value cutoff of 10^{-4}. The obtained sequences for the shared proteins were subjected to tblastn (Mount 2007; Altschul et al. 1990) against *Ostreidae* with an E value cutoff of 10^{-4}. The obtained homologous protein set was eliminated. Further, a blast search was performed against the Database of Essential Genes (DEG) (Peng et al. 2017; Gao et al. 2015) http://tubic.tju.edu.cn/deg/ to identify the set of essential genes mandatory for the survival of the selected pathogen, *V. splendidus* LGP32.

The metabolic pathways (Altman et al. 2013; Aoki-Kinoshita and Kanehisa 2007) involving the essential proteins of *V. splendidus* LGP32 were elucidated using the KEGG Automatic Annotation Server (KAAS) (www.genome.jp/tools/kaas/). KAAS (Moriya et al. 2007) performs homology search by BLAST against the KEGG database, which is a manually curated gene database and provides the functional annotations of query genes as output. The output provides details of the KEGG Orthology (KO) assignments and the corresponding KEGG pathways. The pathways predicted were assessed for occurrence of alternate pathways and based on the observed results, the proteins were selected as potential drug targets.

22.2.2 Three-Dimensional Structure Modelling and Validation

Concerning the above target and the process of identification, this research has reached the process of validation by giving a detailed analysis of proteomes of the *Vibrio splendidus* LGP32, three-dimensional structure of the 3-oxoacyl-(acyl carrier protein) synthase. Due to a lack of template in the PDB, the protein was modelled using I-TASSER, an ab initio protein modelling tool. The I-TASSER server is freely available to the academic community at https://zhanglab.ccmb.med.umich.edu/I-TASSER/. A confidence score (C-score) based on the relative clustering structural

density and the consensus significance score of multiple threading templates is introduced to estimate the accuracy of the I-TASSER predictions (Yang et al. 2015).

The next step followed by the validation process is the predicted protein that was performed by the PROCHECK suite and ANOLEA (Atomic Non-Local Environment Assessment). The PROCHECK suite provides a detailed check on the stereochemistry of a protein structure (Laskowski et al. 1996). ANOLEA (Atomic Non-Local Environment Assessment) is a www server that performs energy calculations at the atomic level in protein structures (Melo et al. 1997).

22.2.3 Docking, Drug Likeliness, and Toxicity Analysis

The analysis of the research carried over consists of around two lac synthetic chemical compounds that were obtained from various literature and databases including the PubChem (http://pubchem.ncbi.nlm.nih.gov/) and drug-like compounds from the NCI database (http://ligand.info).

The Molegro Virtual Docker (MVD) was used to perform docking. The MVD has been shown to yield a higher docking accuracy than other state-of-the-art docking products (Thomsen and Christensen 2006; De Azevedo 2010). It possesses two docking algorithms, the MolDock Optimizer and Simplex Evolution (SE). The receptor-ligand docking was initiated by the selection of receptor from the molecule tab, followed by the cavity preparation. The structure data format (sdf) file of ligands was uploaded and the docking option from the docking wizard was selected.

The Absorption, Distribution, Metabolism, and Excretion (ADME) properties were assessed using Molsoft ADME property (http://www.molsoft.com/mpropdesc.html). ICM is the facility of the Molsoft application. When drawing a compound in ICM, we can monitor important ADME-toxicity and drug-likeness properties. The ICM browser provides researchers with direct access to the rich structural biology resources and protein families. The method is very robust and fast (about 5 K of compounds per second).

22.3 Results and Discussion

The *Vibrio splendidus* strain LGP32 is a pathogen of the *Crassostrea gigas* oysters and associated with their summer mortalities affecting the overall annual production worldwide. This has led to an increased demand for a new drug against the pathogen; however, the number of new drugs identified is low, as the development of a new drug needs a higher investment against a lower market (Duperthuy et al. 2010).

22.3.1 Identification of Drug Targets

Identification of drug targets revealed that the *Vibrio splendidus* LGP32 consists of 4432 reference proteins. When subjected to tblastn for ESTs search and a nucleotide

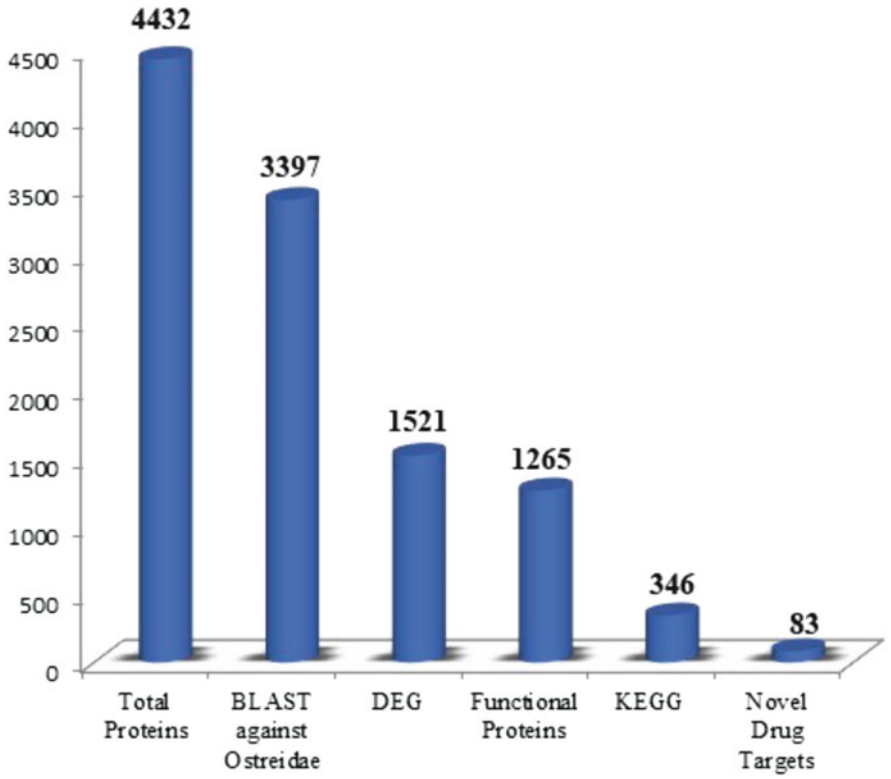

Fig. 22.1 Summary of target identification

blast against the *Ostreidae* species, it was observed that 3397 proteins showed significantly unique hits. Further, out of the 3397 proteins, 1521 were observed to be essential genes as per the DEG analysis. From these identified 1521 proteins, 256 hypothetical proteins that lacked experimental evidence for their in vivo expression were eliminated for further pathway analysis, thus leaving a total of 1265 essential proteins.

The involvements of drug targets, namely, the identified 1265 essential proteins in metabolic pathways, were analyzed using the KAAS server. The 353 essential proteins out of 1265 could not be predicted by the KAAS server. There were 381 proteins predicted by KAAS server, but not contain pathway ID so, could not possible of detailed analysis of those proteins. Detailed pathway analysis of the remaining 531 proteins revealed that 185 proteins were such that even after targeting them, the organism could survive. Hence, these proteins were omitted, and the remaining very crucial 346 proteins were taken for further downstream processing. Some proteins contain the similar KO pathway ID in single pathway; hence, after eliminating of identical proteins, the remaining 83 proteins were acting as an identical and novel drug target (Fig. 22.1).

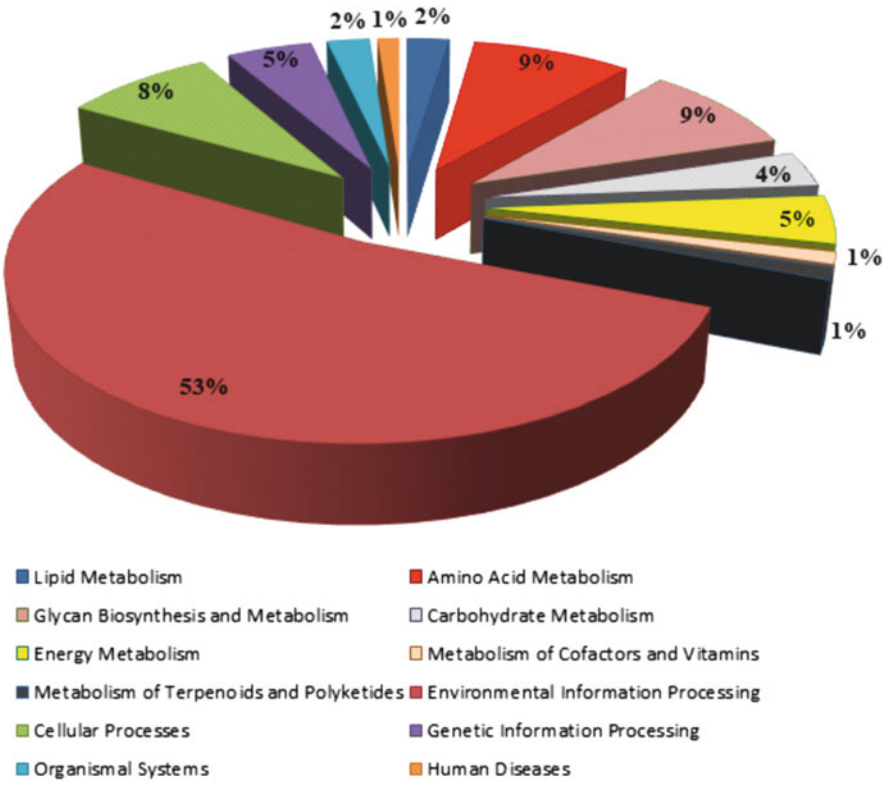

Fig. 22.2 Percentage distributions of novel drug targets involved in different metabolic pathways/biological process

The identified 83 novel drug targets were found to be involved in 25 different pathways/biological processes. The pathways were further divided into 12 classes including the amino acid, lipid, and carbohydrate metabolism; energy metabolism; glycan biosynthesis and metabolism; metabolism of cofactors and vitamins; terpenoids and polyketides; nucleotide metabolism; genetic information processing; cellular processes; environmental information processing; organismal systems; and human diseases. Figure 22.2 details the distribution of novel drug targets across different metabolic pathways/biological processes.

The study experimented nearly 83 predicted drug targets where the manual analysis observed is two proteins that could be very potential as drug targets (Table 22.1), which involved fatty acid biosynthesis (KEEG Map ID: ko00061). It is of crucial importance to Gram-negative bacteria, as its mutation or removal leads to the death of the organism (Wang and Quinn 2010a, b).

The 3-oxoacyl-(acyl carrier protein) synthase I (fabB) [EC: 2.3.1.41] is also known as Beta-ketoacyl-acyl-carrier-protein synthase I involved in the fatty acid biosynthesis, which is responsible for the chain-elongation step of dissociated (type

Table 22.1 Information about the finalized target therapeutics

GI number	KEEG – KO	KEEG- Map ID	Protein name	Localization	Target
218708897	K00647	ko00061 fatty acid biosynthesis	3-oxoacyl-(acyl carrier protein) synthase I	Cytoplasmic	Research target
218709020	K09458	ko00061 fatty acid biosynthesis	3-oxoacyl-(acyl carrier protein) synthase II	Cytoplasmic	Research target

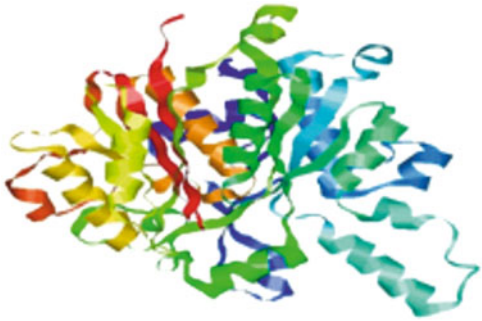

Fig. 22.3 I-TASSER predicted the 3D structure of the 3-oxoacyl-(acyl carrier protein) synthase I

II) fatty-acid biosynthesis. 3-oxoacyl-(acyl carrier protein) synthase I (fabB) plays a key role in the synthesis of fatty acid. This enzyme is mainly located in the cytoplasm as well as in the cytoplasmic membrane. The bacterial pathway offers several unique sites for selective inhibition by chemotherapeutic agents. The antibacterial effect is exerted through the selective targeting of beta-ketoacyl-(acyl-carrier-protein) synthase I (FabB) in the synthetic pathway of fatty acids (Hermans et al. 2016).

22.3.2 Three-Dimensional Structure Modelling and Validation

The 3D structure of the 3-oxoacyl-(acyl carrier protein) synthase I (fabB) was ab initio modelled based on the confidence score, aka C-score (Fig. 22.3). The C-score estimates the quality of predicted models based on the significance of the threading template alignments and convergence parameters of the structure assembly simulations. It typically ranges between −5 to 2, with a higher C- signifying a model with high confidence and vice versa. The predicted structure comprised of 403 amino acids, 3390 bonds, and 3348 atoms. The predicted structure was assessed for its quality by PROCHECK and ANOLEA server. The former uses the Ramachandran plot, and it was observed that the predicted model was of good quality based on the observed values of 93.6%, 5.8%, and 0.6% residues in the most favorable regions, the allowed regions, and the disallowed regions, respectively (Fig. 22.4). A good quality model is expected to have >90% of amino acids in the

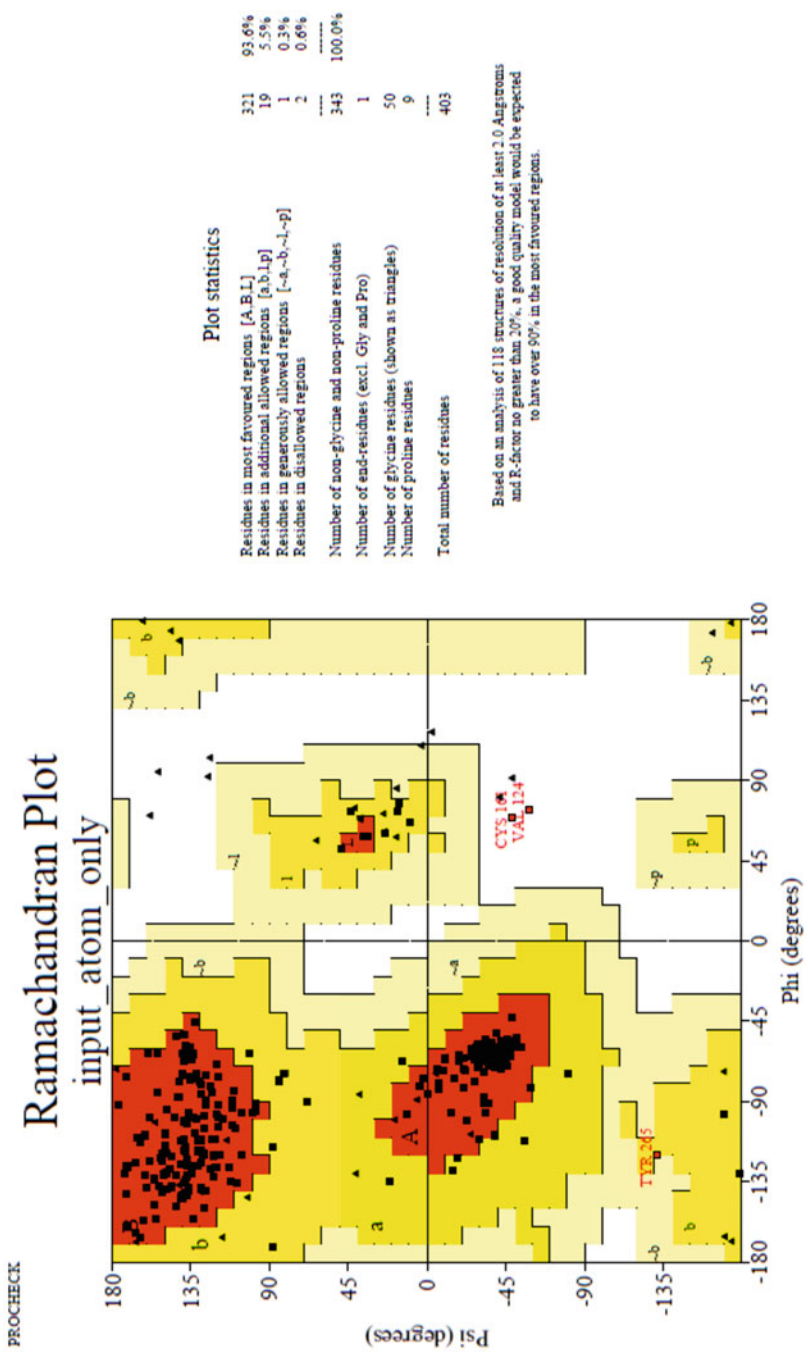

Fig. 22.4 Predicted 3D structure of 3-oxoacyl-(acyl carrier protein) synthase I quality analysis: PROCHECK

most favored regions. All the main chain and side chain parameters in the predicted model were falling under the "better" region. Similar to the C-score is the G-factor (should be > -0.5) that defines the reliability of the predicted model. It is the log-odds score computed by the observed distribution of stereochemical parameters (Morris et al. 1992; Evans 2007). The observed G-factor for the predicted model was 0.00 for dihedral bonds, −1.55 for covalent, and overall −0.67. The distribution of the main chain bond lengths and bond angles were within limits with 74.6% and 80.2%, respectively. Even the planar groups were within limits. The ANOLEA result represents the graphical view of the energy values of each amino acid. Results revealed that most of the amino acids had a negative energy value (Fig. 22.5). For a given amino acid in the targets, negative energy values shown in green represent the favorable energy environment while the unfavorable energy environment is the positive values shown in red (Melo et al. 1997).

22.3.3 Virtual Screening and Docking

The concluding step includes the molecular docking that plays a key role in the identification of binding efficiency between the receptor and ligand. The modelled fabB protein docked with around 2,00,000 chemicals by using MVD. The top ten hits based on docking score of energy were shown in Table 22.2, which can block the targeted therapeutics. The top ten chemicals of 3-oxoacyl-(acyl carrier protein) synthase I contain four natural products, one antifungal, three antiviral, and two anticancer molecules.

The CID 2879872 and CID 2913532 are inhibitors of Sfp phosphopantetheinyl transferase (PPTase) in the bacteria (Yasgar et al. 2010). CID 330973 and CID 3152845 are inhibitors of RecA-Intein splicing activity, DnaB-Intein splicing activity, and GFP chromophore formation in bacteria (Lew and Paulus 2002). CID 16403955 is inhibitors of VIM-2 metallo-beta-lactamase in bacteria (Yamaguchi et al. 2007). CID 5389752 and CID 5389951 are inhibitors of PMK (phosphomevalonate kinase), MK (mevalonate kinase), and DPM-DC (diphosphomevalonate decarboxylase) of the mevalonate pathway in *Streptococcus pneumonia* (Kudoh et al. 2010). CID 5739314 is an inhibitor of streptokinase A precursor in *Streptococcus pyogenes* M1 GAS. CID 5389834 is an inhibitor of pyruvate kinase in bacteria (Suzuki et al. 2008). CID 44142679 is an inhibitor of AddAB recombination protein complex and putative recombination protein RecB in bacteria (Marsin et al. 2010; Wang and Maier 2009).

22.4 Conclusion

The research undertaken on genomics has justified its analysis by finding a solution to ending infections in pathogens. The research gives a systematic analysis of experiments and validations through a comparative genomics approach that can be efficiently applied to retrieve valuable information about the drug target molecule to

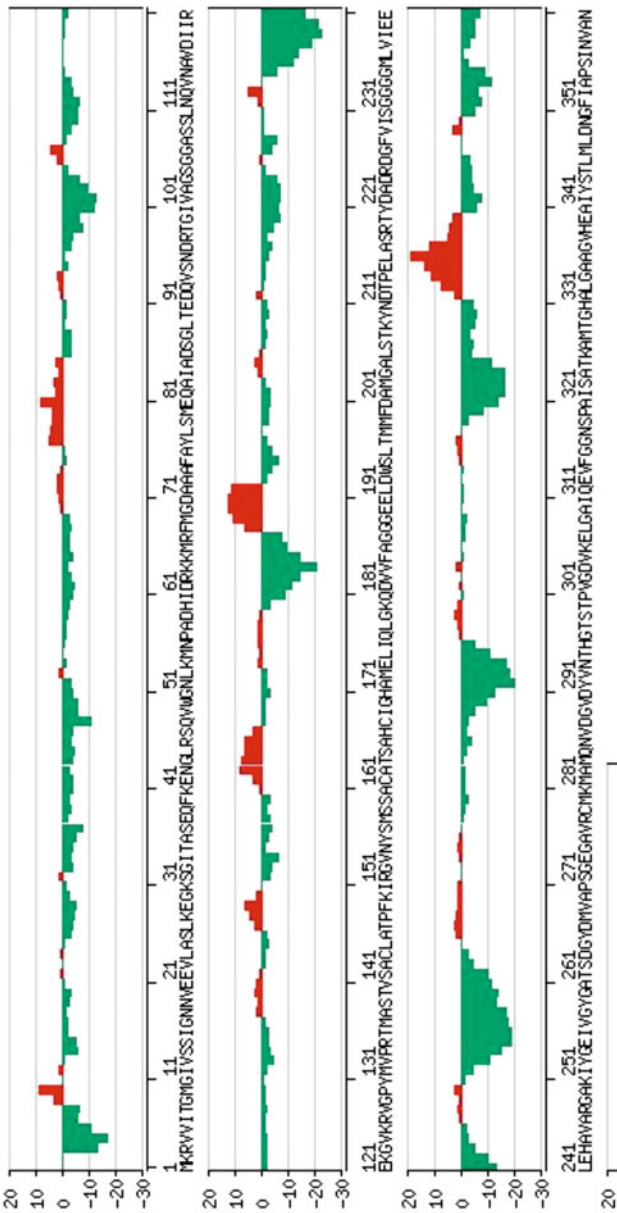

Fig. 22.5 Predicted 3D structure of 3-oxoacyl-(acyl carrier protein) synthase I quality analysis: ANOLEA

Table 22.2 Details about the docking energy value of the top ten drug-like molecules for the target 3-oxoacyl-[acyl-carrier-protein] synthase I

Sr. No.	IUPAC Name of the molecule	Pubchem CID	MVD energy	MVD rerank
1	2-[1-(3-ethoxycarbonyl-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)-2, 5-dioxopyrrolidin-3-yl] sulfanylbenzoic acid	2879872	−177.751	−122.423
2	4-[[5-(4-methoxy-2-nitrophenyl)furan-2-yl]-(3-methyl-5-oxo-1, 2-dihydropyrazol-4-yl) methyl]-5-methyl-1,2-dihydropyrazol-3-one	2913532	−174.988	−109.254
3	Bis[(3aR,5R,5aR,8aR,9S,9aR)-5,8a-dimethyl-1-methylidene-2,8-dioxo-3a,4,5,5a,9,9a-hexahydroazuleno[6,5-b]furan-9-yl] propanedioate	330973	−173.802	−57.2222
4	Methyl 3-[5-[bis(3-methyl-5-oxo-1,2-dihydropyrazol-4-yl)methyl]furan-2-yl] benzoate	3152845	−168.388	−129.158
5	(2S)-1-[2-[[(2Z)-2-[(2,4-dimethoxyphenyl) methylidene]-3-oxo-1-benzofuran-6-yl]oxy] acetyl]pyrrolidine-2-carboxylic acid	16403955	−146.474	−119.584
6	Ethyl 4-[(E)-[1-[2-(dimethylamino)ethyl]-2-(5-methylfuran-2-yl)-4,5-dioxopyrrolidin-3-ylidene]-hydroxymethyl]-3,5-dimethyl-1H-pyrrole-2-carboxylate	5389752	−185.925	−69.2322
7	Ethyl 4-[(E)-hydroxy-[1-(3-imidazol-1-ylpropyl)-4,5-dioxo-2-pyridin-4-ylpyrrolidin-3-ylidene]methyl]-3,5-dimethyl-1H-pyrrole-2-carboxylate	5389951	−185.095	−67.4083
8	4-[(E)-hydroxy-[1-(2-morpholin-4-ylethyl)-4,5-dioxo-2-thiophen-2-ylpyrrolidin-3-ylidene] methyl]-3,5-dimethyl-1H-pyrrole-2-carboxylate	5739314	−180.997	−68.909
9	Methyl 4-[(E)-hydroxy-[1-(2-morpholin-4-ylethyl)-4,5-dioxo-2-thiophen-2-ylpyrrolidin-3-ylidene]methyl]-3,5-dimethyl-1H-pyrrole-2-carboxylate	5389834	−172.687	−86.257
10	4-N-phenyl-5-N-[3-[[4-(phenylcarbamoyl)-1H-imidazole-5-carbonyl]amino] propyl]-1H-imidazole-4,5-dicarboxamide	44142679	−171.513	−132.6

be used as the treatment of various infections caused by pathogens. It is mainly based on the idea to distinguish the genes between the pathogen and host for narrowing down to the organism-specific genes to be tested as potential drug targets. There is a constant need to keep looking for novel drug molecules as a means of protection against those pathogens that are resistant to available antibiotics. The advances in various in silico-based approaches is allowing the screening of multiple proteins predicted as potential drug targets.

Here we described the entire approach for designing a drug target that can block 3-oxoacyl-(acyl carrier protein) synthase I proteins. It explores the possibilities of

creating new drugs from a list of available chemical molecules. The microbes are attaining resistance against the existing drugs; the usage of the drug is one of the finest discoveries done to detect the problems of pathogens and mammals in aquatic life. Hence, designing novel-effective drugs should be made available to real life as a medicinal aid to research problems. In context to the same, the described chapter as a case study can be the best replacement for available existing treatments.

The present study contributes to the identification of ten potential inhibitors, which can combat the pathogenic microorganisms. Relevant studies reveal that these are the best druglike molecules for the *Vibrio splendidus LGP32*. There should be the biological confirmation of these selected druglike molecules for checking their efficiency against the organism. Therefore, the coming future could be identified through druglike molecules as a biological confirmed drug by using many authentic approaches and analysis. For example, the cup borer method can be used as an innovative approach to understanding the identification of medicinal properties through genomics producing molecules that are druglike.

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Marine Bacteria—A Treasure House of Valuable Products and Functions

23

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Abstract

Immense diversity of prokaryotes is reported for the oceans; however, we probably know only 0.01% of the microorganisms from marine ecosystems. Due to the burning need of new or novel therapeutic and environmental useful compounds, these less explored marine habitats have attracted various researchers since the last five decades. Nearly 16,000 natural products are discovered from marine organisms. Marine microbes are very diverse because they are exposed to wide variations in temperature, salinity, nutrition, and pressure at different levels. These extreme conditions are responsible for the presence of diverse photoautotrophs, chemolithotrophs, heterotrophs, nitrogen fixers, denitrifiers, luminescent as well as sulfur and iron oxidizers and reducing microorganisms. Various culture-dependent and -independent methods are used to explore the hidden microbial diversity and their potency. The chapter discusses about the production of various bioactive compounds, enzymes, neutraceuticals, exopolysaccharides, antibiotics, biosurfactants as well as the potential organisms useful in dye decolorization, microbial enhanced oil recovery, hydrocarbon degradation, and metal bioremediation. The chapter also deals with feature prospects in terms of valuable industrial and environmental significance of the bacterial community of oceans.

Keywords

Marine bacteria · Antibiotics · Exopolysaccharides · Biocatalyst · Biosurfactant · Hydrocarbon degradation

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23.1 Preface

It is very well documented that, from the marine ecosystem, 99% of the cells cannot be cultured or recovered because these ultra-small bacteria cannot be isolated in pure due to the inability to mimic sea environment in the laboratory. The low nutrient composition of seawater possesses simple carbon and nitrogen sources, while most of the media used for the isolation are made up of complex substrates (Schut et al. 1997). Contributing factors to the inability of the majority of bacteria to grow on laboratory agar medium plates could be due to the lack of appropriate growth medium and if the substrate is suitable, it is too high in concentration for oligotrophs as well as induction of lytic cycle of bacteriophages upon nutritional improvement in laboratory conditions (Schut et al. 1997; Joint et al. 2010). Thus, the discovery and production of many competent compounds are hindered by problems associated with reproduction and scale-up. In addition to that, an insufficient amount of obtained pure substance and difficulties in the mariculture of most marine microorganisms further limit their use (Imhoff et al. 2011). Hence, despite being a reach reservoir of biodiversity, these habitats are not much explored (Romano et al. 2017). In early times, marine natural products were derived from macroorganisms, mainly invertebrates. Tyrian dye is the first compound, which was extracted from marine mollusks in 1600 BC. After a long period, marine microorganisms have attracted the attention of the scientific community and researchers and in 1949, the first antibiotic product of marine origin, Cephalosporin C, was obtained from a marine fungal strain *Cephalosporium*. Since then, marine microbiology has emerged as an important and fast expanding area (Pandey 2019).

This chapter begins with the bacterial diversity and various methods to study the diversity, industrially useful products from sea microbes, environmental significance of marine bacteria; and ends with concluding remarks.

23.2 Bacterial Diversity of the Marine Ecosystem

Marine microorganisms are found throughout the marine environment ranging from sea ice in polar regions to temperature of 100 °C at deep-sea hydrothermal vents (Kennedy et al. 2010), and from coastal to the offshore regions (Das et al. 2006). These aquatic microorganisms are thought to be a reason for 98% of primary production (Kennedy et al. 2010; Du et al. 2011) and play a key role in the biotransformation of C-, N-, P-, and S-containing compounds (Strom 2008).

Marine microbes are very diverse because they are exposed to variations in temperature, pH, salt concentration, and pressure at different levels or niches and so are thought to possess novel biochemistry which is likely to be suitable for many industrial processes as well as an answer to many unresolved problems of environmental significance. As per Schneider and Rheinheimer (1988), marine bacteria are generally found as mixed communities in nature and based on their metabolic activities, they can be grouped as in Fig. 23.1.

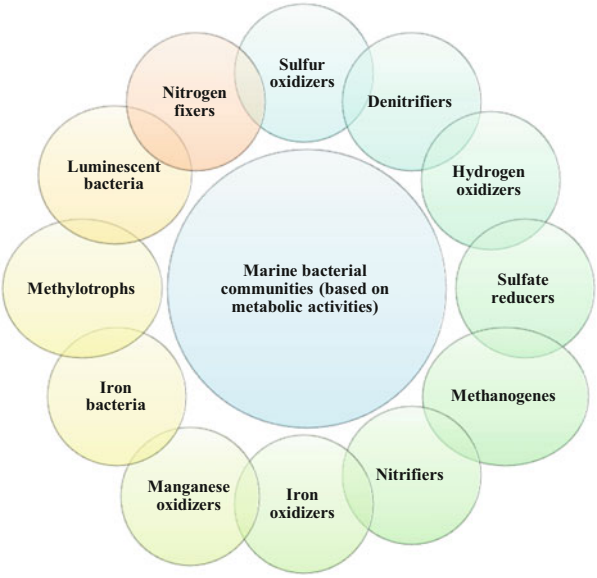

Fig. 23.1 Marine bacterial communities on the basis of their metabolic activities

Table 23.1 Energy sources of some chemolithotrophic bacteria. (Adopted from Jannasch and Mottl 1985)

Electron donor	Electron acceptor	Types of bacteria
S^{2-}, S^0, $S_2O_3^{2-}$	O_2	Sulfur-oxidizing bacteria
S^{2-}, S^0, $S_2O_3^{2-}$	NO_3^-	Denitrifying bacteria
H_2	O_2	Hydrogen-oxidizing bacteria
H_2	NO_3^-	Denitrifying and hydrogen bacteria
H_2	S^0, SO_4^{2-}	Sulfur- and sulfate-reducing bacteria
H_2	CO_2	Methanogenic and acetogenic bacteria
NH_4^+, NO_2^-	O_2	Nitrifying bacteria
Fe^{2+}, Mn^{2+}	O_2	Iron- and manganese-oxidizing bacteria
CH_4, CO	O_2	Methylotrophic- and carbon monoxide-oxidizing bacteria

23.2.1 Photoautotrophs

These organisms derive energy from sunlight to convert CO_2 into simple organic molecules by utilizing water and mineral salts.

23.2.2 Chemolithotrophs

These organisms derive energy from chemical oxidations. Some examples of heterotrophs with their inorganic energy source are given in Table 23.1.

23.2.3 Heterotrophs

These organisms have a complete dependency on complex organic compounds. Cellulose, chitin, and agar decomposers form an important community being strict aerobes requiring mesophilic temperature and showing gliding movement.

23.2.4 Nitrogen-Fixing Bacteria

Nitrogen-fixing bacteria, i.e., Azotobacteriaceae group, are aerobic, heterotrophic bacteria which can fix nitrogen in an environment, which is poor in nitrogen compounds. For enrichment of Prosthecate bacteria like *Hyphomicrobium* sp., *Pedomicrobium* sp., and *Hyphomonas* sp., C_1 compounds can be incorporated into the medium.

23.2.5 Denitrifiers

Denitrifiers (e.g., *Thiobacillus denitrificans*) can oxidize organic compounds by transferring electrons to nitrites and nitrates and are generally facultative anaerobes (Schneider and Rheinheimer 1988).

23.2.6 Sulfate Reducers

Desulfovibrio sp., *Desulfotomaculum* sp. and *Desulfomonas* sp. are examples of sulfate reducers which are found abundant in saline waters where sulfate concentration is high and constant (approximately 29 mM at a salinity of 3.5%). Sulfate is utilized by sulfate-reducing bacteria (SRB) as a terminal electron acceptor during anaerobic respiration. With the production of H_2S, this gives black color to the SRB, thus making it distinguish from non-sulfate reducers. However, these organisms require redox potential of around −100 mV, they can also survive in oxygenated seawater in numbers 10–100/mL because of the possession of oxygen protective enzymes like superoxide dismutase and catalase (Battersby 1988).

23.2.7 Iron Bacteria

Iron bacteria like *Gallionella* sp. and *Siderocapsa* sp. are found in freshwater, seawater, and thermal springs as well and can be recovered easily from waters showing brown coloration.

23.2.8 Luminescent Bacteria

Photobacterium sp. and other marine luminescent bacteria like *Xenorhabdus* sp. and *Vibrio* sp. can be recovered as free-living, symbiotic, or parasitic. The main

distinguishing feature of this group is their ability to produce luminescence, which is attributed to the presence of enzyme luciferase. This character can be employed for environmental monitoring. Microtox® is one such instrument which has been successfully adopted (Schneider and Rheinheimer 1988; Nawaz and Ahmed 2011) and is based on the use of luminescent bacterium *Vibrio fischeri* as an indicator in aquatic toxicity testing since the 1980s (http://www.leederconsulting.com/pdf/microtox. pdf).

23.2.9 Nitrifying Bacteria

They are strictly aerobic chemolithotrophic bacteria, which are studied under two separate heads, namely ammonia oxidizers and nitrite oxidizers. Ammonia oxidizers (*Nitrosomonas* sp., *Nitrosococcus* sp.) oxidize ammonia to nitrite, which is indicated by the formation of the acidic environment by changing the color of the indicator-containing medium from orange to yellow. Nitrite will not be formed in the presence of nitrite oxidizers because these organisms will convert nitrite to nitrate and so in such cases, the presence of nitrate should be checked. Nitrite oxidizers can be enriched by supplementing the medium with a high concentration of nitrite. Decrease in nitrite concentration even from the highest dilution is indicative of the presence of nitrite oxidizers.

23.2.10 Sulfur Oxidizers

Sulfur oxidizers like *Thiobacillus* and *Thiospira* are autotrophs, which share the common feature of oxidizing inorganic sulfur to obtain energy. These organisms are common in habitats containing H_2S and iron (Schneider and Rheinheimer 1988).

23.2.11 Methanogens

Methanogens can grow at a broad range of temperature, salinity, and pH tolerance. Methanogenic species mostly utilize H_2/CO_2 or formate as a source of energy and produce methane, which is a very important biogenic process contributing partly to a methane concentration of the atmosphere, which is increasing by ca. 2% per annum. Apart from these, other substrates like carbon monoxide, methanol, 2-propanol, and methylamines have also been found to be utilized by some methanogens. Methanogenic colonies may easily be recognized by fluorescence, which is due to the presence of fluorescent compounds F420 and F350. These compounds are unique to methanogens but great care is needed to distinguish it from autofluorescent particles and other microorganisms with compounds having similar fluorescent properties (Conrad and Schütz 1988).

Seasonality plays a very important role in diversity. We know that generally, microbial diversity is high during summer probably due to the rise in metabolic

activities and increased nutrient concentration due to high evaporation rates. Monsoon may lead to dilution and hence may decrease diversity. This hypothesis was also confirmed by Giovannoni and Vergin (2012), where they found a high number of microbial communities in summer as compared to winter and monsoon as a result of stratification and concentration of nutrients like dissolved organic carbon, phosphate, and nitrite plus nitrate. As pointed out by them, a collection of time-series data is generally found feasible to understand the impact of weather, latitude, pollution, pH, nutrients, etc., on microbial communities.

23.3 Methods to Study Microbial Diversity

Microbial life in the sea is ubiquitous. Sea microbes were the ancestors of all life forms on earth. Microscopic life in the sea constitutes a seemingly endless array of beautiful and fascinating microorganisms whose processes hold the key to understand the function of the community or ecosystem. But before ecological problems and principles can be defined and understood, the sea microbes must first be observed and identified. Two separate approaches can be used to assess the diversity of bacteria in the natural community: culture-dependent and culture-independent. The culture-dependent approach is a traditional way, which is based on the ability of bacteria to grow in culture media to form a colony-forming unit so that organisms can be isolated and be available for further study. If the organism seems unable to form a colony-forming unit, even then, it should not be reported as an uncultivable organism because it may have some unknown growth requirements (Das et al. 2006). Another approach is culture-independent. It works without necessitating the cultivation of bacterial species; instead, diversity can be accessed from the extraction of genomic material, especially DNA. However, there are some disadvantages like difficulty in lysing all bacteria from the natural community, extraction of DNA from dead bacteria, and difficulty in the quantification of important species from the habitat (Das et al. 2006).

23.3.1 Culture-Dependent Methods

Physico-chemical analysis of samples is beneficial to formulate media, which mimic natural environmental conditions to support the growth of native species. Martin and MacLeod (1984) suggested various media with several modifications for isolating new species. Schut et al. (1993) have stated the importance of dilution culture for isolation of typical marine bacteria that were cultured using the approach “dilution to extinction”. Apart from this approach, many researchers use marine agar medium, artificial seawater agar medium supplemented with carbon source, seawater yeast extract peptone medium, filtered and autoclaved seawater agar medium, basal salt medium with carbon source, etc., for isolation of a variety of microorganisms. But in laboratory conditions, the majority of organisms fail to grow on artificial media in pure and form colonies in the presence of other microorganisms, as pointed out by

Kaeberlein et al. (2002). They may require some specific signals from their neighbours, indicative of a familiar environment. In recent years this phenomenon, known as quorum sensing, is gaining interest among researchers, as has been noted by Burgess (2012). It is a cell-cell communication method by which bacterial cells perform collective decisions for their benefit. It is known to regulate a variety of physiological responses and was discovered in marine luminescent bacteria (Waters and Bassler 2005). Burgess (2012) designed a diffusion chamber, which was formed by a washer sandwiched between two polycarbonate membranes of 0.03 μm porosity and was incubated on the surface of the marine sediment. By this approach, in that simulated environment, he noted the growth of those microorganisms which were previously uncultivable.

After the growth of microorganisms on media, they must be identified by their colony characters and morphological characters. Tentative identification can be done according to Bergey's Manual, which is based on the results of various biochemical tests. Performing Biolog® is also a common practice to know about the substrate utilization profile of the isolate. Apart from these methods, a more sophisticated and accurate method is 16S rRNA gene sequencing and from the obtained sequence phylogenetic tree can be constructed using software programs like BLAST and MEGA, PHYLIP, PAUP, Dendroscope, GenGIS, etc.

23.3.2 Culture-Independent Methods

Amann et al. (1995) have written an extensive review on the detection and identification of an individual microorganism with the uncultivable approach. Molecular techniques can be applied for the analysis of both, cultivable as well as the non-cultivable type of community. These approaches have also been reviewed by MacGregor (1999) in the context of studying aquatic microbial communities. To study bacteria by the culture-independent approach, samples should be first concentrated by centrifugation and the resulting pellet can be used for various molecular methods.

Determination of %G + C Content To determine %G + C content for prokaryotic diversity, contamination of eukaryotic DNA must be avoided which can be done by fractionated centrifugation method before bacterial lysis (Faegri et al. 1977). By estimating %G + C content, the total genetic structure and diversity in bacterial communities can be determined. Total community DNA contains DNA from various types of bacteria in different proportions. This diversity can be found by analyzing the melting curve of DNA, which can be constructed from DNA denaturation and renaturation characteristics.

Chemotaxonomic Analysis Chemotaxonomic characteristics can be used to analyze community structure because of significant differences in their biochemical compositions. Mordarska et al. (1972) have used chemotaxonomic characters for classifying nocardioform bacteria. Characters such as fatty acids, polar lipids, and quinones were also estimated by Srinivas et al. (2012) for their work on isolation of

marine bacterium from coastal surface seawater. The method for their estimation has been described by Wardell (1988).

PCR Based Methods DNA extraction from samples is essential and a crucial step. Depending upon the type of samples to be used for extraction, various methods of DNA extraction have been reported and are compared by Purohit and Singh (2009), that involved soft lysis, hard lysis, and combination of soft and harsh methods among which the best method was found to be bead beating with lysis buffer. DNA extraction processes are generally followed by PCR amplification of the 16S rRNA gene after confirming purity, as also confirmed by Purohit and Singh (2009) and Siddhapura et al. (2010). DNA extracts can also be purified using a commercially available kit according to the manufacturer's recommendations (Piza et al. 2004). After purification, this DNA preparation can be used as a template for the amplification of the 16S rRNA gene. Various PCR amplification procedures have been described by various researchers (Piza et al. 2004; Sun et al. 2010; Du et al. 2011), the product of which is separated by denaturing gradient gel electrophoresis (DGGE). To remove some biases introduced by PCR amplification, certain modifications are suggested by Bonnet et al. (2002), Suzuki and Giovannoni (1996), Polz and Cavanaugh (1998), and Wagner et al. (1994), which can be taken into consideration. Luna et al. (2012) have utilized the new approach of qPCR for fecal-contaminated marine sediment, which when applied to extracted DNA, allows the quantification of those viable pathogenic bacteria which were not in the cultivable state. In DGGE, PCR products get separated according to their melting point under the effect of urea and formamide and bands can be identified even from the numerically dominant bacteria (Torsvik et al. 1998). Cloning is a method by which DGGE-separated product of the desired length should be inserted into a suitable vector and which in turn is to be inserted into the suitable host cell. Cloned transformants then can be selected by the presence of the marker gene and checked for purity again by PCR amplification. RFLP, ARDRA, etc., are some tools employed by researchers to assist in cloning (Sun et al. 2010; Du et al. 2011). Acevedo et al. (2008) used the genome walking technique for cloning of the complete gene possessing a novel enzyme. The desired product then can be sequenced and doing BLAST search of the obtained sequence, the identity of the organism can be revealed. Using the neighbor-joining method of MEGA software, the phylogenetic position can also be inferred for the isolate by constructing a phylogenetic tree (Torsvik et al. 1998; Du et al. 2011).

Functional Metagenomics The most astonishing feature of this approach lies in the fact that it does not require sequencing of the desired gene but based on the functionality directly identify entirely new classes of genes possessing novel functions (Kennedy et al. 2007; Handelsman 2004). This approach excludes the problem of incorrect annotation of sequences with weak similarity or a similar sequence showing multiple functions, which may be encountered during a sequence-based approach (Kennedy et al. 2010). Thus, this approach is useful for exploiting the biochemistry of microbial communities but unable to assess the metabolic capabilities of specific microorganism within these communities (Imhoff et al. 2011). In this method, an environmental sample is collected and from that total

community, DNA is extracted. After DNA isolation, the isolated DNA is used to generate a metagenomic library using a suitable vector, which in turn is transferred to a suitable host strain, usually *E. coli*. Individual clones then can be screened for the presence of enzymatic or other bioactivities encoded by the environmental DNA fragment. *Streptomyces lividans*, *Ps. putida*, and *Rhizobium leguminosarum* have also been assessed for their potential as expression hosts (Kennedy et al. 2010).

23.4 Industrially Useful Products of Sea Microbes

23.4.1 Enzymes

Enzyme prospecting is a task or a step toward finding novel biocatalysts. Some marine microorganism-derived enzymes are listed in Table 23.2. Marine microbes are thought to possess enzymes with many industrially beneficial features due to their stressed habitat, which gives them salt tolerance, hyperthermostability, barotolerance, and cold adaptivity (Trincone 2011).

Apart from these most commonly used enzymes, some carbohydrate metabolizing enzymes have been studied by Beleneva et al. (2010). Marine bacterium *Pseudoalteromonas* sp. KMM 701 has been explored by Balabanova et al. (2010) for the enzyme α-galactosidase, which, at neutral pH, is capable of converting RBCs into the universal blood type cells. Further, Lee et al. (2010) have reviewed various approaches for the discovery of novel enzymes.

23.4.2 Bioactive Compounds

Marine bacteria are known to produce bioactive substances, specifically to protect themselves from predation, by showing antibacterial, antiviral, antitumor as well as an anticoagulant and cardioactive properties (Asha Devi et al. 2011). Jayanth et al. (2002) isolated bacteria from various sources of samples and checked to find their antagonistic effect against human pathogens associated with seafood. Similar work has been done by Gnanambal et al. (2005) and the antagonistic effect was checked against human pathogens as well as fish pathogens. Asha Devi et al. (2011) obtained two bioactive compounds, namely Drechslerine A and cis-Sativen-ediol from *Alteromonas* sp. and *Rhodopseudomonas* sp., respectively. They hypothesized that the antibacterial component of Gram-negative marine bacteria was responsible to produce bioactive substances. One such review has been reported by Holmström and Kjelleberg (1999) on bioactive molecules by marine *Pseudoalteromonas* sp. generally found associated with marine eukaryotes. Mayer et al. (2007) have reported the effect of pharmacologically active marine compounds on the immune and nervous system also. Yada et al. (2008) isolated marine bacteria producing purple pigment violacein, which possess anti trypanosome and antitumor properties.

Multidrug resistance is a burning issue nowadays, which necessitates the invention of new and novel antibiotics. One such antibiotic, Korormicin A, is obtained

Table 23.2 Some enzymes produced by marine microorganisms with their application

Enzymes	Mode of action	General application	Enzyme producers	References
Protease	Catalyze the hydrolysis of peptides, amides, and esters	Detergent, digestive drugs, inflammatory drugs, leather industry	*Oceanobacillus iheyensis*, *Sphingomonas paucimobilis*, *Aureobasidium pullulans*, *Bacillus mojavensis* A21	Purohit and Singh (2011) Turkiewicz et al. (1999) Chi et al. (2007) Haddar et al. (2009)
Lipase	Catalyze the breakdown of fats and oils with the release of free fatty acids and glycerols	Esterification, transesterification, aminolysis, detergents, paper production, cosmetic production, food flavoring, organic synthesis	*Moraxella* sp. *Candida*, *Pichia*, *Yarrowia*, *Lodderomyces*, *Rhodotorula* and *Aureobasidium pullulans* HN2.3 *Streptomyces* sp.	Feller et al. (1990) Wang et al. (2007) Mo et al. (2009)
Amylase	Breaking down of complex sugars like starch into simple sugars	Liquefaction, ethanol fermentation, manufacture of maltose and syrups, detergents, digestive aids	*Aureobasidium pullulans*N13d, *Streptomyces* sp. D1, *Mucor* sp. *Wangia* sp. C52	Li et al. (2007) Chakraborty et al. (2009) Mohapatra et al. (1998) Liu et al. (2011)
Cellulase	Break down cellulose through hydrolysis into smaller polysaccharides	Various industries like fermentation, textile, paper and pulp, food, agriculture, detergent	*Saccharophagusdegradans* strain 2–40, *Saccharophagusdegradans*	Taylor et al. (2006) Suvorov et al. (2011)
Chitinase	Cleave the bond between the C1 and C4 of two consecutive N-acetylglucosamines of chitin	Role in immunity enhancement, promote digestive function, toxins	*Vibrio fluvialis*, *Vibrio mimicus*, *Vibrio alginolyticus*,	Osawa and Koga (1995)

		removal from the body, and inhibit tumor cell growth	*Listonellaanguillarum*, *Aeromonashydrophila*	
Agarase	Catalyze the hydrolysis of agar	Beverages, bread, cosmetic additive, hair conditioning effect	*Pseudomonas droebachense* *Vibrio* sp. JT0107, *Bacillus* sp. MK03	Stanier (1941) Susgano et al. (1993) Suzuki et al. (2003)
Carrageenases	Hydrolyze carrageenan to form tetrasaccharide of the k-carrageenan ideal structure with galactose 2,6-disulfate at the reducing end	Coagulant, adhesive, stabilizer, emulsifier, cosmetics, pharmaceutical	*Cytophaga* IK-C783 *Pseudoalteromonas* CL19	Sarwar et al. (1987) Ohta and Hatada (2006)
Xylanases	Catalyze the endohydrolysis of 1,4-β-D-xylosidic linkages in xylan and involved in the production of xylose	Treatment of agriculture, municipal and food industry waste, industries like paper and pulp, textile, baking, fruit and vegetable processing	*Bacillus* sp. YJ6 *Penicillium*FS010	Yin et al. (2010) Hou et al. (2006)

from some marine species of *Pseudoalteromonas*. This antibiotic acts specifically on an enzyme that is crucial for the growth of many Gram-negative human pathogens like *Vibrio cholerae*, *Pseudomonas aeruginosa*, and *Aliivibriofischeri*. One very striking feature of this antibiotic is that it has no effect on beneficial microflora of the human intestine (Maynard et al. 2019).

23.4.3 Nutraceuticals and Cosmetics

Active ingredients from marine microorganisms are used as food additives, coloring additives, supplements, antioxidants, essential oils, vitamins, and co-factors (Imhoff et al. 2011; Margesin and Schinner 2001). Polyunsaturated fatty acids (PUFA) are generally obtained from fish oil but there are certain problems like undesired odor and difficulties in purification step and hence this source can be replaced with marine bacteria (Margensin and Schinner 2001). Russell and Nichols (1999) found marine bacteria as a potential source for the production of PUFA like docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, and γ-linolenic acid, which can be used as a supplement to treat deficiency in essential fatty acids. Various types of pigments are produced by marine heterotrophic bacteria which include carotenoid, flexirubin, xanthomonadine, and prodigiosin (Stafsnes et al. 2010). Asker and Ohta (1999) first reported canthaxanthin production by extreme halophile, which can be used in cosmetics because of its property to reduce the necessary exposure time in sunlight to acquire a tan. A patent has been granted to Matsunaga and Shibazaki (1994) for establishing a method of reuse of microbial cells for pigment production without their breakage, which ultimately reduces the production cost.

23.4.4 Exopolysaccharide (EPS) Production

Microbes produce EPS in tightly bound, loosely adhered, or freely dissolved form (Bhaskar and Bhosle 2005). It is an important process contributing to the binding capacity of sediment particles and hence influencing sediment stability (Yallop et al.2000). Kumar et al. (2007), in their extensive review on bacterial exopolysaccharide, have mentioned EPS production from marine bacteria like *Hahellachejuensis*, *Vibrio*, *Pseudomonas*, and *Zooglea* spp. Okutani (1984) have studied antitumor and immunostimulant activities of EPS from marine bacterium *Vibrio*. Iyer et al. (2005) have studied rheological properties of EPS produced by marine *Enterobactercloacae*. Delbarre-Ladrat et al. (2014) have described microbial polysaccharide diversity from shallow and deep-sea hydrothermal vents by using organisms like *Sulfolobus*, *Thermococcus*, *Thermotoga*, *Geobacillus*, *Bacillus licheniformis*, *Bacillus thermodenitrificans*, *Alteromonas,* and *Pseudoalteromonas*. Upadhyay et al. (2016) have also studied the EPS production potential of marine isolates from coastal sites of Alang. Metal-binding properties of EPS by *Halomonas* spp. and its role in enhancing trace element bioavailability were studied by Gutierrez et al. (2012). Antarctic marine bacteria have also been investigated for EPS

production potential (Nichols et al. 2004) and chemical characterization revealed diverse types of EPS even among six closely related *Pseudoalteromonas* isolates (Nichols et al. 2005). Decho (1990) has written an extensive review explaining the role of EPS in food webs and a variety of processes occurring in oceans. He has also reported on the possibility of stress as a reason for increased EPS production. Matsunga et al. (1996) reported that sulfated EPSs interfere with viral penetration into host cells. Effect of EPS on corrosion of mild steel has been investigated by Majumdar et al. (1999). They found that biofilm on mild steel inhibits its corrosion in natural marine waters. Bhaskar and Bhosle (2005) reviewed the metal-binding ability of EPS, which include heavy metals like PD, Co, Sr, Cr, Th, and Cd. Other than this, they have also discussed the negative impacts of EPS. Accumulation of aggregates due to EPS production increases oxygen demand by forming anoxic conditions and organic matter accumulation at the bottom leads to anaerobic degradation and hence the resultant increase in greenhouse gases like H_2S and N_2O. Apart from this, the accumulation of toxic metals and their subsequent grazing results in the entry of toxic metals into marine food webs.

Microbially enhanced oil recovery (MEOR) is a very promising field for the application of haloalkaliphiles. EOR (enhanced oil recovery) is a forced extraction of oil which is retained in the strata with the help of water with modified properties. The water used in extraction is usually seawater and condition in reservoir wells is alkaline and saline and hence haloalkaliphiles from the marine system may serve as a promising candidate (Horikoshi, 1999).

23.5 Environmental Significance of Sea Microbes

23.5.1 Dye Decolorization

Azo dyes are the largest group of dyes used in the industry. They resist chemical and microbial attacks and are found stable in light and during washing. Many of them are carcinogenic and may trigger allergic reactions in humans. Estimates say that 10% of the textile dyes do not bind to fibers during processing and so released to the environment (Ozdemir et al. 2008). Dye decolorization may take place in two ways, one being its adsorption on microbial biomass and the other being its degradation by the cells (Zhou and Zimmermann 1993). Some marine microorganisms viz. *Shewanella* sp., *Photobacterium* sp., and *Vibrio* sp. have been shown to possess dye decolorizing ability (Caccamo et al. 1999). Ozdemir et al. (2008) also showed 94% decolorization of Acid Black 210 at 100 ppm dye concentration by the luminescent *Vibrio harveyi* TEMS1 under static condition after 24 h of the incubation period. The concept of "waste to wealth" is very well reported by Lum et al. (2019) by using ash which is an industrial and agricultural waste by-product. Prepared ash-based photocatalysts possess greater absorption ability, ion exchange ability, specific surface area, and reusability. Such reusability is also an attribute of nanoparticles. Shao et al. (2019) have studied the effect of the moderate-intensity static magnetic field (SMF) on dye biodecolorization performance of the marine

microbial community and found that 45.3 mT SMF increases the relative abundance of some potential microorganisms like *Shewanella*, *Vibrio*, and unclassified genera Pseudeurotiaceae in the consortia. The field of nanotechnology has emerged as a potential tool to resolve many environmental concerns. Easy synthesis and separation process of nanoparticles or nanocomposites like MoS_2/Fe_3O_4, TiO_2, Ag_3PO_4/Bi_2S_3-HKUST-1-MoF, Fe_2O_3/Al_2O_3, $ZnFe_2O_4$, Cu_2O, and CuO make them attractive to be used by many researchers for dye degradation (Fadlalla et al. 2019). Elango and Govindasamy (2018) used an ecofriendly approach for dye decolorization. They used activated carbon, prepared from temple waste flowers, and achieved maximum color removal efficiency of 98.17%.

23.5.2 Biosurfactant Production

Surfactants are known for their ability to reduce surface and interfacial tension at the interface between liquids, solids, and gases. Thus, these surface-active compounds allow them to disperse readily as emulsions. Biosurfactants are amphiphilic compounds, produced by microorganisms and possess properties like biodegradability and effectiveness at high pH or temperature. These are widely used in cosmetics, pharmaceuticals, detergents, food processing, paper and pulp processing, and many more (Banat et al. 2000; Kubicki et al. 2019). They exhibit properties like low critical micelle concentrations (CMC), can form metal-ion complex, lowering of surface tension, low eco-toxicity in addition to antifungal, antibacterial, and antitumor effects (Kubicki et al. 2019). Oil spill accidents result in significant contamination of oceans. The largest possible market for biosurfactants is for bioremediation in the oil industry, which includes oil spill at inland and at sea, mobilization of oil sludge from storage tanks, and enhanced oil recovery from oil reservoirs (Georgiou et al. 1992; Khire and Khan 1994a; Khire and Khan 1994b). In the review, Maneerat (2005) have listed various marine organisms like *Alcanivoraxborkumensis*, *Alcaligenes* sp., *Arthrobacter* sp., *Myroides* sp., *Yarrowialipolytica*, *Pseudomonasnautica,* and types of biosurfactants produced by them like glucose lipids, trehalose lipids, ornithine lipids, bile acids, polymeric biosurfactants, and particulate biosurfactants. Kubicki et al. (2019) have also supported the view of many researchers that *Alcanivorax*, *Cycloclasticus*, *Marinobacter*, *Oleispira*, *Pseudomonas*, *Paracoccus*, and *Rhodococcus* are among the topmost genera which can be isolated from oil-spill areas. Biosurfactant production in extreme conditions has been reviewed by Cameotra and Makkar (1998) in detail. Satpute et al. (2008) have assessed a variety of screening methods, namely hemolytic assay, modified drop collapse, tilted glass slide, emulsification index, emulsification assay, oil spread method, hydrocarbon overlay agar plate, and blue agar plate to detect biosurfactant production in marine bacteria. Among other methods tested, the best method found was hydrocarbon overlay method (HOA). An exhaustive review on various applications of biosurfactant has been provided by Banat et al. (2000). Banat et al. (2014) have very well documented problems encountered in biosurfactant production as well as solutions on how to increase production yield. Availability of

economic and renewable substrate for media formulation, process parameters, development and/or isolation of potential microorganisms or consortia and strain improvement by changing substrate type and/or incubation conditions are major points which need to be taken care of if one aims to attain high yield and desired products. A promising and very less explored application of biosurfactant in the field of agriculture has been studied by Ram et al. (2019). To obtain desired control, pesticides are being used in high concentration because of their low wettability. To bring down the contact angle of droplets and to attain high wettability on plant leaves, biosurfactant can be effectively employed. This approach will also reduce the amount of pesticides being used in the field and that too without any compromise in pest control efficiency. In addition to this, biosurfactants, being amphipathic in nature, facilitates the entry of water in seeds, which in turn increases metabolism and thereby promotes seed germination (Araujo et al. 2019).

23.5.3 Metal Bioremediation

Heavy metal pollution is becoming a major threat to the marine environment as it is increasing day-by-day with the increased utilization of various metals in several industrial processes and their disposal in several rivers, which ultimately end in the sea and accumulate without decomposition (Matsunaga et al. 1999; Ayyam et al. 2019). Mercury, chromium, arsenic, cadmium, zinc, lead, copper, nickel, etc., are some of the commonly used metals. For growth and development, microorganisms use these metals as a source of energy by utilizing various metabolic pathways. Electrostatic forces help metal cations to bind to the anionic cell wall of microbes. Apart from this, pH, temperature, and moisture conditions also play an important role in metal bioremediation (Sen Gupta et al. 2019). Other mechanisms involve cell membrane transport, physical adsorption, bioleaching, bioaccumulation, biomineralization as well as biosorption by various groups of microorganisms (Banerjee et al. 2019). In their extensive reviews, Banerjee et al. (2019) and Yin et al. (2019) have discussed various mechanisms of bioremediation and microbial resistance to metals. Members of the group Desulfosarcina-Desulfococcus were found in a variety of polluted environments, e.g., metal-contaminated sediments (Gillan et al. 2005) as well as in petroleum-contaminated marine sediments (Ravenschlag et al. 2000). Correlation between metal pollution and community tolerance has been investigated by Ogilvie and Grant (2008) and it was found that metal pollution increased the abundance of some tolerant species as compared to pristine environment. Marine microalgae Chlorella sp. NKG16014 was checked by Matsunaga et al. (1999) to test its bioremediation potential against cadmium-contaminated seawater.

23.5.4 Hydrocarbon Degradation

It is estimated that each year approximately 1.3 million tons of petroleum enters into the marine environment (McGenity et al. 2012). Niger Delta oil spill, oil spill case in

the Gulf of Mexico, Arctic oil spills, and North-western oil spill in the Persian Gulf are some of the well-known oil spill incidents that have occurred till date across the globe (Jernelov 2010). The Exxon Valdez oil spill of 1989 of Alaska is one of the most destructive human-induced disastrous oil spills. As a result, 11 million gallons crude oil was estimated to be lost. To overcome this problem, 1,70,000 liters of dispersant had been sprayed but it did not prove much effective. Approximately, 15,000 gallons of the oil was collected and ignited. Due to the storm, shoreline clean-up operations were started which involved around 10,000 workers, 1000 vessels, 100 air crafts and helicopters for 4 years with the estimated cost of $2.1 billion. Despite these actions, nearly 10% of the spilled oil was removed or treated. The major portion was naturally weathered or degraded by native microorganisms (Shigenaka 2014). Wolfe et al. (1994) have discussed the fate of the oil spilled from the Exxon Valdez. They stated that 50% was biodegraded, 20% of the oil evaporated and underwent photolysis, 14% was recovered, 13% remained in sub-tidal sediments, 2% on the intertidal shoreline, and less than 1% remained in the water column. Prof. Ananda Mohan Chakrabarty is very well known for his contribution in the field of oil or hydrocarbon bioremediation. He has genetically engineered a species of *Pseudomonas* bacteria, which is known as Superbug or *Pseudomonas putida* that could digest approximately two-third of the hydrocarbon found in the typical oil spill. When petroleum is spilled, it undergoes certain changes with time called "weathering". During this process, low molecular weight fractions evaporate, oil droplets get mixed with seawater, photochemical oxidation and biodegradation take place (Harayama et al. 2004). Leahy and Colwell (1990) have written a detailed review on explaining the role of temperature, pH, oxygen, nutrients, salinity, pressure as well as some biological factors influencing hydrocarbon degradation. Interspecies interactions also play a significant role during crude oil biodegradation, as has been explained by McGenity et al. (2012).

23.6 Conclusion

This chapter has discussed an important concern of marine bacterial diversity. Many reports have indicated that polluted or stressed environment results in loss of bacterial diversity, though some population become dominant and thus decreasing species evenness. Further, it was also found that diversity increases when stress or pollution is removed. Native species should be preserved from perturbation because species richness prevents invasion from other new species and hence subsequent loss of biodiversity can be prevented. Because of the unique environment of marine ecosystem, marine bacteria possess some unique biodegradative abilities as compared to their terrestrial counterparts. Moreover, much of the research has been carried out to find the industrially significant products from terrestrial microorganisms, and marine bacterial diversity is not much explored. Understanding this great reservoir of enormous useful products, many researchers are paying attention to exploit the potential of marine microbes. Therefore, in this chapter, we

tried to incorporate various groups of marine microorganisms that can be used in restoration of habitats, environmental clean-up and even in gaining economic growth.

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Current and Potential Uses of Marine Collagen for Regenerative Medicines

24

Kirti and Samanta S. Khora

Abstract

Marine ecosystem is a rich source of a variety of organisms that are a source of immeasurable value that can be used in drug development, cosmetics, and other value-added products. Regenerative medicine is a new medical multidisciplinary field whose main target is to repair or replace diseased body parts. It basically combines three components: cell, nutrient, and scaffold. Basically, the lost or damaged tissues are replaced by cell-supporting biomaterials using regenerative medicines. Collagen has a special advantage in this regard because of its structural versatility and biocompatibility. Moreover, marine collagen obtained from the scales, skin, and bones of fishes has been widely used as a scaffold for tissue regeneration due to their excellent bioactive properties such as low antigenicity, high biodegradability, and cell growth potential. Collagens from marine sources are free of risk of disease transmission. The knowledge about the molecular structure, biosynthesis, and assembly of collagen molecules is important to understand various biological processes and pathological conditions related to human diseases. The current chapter covers the basic knowledge of marine collagen and its potential applications for regenerative medicines.

Keywords

Collagen · Biocompatibility · Biodegradation · Regenerative medicines · Scaffolds · Tissue engineering

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24.1 Introduction

The most exciting prospect for the development of biomaterial for regenerative medicines is the exploration of rich biodiversity hidden in the marine ecosystem. Many of the natural marine resources are enormously unexploited. The materials obtained from marine sources are biocompatible, biodegradable, safe, and also superior to the currently available materials used for grafts or scaffolds (Lalzawmliana et al. 2018). Marine organisms such as fish, fish wastes, starfish, sponges, and jellyfish have recently been explored as alternative sources (Addad et al. 2011). Marine collagen and peptides obtained from the wastes (skin/scales) of marine fishes are very useful possessing various health benefits (Kumar et al. 2019). Fish collagen has many benefits over collagen obtained from mammalian sources and hence fish waste can be used as a potential and cost-effective alternative to isolate collagen. Here, we provide an overview of current and potential applications of collagen obtained from marine sources for regenerative medicines.

24.1.1 Structure of Marine Collagen

Collagen is a large protein molecule which produces very small bioactive peptides with various biological functions upon enzymatic hydrolysis. The word collagen has Greek origin, *cola* which means glue and *genno* means birth. Thus, collagen glues cells to form the basis for the body's tissues and organs. It comprises one-third of total protein in humans and accounts for three-quarters of the dry weight of skin. It is the most prevalent component of the extracellular matrix (Brinckmann et al. 2005).

It is present abundantly in connective tissues including skin, bones, joints, cartilage, tendon, and other organs (Rodriguez et al. 2018). The biological functions, mechanical strength, and stability of collagen basically depend on its structure. The fundamental aspects of the tri-helix structure of collagen were deduced in the mid-1950s from fiber X-ray diffraction of tendons. However, the appearing models could only attempt an ordinary description of the molecular conformation. Then around 20 years later with the chemical synthesis of sufficiently long and homogeneous peptides with collagen-like sequences brought new insights. A large number of studies based on biochemical, crystallographic, and NMR techniques revolutionized our understanding toward collagen structure (Bella 2016). The first correct structure for collagen was proposed in 1954 by Ramachandran and Kartha and consists of three left-handed helices (Ramachandran and Kartha 1954).

The basic unit of collagen protein is tropocollagen. Tropocollagen is a subunit of larger collagen fibril aggregates. It is a cylindrical protein of diameter 1.5 nm and the length of each subunit is around 300 nm and is formed of three left-handed polypeptide chains or α-chains of molecular weight 100,000 daltons each (Schmidt et al. 2016). The three polypeptide chains are twisted together into a right-handed triple helix linked by numerous hydrogen-bonds and van der Waals interaction (Brinckmann et al. 2005) as well as some covalent bonds (Harkness 1966). It provides rigidity to the structure and less solubility. Hence collagen is produced

from the interaction of tropocollagen molecules (Sionkowska et al. 2017). There are different types of collagen protein and they can be made up of either three alpha chains (homotrimers) which are identical, or two or three alpha chains (heterotrimers) which are different. The characteristic feature of collagen protein is the sequence of amino acids in each of the three polypeptide chains of collagen subunits. The sequence of amino acid in the polypeptide chain has repetition of Gly-X-Y, where X and Y are proline (Pro) and hydroxyproline (Hyp), respectively (Shoulders and Raines 2009). Glycine and hydroxyproline are mainly responsible for the structure and stability of the collagen molecule.

24.1.2 Types and Characterization of Marine Collagen

Collagens are of various types. Earlier, 19 types of collagen were reported, named as type I to XIX (Benjakul et al. 2009). Currently, at least 29 distinct types of collagens have been identified which are produced by more than 30 genes (McCormick 2009).

The classification of these collagens has been done on the basis of their complexity, diversity in their structure, the presence of additional, non-helical domains, their assembly, and their functions. Collagen can be classified as (i) fibril forming collagen, (ii) fibril-associated collagens, (iii) network forming collagens, (iv) anchoring fibrils, (v) transmembrane collagens, (vi) basement membrane collagens, etc., on the basis of structure and supramolecular organization (Gelse and Aigner 2003).

The most abundant collagen in all connective tissues is Type I collagen accounting for 30% of all protein (Muyonga et al. 2004). It consists of two α1 chains and a single α2 chain. Collagen type I, II, and III share large sections of homologous sequences which are independent of species (Timpl 1984).

Different types of collagen serve different functions. The basic qualities of collagen essential to work as biomaterial depend on collagen structure, size, and the specific sequence of amino acids (Friess 1998).

As collagens are extracted from many sources and are of different types, it is very important to study their characteristics. Characterization is required to get a better knowledge of sample, so that it will be easier to understand their biological response and results and features of extracted protein (Abraham et al. 2008). This will be helpful in improving the results and to get the desired biological response. Some of the methods of collagen characterization are explained as follows:

24.1.2.1 SDS-Page

This method is used to separate proteins and their fragments based on their molecular weight and size. Proteins are loaded in the small wells of the gel and under applied electric field, they get separated based on their size; the smallest go further than the larger ones. This will be helpful in the identification of different collagen types (Abraham et al. 2008).

24.1.2.2 Western Blot

This method is used to find functional proteins. Mixture of proteins separated by electrophoresis on the basis of their molecular weight are transferred to a biologically active membrane and then antibodies that are specific to our protein of interest are applied on the membrane and then these complexes are detected by chemiluminescence and film-development (Ikenoue et al. 2003).

24.1.2.3 Hydroxyproline Determination

Amino acid, hydroxyproline is almost exclusive to collagen and it is present in insignificant amount in other proteins. Hydroxyproline determination is required to measure the collagen extraction yield and it is calculated by taking the ratio of extracted hydroxyproline to that of its initial concentration in fish skin (Skierka and Sadowska 2007).

24.1.2.4 Amino Acid Analysis

This process involves protein hydrolysis which is followed by amino acid separation, then identification and quantification using different chromatographic techniques (Jámbor and Molnár-Perl 2009). It is expressed as the number of each amino acid residue per thousand residues or as the number of moles of each amino acid in the sample.

24.1.2.5 Determination of Denaturation Temperature

It is an important factor in determining the thermal stability of collagen. Thermal stability of collagen can be assessed by differential scanning calorimetry (DSC) (Komsapenkova et al. 1996) in which heat flow is measured between sample and reference zone and it provides information about the thermal transition of proteins. The maximum transition temperature differs between fishes and it is different for different types of collagen (Kittiphattanabawon et al. 2010a, b).

24.1.2.6 Fourier Transform Infrared Spectroscopy

It is based on molecular vibrations. Chemical bonds undergo different types of vibrations such as twisting, rotating, and stretching. This technique is very helpful in providing information on conformational changes in protein on the basis of differences in spectra (Braiman and Rothschild 1988). These spectra can be obtained for proteins in a wide range of environments.

24.1.2.7 Characterization of Morphological Features

The presence and organization of collagen fibrils can be observed by acquiring images of collagen samples by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (Starborg et al. 2013).

24.1.3 Regenerative Medicines—An Overview

Regenerative medicines not only aim to heal tissues due to traumatic injury but also help in restoring the tissue's native function. Various biomaterials are designed to fill

the gap between therapeutic agents and the body's natural healing process (Pawelec et al. 2016). Some of the examples of natural bioactive biomaterials are collagen, gelatin, matrigel, fibrin, alginate, cellulose, chitosan, hyaluronic acid, and silk fibroin. It is the major component of the extracellular matrix (Lin et al. 2009; Tayebjee et al. 2003). In recent years, a very diverse mechanical and regulatory functions of collagen fibers have been reported (Bretaud et al. 2018). Collagen has an important role in the formation of tissue and organs and is involved in various functional expressions of cells. Various studies have shown the relation between structural and mechanical features of collagen fibrils and the framework of functional artificial tissues (Roeder et al. 2002). Collagen type I is advantageous as it provides multiple cell attachment sites by stimulating cell adhesion and growth. Its triple helical structure has various integrin receptors, such as α1β1, α2β1, α3β1, α10β1, and α11β1 (Khew and Tong 2007; Knight et al. 2000). Langer and colleagues first of all elucidated the concept of tissue engineering in which cells, genes, and proteins are delivered via a degradable material, called scaffold, in order to regenerate tissue (Langer 1990; Langer and Vacanti 1993; Langer and Vacanti 1999). The basic requirements for scaffolds are as follows:

(i) The biomaterial must be biocompatible.
(ii) The material must be chosen based on the structural and metabolic demands of the particular tissue.
(iii) Both in vitro and in vivo evaluation should be performed to check the performance of the matrix.

The various functions of scaffolds are as follows (Yamada et al. 2014):

(i) They provide structural integrity.
(ii) They help in the proliferation of donor cells and the growth of the host cells.
(iii) They maintain a distance between parenchymal cells so that the diffusion of gas and nutrients can take place.
(iv) They transmit tissue-specific mechanical force to guide the behavior of cells (Cima et al. 1991).

24.2 Sources of Marine Collagen

Collagens can be obtained from both terrestrial and aquatic organisms. The marine organisms available as the source of collagen can be roughly divided into three categories: marine invertebrates such as cuttlefish, jellyfish, starfish, sea urchin, marine fishes like jawless fish, cartilaginous fish, bony fish, and sea mammals such as seal and whale and fishes (Eastoe and Leach 1977). Researchers have found that scale, fin, skin, bone, and cartilage of freshwater and marine fish, scallop mantle (Shen et al. 2007), adductor of pearl oyster (Mizuta et al. 2002a), muscle layer of ascidian (Mizuta et al. 2002b) and from marine sponge *Spirastrella inconstans* have been isolated and can be used as new sources of collagen. Marine

Table 24.1 Different sources of marine collagen

Organisms	Tissue/organ	References
Sponges		
Chondrosia reniformis	Sponge material	Swatschek et al. (2002)
Spirastrella inconstans	Sponge material	Sudharsan et al. (2013)
Axinella cannabina and Suberites carnosus	Sponge material	Tziveleka et al. (2017)
Jellyfish		
Cyanea nozakii	Umbrella	Zhang et al. (2014)
Acromitus hardenbergi	Tissues	Khong et al. (2018)
Squids		
Doryteuthis singhalenis	Skin	Veeruraj et al. (2015)
Ommastrephes bartrami	Skin	Yan et al. (2009)
Crustaceans		
Penaeus chinensis	Muscle	Minamisako and Kimura, (1989)
Starfish		
Asterias amurensis	Body tissues	Lee et al. (2009)
Fish		
Parupeneus heptacanthus	Scales	Matmaroh et al. (2011)
Lagocephalus gloveri	Skin	Senaratne et al. (2006)
Diodon holocanthus	Skin	Huang et al. (2011)
Katsuwonus pelamis	Bones	Nagai and Suzuki (2000)
Carcharinus albimarginatus	Cartilage	Jeevithan et al. (2014)
Mammals		
Balaenoptera acutorostrata	Body pieces	Nagai et al. (2008a, b, c)

fishes are the prominent source of collagen. Based on their living environment fish are usually subdivided into four groups: hot-water fish, warm-water fish, cold-water fish, and ice-water fish (Eastoe and Leach 1977). Collagen has been isolated and characterized from various marine sources such as the scales of marine fishes (Thuy et al. 2014), skin of flatfish (Heu et al. 2010), scales of spotted golden goatfish (Matmaroh et al. 2011), skin and bone of bigeye snapper (Kittiphattanabawon et al. 2005), and skin of *Lagcephalus gloveri* (Senaratne et al. 2006). The different sources of marine collagen are listed in Table 24.1.

The general methodology for the extraction of collagen from fish by-products and other marine sources involves three important steps: preparation, extraction, and recovery (Silva et al. 2014). Different techniques have been proposed to obtain collagen from different marine sources. However, marine collagens have a lower denaturation temperature than that of porcine skin collagen. This shows that fish collagen is less stable than collagen obtained from mammals (Ogawa et al. 2003). Starving fish or fish that undergo a poor diet produces more collagen than well-fed

fish. There are different techniques proposed to obtain collagen-based molecules depending on the collagen source.

24.3 General Properties of Marine Collagen for Regenerative Medicine

Regenerative medicines not only heal tissues after injury but also help in restoring their native functions. The process of healing involves communication with cells through porous scaffolds of biomaterials. These porous scaffolds not only support or direct cell growth but also function for growth factors or drug delivery (Mullen et al. 2010). Regenerative medicines often utilize scaffolds to serve these purposes.

The materials and methods used for scaffold preparation vary a lot. Natural biopolymers are preferred as they are less immunogenic. Among these biopolymers, collagen is widely used as it is the major structural component of extracellular matrix in living tissue. By varying the structure of these collagen scaffolds, different functions can be performed (Lynn et al. 2010).

Marine collagen has wide applications due to its various properties like water solubility, biocompatibility, biodegradability, less immunogenicity, safety, and low cost in production (Cho et al. 2014). The lower denaturation temperature and viscosity of marine collagen in comparison to collagen from terrestrial sources produce hindrance in the manufacture of scaffolds (Nomura et al. 1996). In vitro cross-linking can be achieved in collagen to increase its stability. Cross-linking methods can be divided into physical treatments (UV irradiation, gamma irradiation) and chemical treatments (using glutaraldehyde, carbodiimide, etc.). Though chemical treatments provide high strength and stability but they can cause cytotoxicity or poor biocompatibility whereas physical treatments have no cytotoxicity (Lim et al. 2019).

One of the most important limitations of using collagen as a biomaterial for regenerative medicine is related to their mechanical properties in case if high pressures and stresses have to face in the blood vessel (Achilli et al. 2010; Meghezi et al. 2015). Hence, many researches have been conducted to enhance mechanical strength by controlling polymerization of helices, stability of collagen in solutions, enzymatic degradations, and also by controlling the pore size of scaffolds. One of the approaches to increase the mechanical strength of scaffolds is by chemically, physically, or enzymatically crosslinking the biopolymer (Davidenko et al. 2015; Liu et al. 2019). Cross-linking modifications can be done in collagen as they have a limited number of functional groups (Ryglova et al. 2017).

24.4 Few Examples of Marine Biomaterials for Use in Regenerative Medicines

A biomaterial is a substance of biological origin which is designed to interact with a biological system in order to support cells, enhance a biological function and also to repair or replace damaged tissue. Numerous researchers have made many efforts in developing biomaterials for use in regenerative medicines. Marine organisms are a rich source of biologically active and novel compounds.

24.4.1 Alginate

It is one of the most abundant biosynthesized materials in nature. It occurs as a structural component in marine brown algae (*Phaeophyceae*) like *Laminaria hyperborean*, *L. digitate*, etc. It is a polysaccharide made up of (1–4) linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers (Sun and Tan 2013). Alginate has a huge application as a biomaterial. It is used as supporting matrix or delivery systems for tissue repair and regeneration. This is due to its hydrogel-forming ability, biocompatibility, biodegradability, and non-antigenicity (Bouhadir et al. 2001). Alginate and its derivatives can easily be processed to various shapes and structures for fabricating the biomaterial forms like hydrogels, microspheres, porous scaffolds, and nanofibers (Sun and Tan 2013). Alginate has also cell therapy applications.

24.4.2 Chitosan

It is a marine polysaccharide obtained by deacetylation of naturally occurring chitin extracted from the exoskeleton of mainly crabs and shrimps. It is a polymer of two monomers; β-(1–4)-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose (Burrows et al. 2007). Chitosan is used as a biopolymer for tissue engineering due to its ability to form the porous structure by freezing and lyophilizing its solutions (Suh and Matthew 2000). Chitosan has been approved by the FDA for use in wound dressing (Wedmore et al. 2006) due to its good biocompatibility (Thanou et al. 2001). Multifaceted strategies are required to develop to utilize chitosan for cell therapy application. Chitosan has also been exploited for application in bone tissue engineering (Suh and Matthew 2000).

24.4.3 Carrageenans

These are sulfated polysaccharides produced in several species of red seaweeds (Rhodophyceae) such as *Chondrus crispus, Gigartina*, etc. On the basis of number and position of sulfate group in the repeating galactose units, it has been characterized into three main families: kappa, iota, and lambda (Pereira et al.

2009). Among them, kappa carrageenan has primarily been exploited in the cell therapy application due to its distinguishing properties. Kappa carrageenan can be processed into various shapes and structures and its chemical structure resembles glycosaminoglycans which provide distinctive advantages for various applications (Popa et al. 2014). Carrageenan has also potential in cartilage tissue engineering (Bhattacharyya et al. 2010). Carrageenan along with alginate is used for preparing hydrogel beads and fibers which have wide applications in cell delivery and tissue engineering applications (Popa et al. 2011).

24.4.4 Collagen

Collagens from marine sources are in abundance and they can be easily extracted. Also, marine collagens are water soluble, have better physical and chemical durability, and are free of risk of animals' disease transmission (Yamada et al. 2014). Marine collagen has excellent biocompatibility due to which it has an important role in tissue engineering and in regenerative medicine for the design of biomaterial scaffolds. Marine collagen peptides (MCP) have an important role in bone tissue engineering and regeneration. MCPs have been found to have a substantial osteogenic activity (Pallela et al. 2013). MCP extracted from bone and skin of cods increased cell proliferation, expression of osteogenic markers, and also increased alkaline phosphatase and mineralization activity while treating human osteoblastic cells (NOS-1) (Yamada et al. 2013). This clearly indicates the potential of MCP as a biomaterial for bone tissue engineering.

MCP has also potential uses in cartilage tissue engineering and regeneration. MCP obtained from the skin of deep-water ocean fish showed enhanced chondrogenic differentiation of primary adipose-derived stromal cells (Raabe et al. 2010). Fish collagen has a potential application in cartilage repair.

Marine collagen has a potential application in skin tissue engineering. From the past many centuries, there is an increasingly strong demand for skin substitutes for the replacement of skin defects which can be due to burns, infection, graft rejection, genetic diseases, and other skin diseases.

24.5 Safety Analysis of Marine Collagen for Regenerative Medicine

Earlier collagens from mammalian sources were extensively used as a scaffolding material in regenerative medicine (Ramshaw et al. 2009). However, the pathological risk of mammalian collagen in transmitted diseases cannot be ignored. A variety of marine biomaterials have been found as safe for various biological applications. A comparative study between mammalian and marine collagen has been shown in Table 24.2.

The three main components of regenerative medicines are cells, nutrients (growth factors, cytokines, and chemicals, etc.), and scaffold materials (Hayashi et al. 2012).

Table 24.2 Differences between marine and mammalian collagen

S No	Aspects	Mammalian collagen	Marine collagen
1	Cost	Expensive	Cheap
2	Meting point	High	Low
3	Viscosity in solution	High	Low
4	Extraction procedure	Difficult	Easier comparatively
5	Solubility	Soluble in organic solvent	Soluble in water
6	Risk of transmitted disease	High	Low
7	Amino acid composition	Low contents of GLY and ALA with high PRO	High contents of GLY and ALA with low PRO

(Source: Adopted and modified from Subhan et al. 2015)

All these components play an important role. In the case of scaffold preparation, collagens obtained from marine sources are safe comparatively as they are not associated with transmissible diseases and severe infections like bovine spongiform encephalopathy, avian and swine influenza, tooth and mouth diseases which occur worldwide in bovines, pigs, and buffalo. Marine collagens have been found to have excellent biocompatibility, low antigenicity, high biodegradability, and cell growth potential due to which they have a potential application as a scaffold material (Dillow and Lowman 2002).

24.5.1 Biocompatibility and Allergy

The applications of marine fish collagen as a scaffold material for tissue engineering purposes were evaluated (Nagai et al. 2008a, b, c; Sugiura et al. 2009). It was found that collagen from tilapia (fish) showed very mild reactions in rat pulp when induced even at the initial stage of the experiment (Yamada et al. 2014). Atelocollagen is a processed biomaterial synthesized from bovine type I collagen. It shows low inflammatory response, high biocompatibility, and biodegradability (Hanai et al. 2006). Low immunogenicity of atelocollagen is due to the removal of some telopeptides causing immunogenic response at the time of atelocollagen production (Sano et al. 2003). Jellyfish collagen has a highly porous and interconnected pore structure which is useful for nutrient and oxygen supply to cells cultured in three-dimensional matrices. The immunogenicity of jellyfish collagen was compared with bovine collagen by measuring the levels of proinflammatory cytokines and antibodies following in vivo implantation. Jellyfish collagen was found to induce immune response comparable to that induced by bovine collagen (Song et al. 2006). However, salmon collagen vascular grafts when placed subcutaneously in rat tissues induced little inflammatory response (Nagai et al. 2008a, b, c).

24.5.2 Biodegradability

When in vitro degradation studies were conducted using a collagenase solution, it was found that cross-linked collagen scaffolds from freshwater fish scale showed only a 50% reduction in mass after 30 days whereas uncrosslinked scaffold degraded completely within 4 days. Also minimal immunological reactions were observed when the collagen solution was injected in mice (Pati et al. 2012). This study shows the biocompatible nature of fish scale collagen in humans. Many subcutaneous grafts gradually biodegrade when placed in subcutaneous tissues in rats. It is also reported that various types of treated collagen do not disappear even at 4 weeks after implantation (Sugiura et al. 2009).

24.6 Potential Applications of Marine Collagen for Regenerative Medicines

Collagen has an important role in vital fields. It shows excellent biocompatibility, weak antigenicity, high biodegradability, and cell adhesion capacity compared to other natural polymers such as chitosan, albumin, and other synthetic polymers. Collagen and collagen-based scaffolds offer distinctive advantages when chosen as biomaterial to use as regenerative medicine purposes (Cunniffe and Brien 2011). Regenerative medicines are basically based on biomaterials science and cell biology. Marine collagen is a versatile biomaterial and can exist in various formulations for their respective applications. The potential applications of marine collagen for regenerative medicines are as follows:

24.6.1 Tissue Engineering and Regeneration

It is an emerging and growing interdisciplinary field of life science. It combines both engineering and biological principles to synthesize new tissues and organs in order to regenerate damaged or diseased tissue/organ by combining cells from the body with highly porous scaffold biomaterials (Lim et al. 2019). Few applications of marine collagen in tissue engineering are listed in Table 24.3.

24.6.1.1 Bone Regeneration

Collagen-like biomaterial enhances repair of tissues such as bone, tendon, ligaments, skin, vascular and connective tissues. Bone regeneration is a physiological process which involves bone formation and bone resorption. Bone regeneration is required for skeletal reconstruction in the case of defects and damage due to injury, infection, and osteoporosis (Dimitriou et al. 2011). Several studies have shown that marine collagen peptides have osteogenic activity (Pallela et al. 2013). The biological effects of marine collagen peptides obtained from the bone and skin of cods on human osteoblastic cells were studied and it was found that marine collagen peptides are responsible for increased cell proliferation, osteogenic markers expression,

Table 24.3 Applications of marine collagen as regenerative medicines

Marine origin collagen	Application	References
Collagen from codfish skin	Cell adhesion of lung fibroblast cells MRC-5	Carvalho et al. (2018)
Collagen from Blueshark (*Prionace glauca*) skin	Osteogenic activity	Elango et al. (2018)
Collagen from marine sponge (Spongin)	Bone tissue engineering	Parisi et al. (2019)
Collagen from marine sponge (*Chondrosia reniformis*)	Capability to generate crosslinks in membranes	Pozzolini et al. (2018)
Collagen from blue shark	Bone tissue engineering	Elango et al. (2016)
Collagen from marine sponge (*Ircinia fusca*)	Bone grafting application	Pallela et al. (2012)
Collagen from shark skin (*Prionace glauca*)	Bone tissue engineering	Diogo et al. (2018)
Collagen seabass scale	Cell growth, proliferation, and migration	van Essen et al. (2013)
Collagen from silver carp skin	Wound healing	Cao et al. (2015)
Collagen from tilapia scale	Osteogenic gene expression, cell viability, and cell attachment	Tang and Saito (2015)

alkaline phosphatase activity, and mineralization (Yamada et al. 2013). It clearly shows the relevance of marine collagen peptides in bone tissue engineering. Salmon skin collagens have also potential applications in bone tissue engineering on the basis of biomimetic mineralization principle (Hoyer et al. 2012).

24.6.1.2 Wound Healing

Wound healing involves four phases: hemostasis, inflammation, proliferation, and remodeling. Collagen has an important role in all these phases mainly due to its chemotactic properties. It promotes the production and migration of keratinocytes and fibroblasts to the wound site in the proliferation phase. It is found that jellyfish collagen peptides accelerate the wound healing process by promoting chemotactic factors and fibroblast production (Felician et al. 2018). The wound healing effects of collagen peptides from chum salmon in cesarean sectioned rats (Wang et al. 2015) and collagen peptides from nile tilapia in rabbits (Hu et al. 2017) have also shown similar results. The exact molecular mechanism of wound healing by collagen peptides is still unclear (Felician et al. 2018).

24.6.1.3 Drug Delivery

Marine collagens have been widely used for drug delivery. There are larger numbers of biopharmaceuticals which are administered by subcutaneous injection or intravenous infusion with advancements in biopharmaceuticals. Many of these biopharmaceuticals are susceptible to proteolysis and degradation as well as parenteral administration is preferred due to their large sizes. Though implanted system

provides precise dosage, however injectable systems are more preferable as they do not require surgical implantation. There are some limitations of synthetic polymer matrices in parenteral delivery (Jeevithan et al. 2013). The major advantage of using marine collagen-like biomaterials for controlled protein delivery is due to their excellent biocompatibility, compared to that of synthetic polymers (Freilberg and Zhu 2004). Also, these collagens are biodegradable upon the actions of collagenase (Chapman and Hulmes 1985).

24.6.1.4 Vascular Tissue Engineering

With the increasing number of patients suffering from cardiovascular diseases, peripheral vascular disease, and ischemia, it is required to develop treatments related to vascular grafts or artificial blood and lymphatic system (Serbo and Gerecht 2013). Marine collagen works as an alternative biomaterial for vascular tissue engineering. Collagen from snakehead fish scale developed and modified by chemical processes like methylation and partial esterification and found to be effective for cell interaction by attracting negatively charged proteins (Zhang et al. 2004). Methylated collagen was also found to be water soluble and potential to use as a drug carrier (Liang et al. 1998).

24.6.1.5 Dental Tissue Engineering and Regeneration

The tooth structure combines both hard tissue as well as soft tissue (Aurrekoetxea et al. 2015). First of all it was reported that marine collagen peptides could promote the viability of human periodontal ligament and upregulate the expression of osteogenic markers. It suggested the application of marine collagen peptides as a bioactive agent for alveolar bone regeneration (Liu and Sun 2015). Tilapia (Oreochromis niloticus) scale collagen showed activity in dentin pulp regeneration when treated on rat odontoblast-like cells (MDPC-23) (Tang and Saito 2015).

24.6.1.6 Corneal Tissue Engineering

Any damage in the cornea can lead to blindness (Lamm et al. 2014). Keratoplasty or corneal transplantation is a solid tissue transplant procedure and is used generally when other treatments fail to correct vision (Akpek et al. 2015). Biomaterials such as amniotic membranes, acellular corneal stroma, and natural polymer-based material can be used as cornea repair material as they show excellent biocompatibility (Zhao et al. 2018). Collagen–based biomaterials have also shown corneal repair and scar inhibition (Chae et al. 2015). Marine collagen scaffolds synthesized from fish scales showed good mechanical properties, physical strength, microbial resistance, and swelling ratio required for the purpose of corneal regeneration (Krishnan et al. 2012).

24.7 Problems Regarding the Use of Marine Collagen in Regenerative Medicine

Marine biomaterials have some unique characteristics which make them desired material to be used in biomedical purposes but, on the other hand, their processing raises some obstacles. Suitable efforts are required to overcome these obstacles. This can be done by combining these natural biopolymers with some chemically synthesized polymers to increase their structural stability using an adequate solvent. Various methods have been adopted to improve structural property and denaturation temperature of marine collagen. Marine origin collagens differ in various characteristics from the collagens obtained from terrestrial sources. The denaturation temperature of marine origin collagens is comparatively lower than collagen from terrestrial sources. When these collagens are intended to implement for human health care products, it is essential to maintain the structural integrity of triple-helical marine collagen by chemical derivatizations which result in higher denaturation temperature and increased resistance to enzymatic degradation (Pati et al. 2012). The denaturation temperature in the case of collagen extracted from the Nile Tilapia was increased by using the modified method of acid-solubilized and pepsin-solubilized collagen extraction (Potaros et al. 2009). It is observed that the denaturation temperature of marine collagen can also be increased by forming hybrid with chitosan and hydroxyapatite using freeze-drying and lyophilization method (Pallela et al. 2012).

Marine collagens must be safe for biomedical applications. When used for medical purposes they should pass all biocompatibility tests in accordance with the essential requirements defined in Annex I of the Council Directive 93/42/EFC. They should not be immunotoxic or allergy-causing which may be possible due to large phylogeny differences between human and marine species. It is very important to conduct in vitro and in vivo studies before using marine collagen for clinical applications.

The manufacturing process of marine collagen must be fully validated and reproducible and there should be the removal of harmful pathogens or residues. It must be guided by safety regulations.

Due to a unique microenvironment, a collagen scaffold working well in one specific tissue may have a poor effect on another tissue. The mechanical and biological properties of the scaffold should be according to the target tissue. Also, chemical, mechanical, electrical, and morphological properties of the scaffold are all directly related to cellular behavior and fate. These issues must be taken into consideration to improve scaffold performance by the researchers. It is also required to explore collagens that mimic natural collagen both structurally and functionally (Dong and Lv 2016).

24.8 Future Needs and Prospects

Marine environment is a rich source of bioactive substances, collagen is one of them. Collagen derived from marine organisms has been explored and found to be similar to collagen obtained from terrestrial sources in terms of biocompatibility and amino acid composition.

Before using marine collagen for biomedical purposes it is important to check their safety regarding the presence of pathogens and also for immunotoxicity. There can be metal ions present in fish products and they must be removed before using collagen. Studies are required to know in details about the efficacy of marine collagen. Experiments are to be designed to show the implantation of biomaterial in the host body without significant adverse immunological response. On the basis of positive performance, a clinical trial of marine collagen-based biomaterial can be accepted. Industries should also take initiative in developing marine collagen-based marketable products. The translation of lab-scale processes to industry level is required to improve and innovate extraction and purification processes of marine collagen. Still the use of a marine collagen biomaterial in clinical procedure in the established way will take some time.

24.9 Conclusions

Marine collagen acts as a promising biomaterial for biomedical applications due to its natural origin as well as structural resemblance with mammalian collagen. They are of utmost importance for tissue engineering and regenerative medicines. They can be easily extracted and are water soluble, biocompatible, and free of risk of disease transmission. This provides special advantages to marine collagen for tissue engineering and drug delivery processes. Due to characteristic physicochemical properties, marine collagen has wide applications in tissue engineering, skin regeneration, wound healing, drug delivery, and as therapeutic agent for diseases such as obesity and diabetes. Recent researches are aimed at the enhancement and utilization of collagen-based biomaterials for medical purposes by improving their mechanical strength, biocompatibility, and delivery characteristics.

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