26. Biotechnological Potential of Marine Microbes

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The world's oceans, from the coasts to the abysses, harbor an incredible level of microbial diversity. This marine microbial biosphere is an enormous, untapped resource of biotechnological interest. This chapter reviews the potential of marine microbes in biotechnology. The biotechnological potential is considerable, ranging from the synthesis of bioactive molecules to the production of biofuels, cosmeceuticals, nutraceuticals, and biopolymers; from the engineering of marine microbes for biomedical purposes to the degradation of pollutants, and the use of microbial biosensors as sentinels for environmental quality. Marine viruses have great biotechnological potential, yet the exploration of the marine virome, and the associated gene and protein pool, is only beginning. Marine archaea have so far been exploited for the isolation of enzymes, yet many biotechnological exploitations can be foreseen. Bacteria and microbial eukaryotes, especially fungi and photosynthetic protists, provide an important contribution to biotechnology; the combination of *omics-driven* technologies and improved cultivation techniques is widening the knowledge on their biological diversity, paving the way for new biotechnological exploitations. The exploration of the marine microbial biosphere, and its extraordinary genetic and physiological diversity, will undoubtedly continue to offer chances for the development of new and sustainable blue biotechnologies, helping to solve important societal challenges of the twenty-first century.

26.1 Microbial Diversity in the World's Oceans and Biotechnological Applications of Marine Microbes

The world's oceans are the largest ecosystem on Earth. Life in global oceans is dominated by unicellular microbes, accounting for the largest fraction of biomass and biodiversity [26.1]. Although microbes play a fundamental role in the productivity, ecosystem functioning, and global biogeochemical cycles of the major elements [26.2], we still know little about marine microbial diversity, the patterns and driving forces, the genetic and metabolic repertoire, and the ecological role each microbial species plays in the ecosystem [26.3]. Little is known about the functions of marine microbes in the ecosystem and their metabolic capabilities because of the current inability to assign any function to a large proportion of their genes [26.4]. The vast majority of marine microorganisms cannot be cultured in the laboratory under standard laboratory conditions [26.5, 6]. It was only the development of molecular methods that made it possible to identify marine microbes [26.7–9], but scientists are merely beginning to gain a comprehensive understanding of the full extent of microbial biodiversity and functions in the world's oceans [26.10].

In the United Nations Convention on Biological Diversity, biotechnology was defined as [26.11]:

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

There is, however, no agreed upon definition of biotechnology, and definitions may range from *the uses of biology for the benefit of man* to *the use of biology to make money* [26.12]. Biotechnology is nowadays experiencing an increasing importance at the planetary scale, and is expected to increasingly contribute in addressing important socio-economic issues of our societies. The term *blue biotechnology* is typically utilized to describe the aquatic, either marine or freshwater, applications of biotechnology [26.4].

Marine organisms can be utilized as biotechnological agents to provide products and services that have useful applications for humans and their well-being. Among these, there are new medical technologies, food and feed ingredients (e.g., nutraceuticals), molecules useful in many industrial sectors, and biofuels [26.13]. Marine biotechnology has the potential to contribute significantly to key societal challenges, such as the sustainable supply of healthy and high-quality food, the production of bioenergy (such as biofuel production from many microalgae), the discovery and exploitation of pharmaceuticals or diagnostic tools for improving human health, the isolation of biopolymers useful in the cosmetic, food and health industries, and the synthesis of enzymes, proteins, or biomaterials of interest in several industrial processes. Marine bioresources (organisms and molecules) can be helpful in increasing stock production in aquaculture processes, in controlling infectious diseases of farmed organisms, as well as in addressing key environmental issues such as marine pollution (e.g., by the development of biosensing technologies for environmental monitoring, the use of microbes for degradation of pollutants, or the identification of natural antifouling technologies).

This chapter reviews the potential of marine microbes in biotechnology. Marine microbes include representatives from three domains of life: archaea, bacteria and eukarya [26.14]. Bacteria and archaea are the most abundant cells in all known marine ecosystems. Microbial eukaryotes, among which unicellular microbes (protists) and fungi (including either unicellular or multicellular forms), are ecologically important members of marine microbiota, and many studies are documenting their once-unimaginable diversity [26.15, 16]. This review also covers the biotechnological potential of marine viruses. Viruses do not have an organized cell structure, but represent the most abundant biological entities in the oceans. Phages are believed to be the most diverse biotic component in marine systems, and they are potentially interesting resources for biotechnology.

26.2 Why Do Marine Microbes Matter in Biotechnology?

In the twentieth century, research on marine natural products mainly involved the collection of macroorganisms from the sea, their extraction, and the analysis of the extracts [26.17]. Most frequently, algae and marine invertebrates were investigated. Several molecules with biological activity were isolated, and most of them were typically obtained from corals, sponges, or other invertebrates. The first marine bioactive compounds, spongouridine and spongothymidine, were isolated from the sponge *Cryptotheca crypta* in the Caribbean [26.17]. In the following decades scientists proved that these compounds had anticancer and antiviral activities ([26.18] and references therein). These nucleosides were the basis for the synthesis of Ara-C, the first anticancer agent derived from a marine organism, which is currently used in the treatment of patients affected by leukemia and lymphoma, and the antiviral drug Ara-A [26.19]. An overwhelming number of bioactive compounds of marine origin have subsequently been isolated and described, and more than 15 000 marine products have been described so far [26.20]. Recent estimates report some 20 000 marine high-value-added compounds, including a range of proteins, carbohydrates, and lipids [26.4, 21]. There are many examples in the marine pharmacology literature of antitumor and cytotoxic compounds isolated by marine animals (tunicates, nudibranchs, sponges, octocorals, bryozoans), algae, fungi, and bacteria [26.22]. Some of these substances have created new and exciting means for disrupting tumor specific cell signaling, cell division, energy metabolism, or gene expression, and have the potential to revolutionize cancer treat-

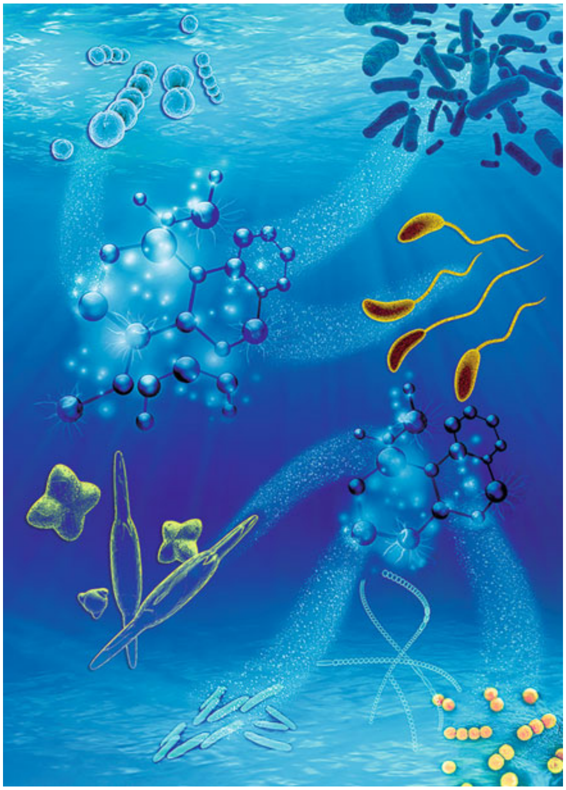

Fig. 26.1 Marine microbes are a source of bioactive molecules and secondary metabolites, many of which offer potential biotechnological opportunities. Photo courtesy of Marta Scandali (Ancona, Italy)

ment [26.22, 23]. Marine organisms can be important sources of useful molecules for treating infectious diseases, such as HIV-AIDS (HIV: human immunodeficiency virus; AIDS: Aquired Immuno Deficiency Syndrom; [26.22] and references therein). However, the application of many substances from marine macroorganisms has been hampered by difficulties regarding their reproduction and scaling up, or problems of supplying sufficient amounts of the pure substance [26.17]. Efforts in trying to cultivate marine macroorganisms, such as in the case of sponges, have been difficult [26.24], despite recent advances in the ability to cultivate organisms, cells, or their microbial symbionts [26.25]. Therefore, although a large number of marine natural products isolated from macroorganisms has been described from marine biota, only a few of them have so far entered preclinical or clinical trials [26.17].

The marine environment is incredibly heterogeneous, being characterized by a wide variability in the main physical and chemical variables. Temperature can range from values close to (or below) 0 °C to up to 370 °C in hydrothermal vents [26.26]. Salinity is typically more uniform across the ocean, but can vary significantly in specific areas, such as coastal transitional environments and estuaries, or reach nearly saturating concentrations in hypersaline environments, such as in the deep-hypersaline anoxic basins in the Mediterranean Sea [26.27]. The seabed, historically considered to be a flat, uniform, and biologically inert territory, where hydrostatic pressure can reach values up to 1100 bar, and light is typically absent, is now known to include a wide variety of habitats [26.28]. Despite these apparently inhospitable conditions for supporting life, the seafloor has been recognized as the largest biome on Earth, and hosts a significant proportion of global biomass and biodiversity.

Marine organisms, including microbes, have evolved to occupy a broad range of ecological niches, and their adaptation across the evolutionary time scale has originated an enormous array of physiological, genetic, and metabolic diversity. The marine biosphere, and particularly its microbial component, is an immense source of biodiversity, of bioactive compounds and secondary metabolites with potential applications in biotechnology (Fig. 26.1). Marine microbes have evolved specific adaptations to survive under different environmental challenges, resulting in different survival mechanisms, growth strategies, and genetic adaptations [26.29]. Some examples have been described recently. The photosynthetic pelagic bacterium *Prochlorococcus marinus* has adopted a minimalist approach and has reduced the size of its genome size, to gain a competitive advantage, given that conditions within its environmental niche vary only a little [26.29, 30]. *Pelagibacter ubique*, a marine α-proteobacterium, has no transposons, extrachromosomal elements, pseudogenes, or introns, and its genome, consisting of less than 1400 genes, is the smallest described for a free-living microbe [26.31].

The ability of marine microbes to adapt to changes in environmental conditions and to thrive under extremes conditions, coupled with their high genetic plasticity, have influenced their prowess to produce compounds and secondary metabolites. The marine microbial biosphere represents an important opportunity for bioprospecting, but the exploration of the potential of marine microbes in terms of the exploitation of molecules is still at an early stage [26.4]. As opposed to macroorganisms, which need to be collected from the sea for sufficient amounts of the searched metabolite to be extracted, marine microbes can be cultivated in the laboratory in large quantities [26.17, 32]. Therefore, microbial blue biotechnology has the advantage of being a more sustainable activity, by avoiding the collection of marine organisms from their habitat.

26.3 Biotechnology of Marine Microbes, from Viruses to Microbial Eukaryotes

26.3.1 Marine Viruses

Viruses are ubiquitous biotic components of pelagic and benthic ecosystems. They influence lateral gene transfer, genetic diversity, and bacterial mortality [26.33, 34]. Marine viruses were not studied until 1989, but are now recognized as the most abundant biological entities in the sea (typically 10^7 viruses ml^{-1}; [26.35]. Phage diversity studies were initially restricted by the requirement for cultivated hosts, but recent applications of culture-independent techniques have revealed an enormous, and unexpected, viral diversity in marine ecosystems [26.35, 36]. Archaeal, bacterial, and eukaryotic viruses are cosmopolitan and abundant throughout the world's oceans [26.37]. Their host range covers all known marine organisms, from prokaryotes to fishes and mammals. Phages are particularly abundant in marine sediments, and viral infection represents a substantial source of mortality in deep-sea benthic ecosystems [26.38]. Many lines of evidence have suggested that phage diversity is virtually immense, but studies describing marine phage diversity are nowadays limited [26.39].

The genetic pool of marine viruses has enormous potential for biotechnology, but the use of marine viruses as biotechnological agents is still at an early stage. One potential application can be in medicine, to provide new solutions to sanitary problems for humans or other organisms. Potential applications of viruses in biomedicine, some of them already explored, include the use of viral vectors (gene transfer, gene therapy and vaccine development) and the use of viruses for protein expression and oncolytic viruses (e.g., virotherapy for treating tumors). There are currently no studies involving marine viruses but, given the magnitude of the diversity of marine phages, potential biotechnological exploitations can be expected.

Phages were used in early forms of biotechnology to control bacterial infections [26.33, 40]. Phage therapy was abandoned due to contrasting results and the discovery of antibiotics. It is, however, currently the subject of revived interest because of the emergence of antibiotic-resistance as either a biomedical and environmental problem. Some authors have explored the biotechnological usefulness of marine viruses as agents for curing coral diseases. The rapid emergence of infectious diseases in tropical stony corals is causing serious damage to coral reef ecosystems [26.41]. *Efrony* et al. [26.42] recently isolated two phages of known coral bacterial pathogens (*Vibrio coralliilyticus* and *Thalassomonas loyana*) and used them in controlled aquarium experiments to explore their utility in coral *phage therapy*. The results showed that diseases could be successfully controlled by the use of pathogen-specific phages, suggesting the possible usefulness of phage therapy. *Cohen* et al. [26.43] and other authors recently confirmed the potential application of marine viruses in the treatment of coral diseases. Other potential biotechnological applications of marine viruses are in the control of harmful algal blooms. *Onji* et al. [26.44] reported on the isolation of two virus-like agents that are able to suppress the growth of *Gymnodinium mikimoto*, a red-tide-forming marine dinoflagellate.

The exploration of the diversity of the marine virome has just begun. The discovery of novel genes and proteins with unknown functions, with potential biotechnological applications, can be expected, and deserves further in-depth investigations.

26.3.2 Marine Archaea

Archaea are evolutionarily unique prokaryotes, as genetically distant from bacteria as they are from eucarya [26.14]. They were historically assumed to live only in *extremophilic* habitats, but two decades of studies have revealed that they are abundant, widespread, and ecologically important members of the marine biota [26.45, 46]. The discovery of archaea was a significant breakthrough in the history of biology, leading to the replacement of the prokaryote/eukaryote dichotomy by a trinity of domains, the archaea, bacteria and eukarya [26.47].

Marine archaea are an interesting source for biotechnology, however only few fields of biotechnological exploitations have so far been successfully investigated. One was the isolation of enzymes from some hyperthermophilic archaea. The vent polymerase was isolated from the marine archaeon *Thermococcus litoralis*, and is currently marketed as a useful alternative to the widely used *Taq* deoxyribonucleic acid (Taq DNA: a thermostable DNA polymerase named after the thermophilic bacterium *Thermus aquaticus*; Pfu DNA: a thermostable DNA polymerase named after the thermophilic bacterium *Pyrococcus furiosus*) polymerase. The *Pfu* DNA polymerase, isolated by the hyperthermophilic archaeon *Pyrococcus furiosus*, is commercially available and possesses a higher fidelity compared with other conventional Taq DNA polymerases [26.48]. Research into high-fidelity enzymes from marine archaea is still ongoing and is continuously yielding new findings [26.49].

Marine archaea have been also exploited in several industrial sectors. A heat and acid stable α-amylase, Valley Ultra-Thin, discovered from a deep-sea hydrothermal vent archaeon, has been developed to facilitate the processing of corn into ethanol [26.17]. Marine archaea can be important producers of biopolymers and bioplastics, such as PHA (polyhydroxyalkanoate), and a source of new secondary metabolites [26.17]. Recent studies have pointed out the importance of aquatic archaea as useful agents for hydrocarbon biodegradation and decontamination of polluted areas [26.50], indicating a potential, underexploited opportunity for biotechnological investigations.

26.3.3 Marine Bacteria

Bacteria are typically the most abundant and diverse members of the microbial biota in pelagic and benthic ecosystems. They typically outnumber archaea, despite exceptions in certain habitats [26.51, 52] and are the key players in biogeochemical processes and the fluxes of energy and matter in the ocean. Many bacterial species are distributed across marine ecosystems worldwide. *Candidatus Pelagibacter ubique*, a marine bacterium, is recognized as being one of the most abundant organisms on Earth [26.31].

Marine bacteria have been largely exploited for several biotechnological applications. Most of the marine bioactive compounds that have been successfully screened originate from bacteria [26.4]. Marine bacteria, especially actinomycetes, are important producers of antimicrobial secondary metabolites and antibiotics (see [26.32] for a review). Marine actinomycetes produce different types of secondary metabolites, a large fraction of which possess biological activities and have the potential to be developed as therapeutic agents [26.53]. Many other marine bacteria display significant antibacterial activity [26.54], and they are believed as an exciting resource for discovering new classes of therapeutics within the areas of oncology and infectious diseases [26.55]. Marine cyanobacteria also produce bioactive and cytotoxic molecules that are useful as drugs [26.56, 57].

Unique biosynthetic enzymes from marine bacteria have begun to emerge as powerful biocatalysts in medicinal chemistry and total synthesis [26.58]. Marine bacteria are important producers of biopolymers and biodegradable plastics [26.59], pigments (including melanin, which is potentially exploitable for sunscreens, dyes, and coloring), cohesive molecules that are useful as marine cements, and extracellular substances that are useful as surfactants ([26.22] and references therein).

The surface of many marine macroorganisms such as, to mention only a few, sponges [26.60], algae [26.61], tropical stony corals [26.62, 63], cold-water corals [26.64], hydroids [26.65], crabs [26.66, 67], and fishes, is a particularly interesting niche to study marine bacteria and other microorganisms with respect to biotechnology. The surface of virtually all marine macroorganisms hosts abundant and diverse communities of microbes [26.68]. For instance, the tissue of stony corals typically harbors an associated and diversified microbial community (Fig. 26.2), consisting of archaea, bacteria, and eukaryotes [26.69, 70]. Similarly, marine sponges typically contain diverse and abundant microbial communities, made up of bacteria, archaea, microalgae, and fungi, which comprise up to 40% of the sponge volume and contribute significantly to the host metabolism [26.60]. Microbial associates are believed to have several functions for the sponge, such as stabilization of the sponge skeleton, nutrient uptake, processing of metabolic waste, and secondary metabolite production. An increasing number of studies is documenting the existence of close associations between prokaryotes and higher eukaryotic organisms, typically forming mutualistic or symbiotic relationships [26.17]. Chemically-driven interactions are thought to be important in the establishment of relationships between epibiotic microorganisms and their eukaryotic hosts. For instance, soft-bodied marine organisms lack obvious structural defense mechanisms and rely on chemical defense, by production of bioactive compounds (either by themselves or the associated microflora), to survive [26.71]. Marine invertebrates are a rich source of bioactive metabolites, yet recent stud-

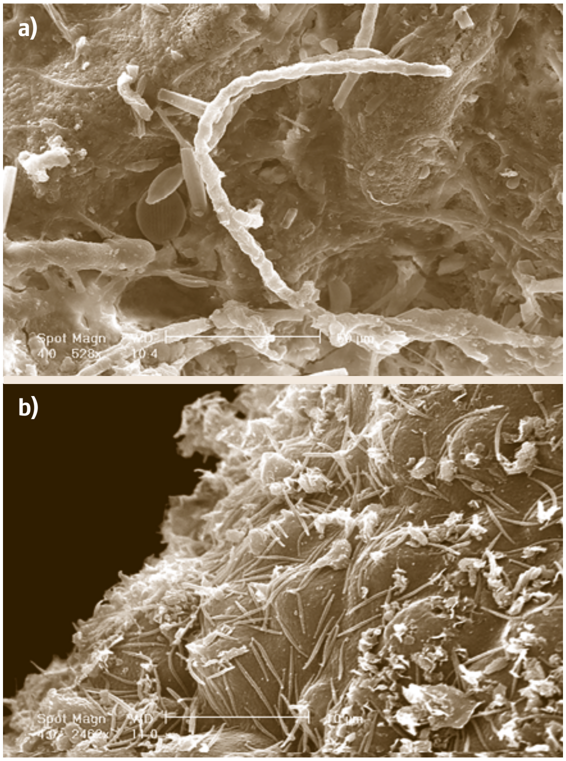

Fig. 26.2a,b Scanning electron microscopy (SEM) images of **(a)** the surface of the stony coral *Merulina ampliata* and **(b)** the colonial hydroid *Ectopleura crocea*. The surfaces of marine macroorganisms are typically hotspots of microbial diversity, and host a myriad of prokaryotic and eukaryotic microbes with interesting, underexplored biotechnological potentialities. Photo in b) courtesy of Dr. Cristina Gioia Di Camillo (Polytechnic University of Marche, Italy)

ies have shown that some bioactive compounds that were previously ascribed to the host are produced by the microbial symbionts [26.72]. Epibiotic microbes can produce a plethora of bioactive compounds, including antibiotics, antiviral, antitumor, and biopolymers. The investigation of the epibiotic microbial communities clearly deserves further study. Similarly, the study of other underexplored niches, such as the intestines of marine organisms [26.73, 74], is expected to provide important contributions to biotechnological developments.

Marine bacteria have enormous potential applications as biotechnological agents to remediate pollution in marine systems. These applications can range from the use of hydrocarbon-degrading bacteria for oil degradation [26.75–77] to the biosynthesis of surfactant and emulsifier molecules to be used in industrial processes, for environmental remediation or as drugs [26.78]. Hydrocarbon contamination is an important environmental issue, especially in coastal areas subjected to high anthropogenic input. Hydrocarbons, and similarly other pollutants such as pesticides, toxic metals, or herbicides, reach the marine environment from a wide variety of anthropogenic sources, including oil spills, urban runoff, shipping, and industrial activities. Hydrocarbons typically accumulate in the sediments, posing serious concerns for both the environment and human health. The success of petroleum bioremediation strategies relies on the ability to provide the optimal conditions to stimulate metabolism of those bacteria that are able to degrade hydrocarbons. Autochthonous bacterial communities can be used in the bioremediation of contaminated sediments, by stimulating their degradation processes with the addition of adequate substrates [26.77].

The aquaculture industry can also benefit from several biotechnological applications deriving from marine bacteria. They can contribute as probiotics, producers of functional foods and additives to increase biomass yields, to fight potentially pathogenic bacteria and to manage infectious diseases, or may serve as biodegraders of aquaculture wastes and organic pollutants [26.79]. Exploring the potential of marine bacteria and their metabolites will help in transforming aquaculture into a more sustainable and efficient industry.

26.3.4 Marine Microbial Eukaryotes

Protists (including photosynthetic protists or microalgae) and fungi are abundant and ecologically relevant members of marine microbial biota. A plethora of studies have documented their functional role, and recent studies are documenting their under-recognized genetic diversity and the spatial distribution across the ocean [26.80, 81].

Marine fungi form a taxonomically heterogeneous group, including *obligate* marine fungi (those able to grow and sporulate exclusively in seawater) and *facultative* marine fungi, which have a freshwater or terrestrial origin but can grow and sporulate in marine ecosystems [26.82]. There are currently about 800 described species of obligate marine fungi, mostly belonging to ascomycetes, anamorphs, and a few basidiomycetes [26.82]. Many fungi, such as thraustochytrids, play an important ecological and biogeochemical role in marine ecosystems, and mediate the

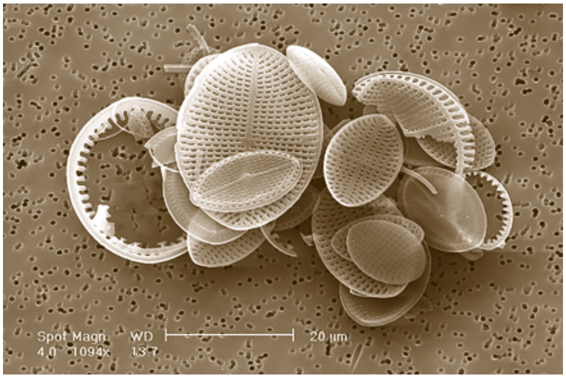

Fig. 26.3 SEM image of benthic diatoms (*Cocconeis* spp.). Marine microalgae can be important targets for biotechnology, especially for biofuel production. Photo courtesy of Dr. Chiara Pennesi (Polytechnic University of Marche, Italy)

degradation of organic matter by the production of extracellular degradative enzymes [26.83]. Historically, fungi have been believed to be rare in marine environments, but recent studies, based on molecular and metagenomics approaches, are revealing an unexpected diversity of fungal communities in coastal and deep-sea ecosystems [26.84, 85]. Recent authors reported on the discovery of 36 novel marine lineages of marine fungi [26.85]. Marine fungal biotechnology, or *blue mycotechnology*, is consequently an exciting and promising area of investigation [26.86]. The number of bioactive compounds isolated from marine fungi is increasing [26.32]. Marine fungi produce a plethora of compounds, among which are anticancer, antibiotic, antiangiogenesis, and antiviral compounds, and molecules having antiproliferative activity [26.87]. Deep-sea fungi have been less described in terms of their abundance, diversity, and ecological role, but are potentially important and productive sources of bioactive products. *Li* et al. [26.88] reported about the isolation of *Phialocephala* sp. in deep-sea sediments, which synthesizes new sorbicillin trimers with cytotoxic properties. *Damare* et al. [26.89] isolated barotolerant fungi in deep-sea sediments, and reported that some of them produce proteases active at low temperatures, with biotechnological potential for waste digestion, food processing, detergents for washing at cold temperatures, and preservation. Marine fungi may also serve as useful pollutant degraders [26.90].

Microalgae have an important biotechnological potential. Microalgal biotechnology is currently based on the production and synthesis of food and feed, additives, cosmetics, pigments (carotenoids), and biofuel [26.91]. They are used as additives in products for human consumption (to enrich the protein content or the nutritional value in food) or as live feed in the aquaculture industry for a variety of farmed organisms. Marine microalgae are important producers of bioactive compounds [26.92], which are useful as pharmaceuticals and cosmetics. An noteworthy line of biotechnological exploitation of microalgae is their use for the production of biofuels [26.93]. Combined with their fast growth rate, microalgae are considered one of the few realistic sources for the production of biofuels and as being superior to agricultural crop-derived bioethanol [26.94]. Many microalgae, such as diatoms and dinoflagellates [26.95] naturally accumulate large amounts of hydrophobic compounds, which can exceed 80% of the algal dry weight. Diatoms are among the most productive and environmentally flexible eukaryotic microalgae on the planet (Fig. 26.3). They are responsible for 20% of global carbon fixation by primary production [26.96, 97] and have several characteristics that make them useful for large-scale biofuel cultivation [26.98]. There has been substantial progress in the development of algal biofuels in the last decade, but more fundamental research is needed to better understand their physiology and metabolism and to develop efficient large-scale culture systems to grow algae and produce biofuel [26.99]. The biotechnological potential of diatoms and other marine microalgae also cover the production of pharmaceuticals, health foods, biomolecules, materials relevant to nanotechnology, and their use as bioremediators of contaminated waters [26.100]. The dinoflagellate *Karlodinium veneficum* synthesizes karlotoxins, a group of potent toxins that cause membrane permeabilization, having possible opportunities to construct new molecules to fight tumors and other human pathologies [26.101, 102].

26.4 Conclusions and Future Perspectives

Oceans are an enormous, untapped and sustainable source of biotechnological opportunities, yet are unexplored for the most part. Marine microbial diversity is almost unlimited, and offers a huge potential for biotechnological exploitations. Marine microbes may pose solutions to a variety of issues relevant for humans, by favoring the discovery of more efficacious drugs, the production of biofuel from sustainable sources, the isolation of enzymes that are useful for the industry, the synthesis of biopolymers and biodegradable plastics, the remediation of environmental pollution, and the development of a sustainable aquaculture. The combination of improved cultivation techniques with new culture-independent, *omics-driven* approaches (such as metagenomics, metatranscriptomics, metaproteomics, and metabolomics) will multiply the possibilities of successfully exploring the biotechnological potential of marine microbes, with the hope of solving important societal challenges of the twenty-first century.

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