

Review

Received 1 Nov 2011
Accepted 23 Dec 2011
Available online 5 Jun 2012

Keywords:

seaweeds
marine natural products
bioprospection

ISSN 0102-695X
<http://dx.doi.org/10.1590/S0102-695X2012005000077>

Bioprospecting for bioactives from seaweeds: potential, obstacles and alternatives

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Abstract: Seaweeds are potential sources of high biotechnological interest due to production of a great diversity of compounds exhibiting a broad spectrum of biological activities. On the other hand, there is an urgent need for management options for a sustainable approach to the use of marine organisms as a source of bioactive compounds. This review discusses the bioprospection for bioactive seaweed compounds as pharmaceuticals and antifouling agents, encompassing their potential and possible obstacles and alternatives. In spite of their potential, research on pharmaceuticals and antifouling agents from seaweeds includes mainly the search for molecules that exhibit these biological activities, but lacks of consideration of fundamental and limiting aspects such as the development of alternatives to sustainable supply. However, for the complete development of pharmaceuticals and antifouling compounds in Brazil, marine bioprospection should be more comprehensive, associating the search for molecules with an analysis of their supply. In this way, it is possible to promote sustainable development and conservation of biodiversity, as well as to assert the economic development of Brazil.

Introduction

Although the marine environment covers about 70% of the planet's surface and holds high biodiversity (Tittensor et al., 2010), very few species have been explored or used for biotechnological purposes. However, the "marine world" is endowed with diverse life forms that constitute a rich resource for the development of a wide array of applications (Demunshi & Chugh, 2010). For example, the marine environment is the source of structurally unique secondary metabolites produced by different organisms such as sponges, tunicates, bryozoans, soft corals, mollusks, microorganisms and seaweeds (Blunt et al., 2011). These metabolites have attracted the attention of chemists and biologists for the past five decades, because several of these chemicals exhibit pronounced pharmacological (e.g., Mayer et al., 2010) and antifouling activities (e.g., Hellio & Yebra, 2009). As such, they are interesting candidates for new drugs and antifouling paint development, respectively.

Secondary metabolites from terrestrial plants have been or are the basis of much of human disease therapy and are often the major component of modern pharmaceuticals. About 60% of the world's population relies almost entirely on plants for medication (Farnsworth, 1994), reflecting the important therapeutic contribution of natural products (Harvey, 2000). Marine natural products

or derivatives thereof are included in this context because they are currently in different phases of clinical trials (Molinski et al., 2009; Mayer et al., 2010). On the other hand, antifouling chemical defenses have long been acknowledged as a protection mechanism for marine organism (e.g. Davis et al., 1989; Steinberg et al., 2001). Today, however, antifouling marine secondary metabolites have been recognized as a promising alternative to commercial paints (e.g., Rittschof, 2000; Bhadury & Wright, 2004; Fusetani, 2011).

In the search for novel bioactive compounds for development of pharmaceutical drugs, many marine species have been collected in countries/regions worldwide such as North America (Harbor Branch Media Lab Project, 2008), Canada (CBIN, 2005), Scandinavia (Aldridge, 2006), Australia (AIMS, 2002), New Zealand (The Treaty of Waitangi, 2006), Fiji (Aalbersberg, 1997), and Brazil (Veiga-Santos et al., 2010). In this bioprospecting context, the search for antifouling compounds included several marine species from different countries/regions such as the Antarctic (Amsler et al., 2000), the Mediterranean (Faimali et al., 2003), the Caribbean (Kelly et al., 2003), the Red Sea (Kelman et al., 1998), Australia (Butler et al., 1996), China (Dobretsov et al., 2005), France (Hellio et al., 2000), Singapore (Koh et al., 2002), Palauan (Sera et al., 1999), Japan (Takada et al., 2001) and Brazil (Da

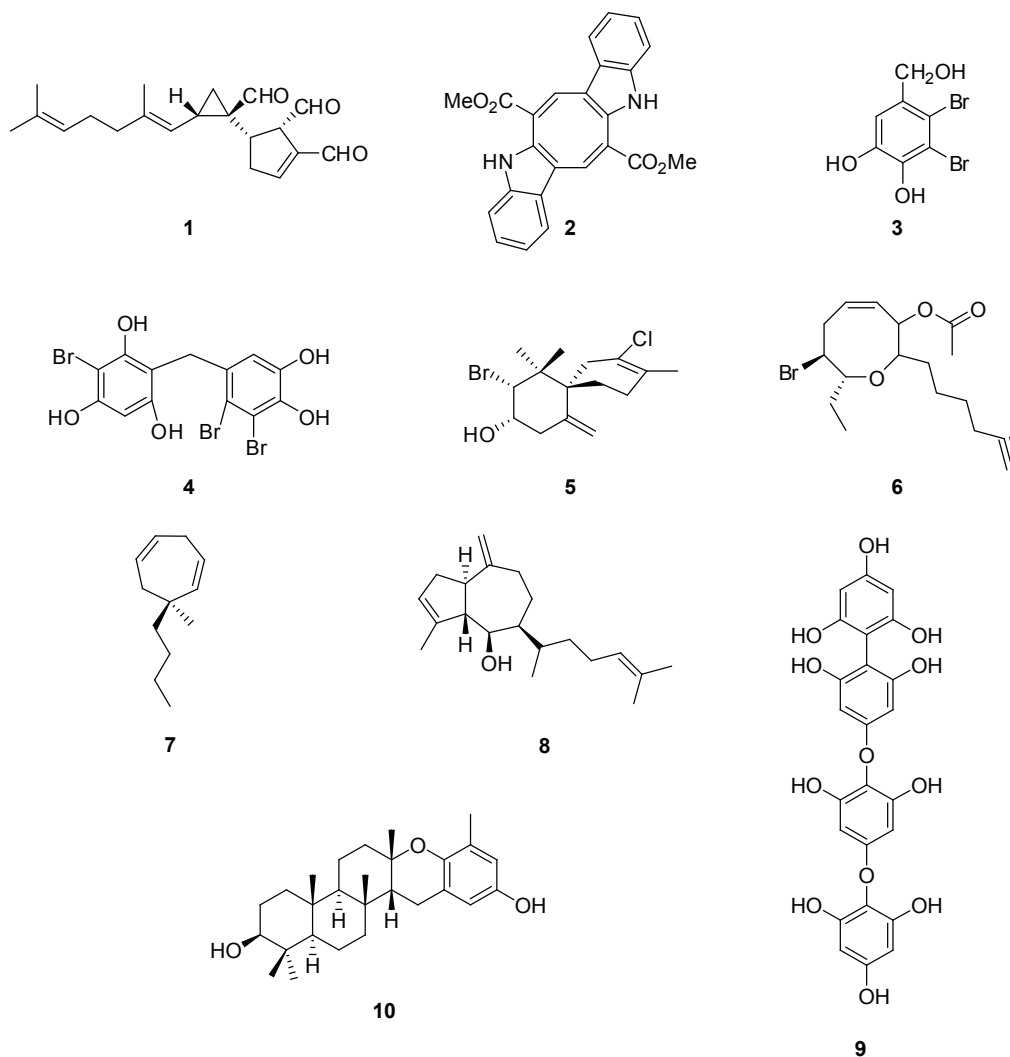
Gama et al., 2008).

Although seaweed exploitation accounts for a billionaire market of over US\$ 6 billion dollars/year (FAO, 2003; Smit, 2004), the development of seaweed secondary metabolites as therapeutic or antifouling agents is still in its embryonic stage. With a total annual use estimated at 8 million tons, seaweeds are the most abundant source of polysaccharides, including alginates, agar and carrageenans (Gomez D'Ayala et al., 2008; Laurienzo, 2010). The common uses are related to the food and cosmetic industries; however, biotechnological applications are rapidly expanding and hydrogels, for example, correspond to 10% of this market (Gomez D'Ayala et al., 2008; Laurienzo, 2010).

Screening for seaweed compounds with antifouling properties can provide effective, environmentally friendly alternatives to currently used antifouling paint booster biocides (Da Gama et al., 2002; Chambers et al., 2006; Fusetani, 2011), but few studies have explored this potential (Hellio & Yebra, 2009). In fact, the core limitations in the development of new

products from marine sources, including seaweeds, has changed throughout the years from the poor resolution of the chemical techniques used in the isolation and structural elucidation to the sustainability of the entire process (Hunt & Vincent, 2006; Bugni et al., 2008a, b; Molinski, 2010).

As one of the world's largest countries, Brazil possesses about 8000 km of coastline and a rich macroalgae flora that is potentially a producer of bioactive secondary metabolites with pharmaceutical (Veiga-Santos et al., 2010) or antifouling (Da Gama et al., 2008) properties. In addition, this biodiversity offers unexplored opportunities and challenges for the discovery of new genes, enzymes, and processes, which might be very useful in the bioprospecting context. This review provides a current view on the bioprospecting of bioactives from seaweeds, highlighting the main metabolites, the potential of the different compounds produced by these marine organisms, the obstacles to development and promising alternatives to obtain bioactive compounds on a large scale.



Seaweed chemicals

Several species of seaweed have the capacity to produce a diverse array of secondary metabolites, which exhibit important and vital ecological roles as defense and/or signal compounds (Amsler, 2008) and are also of biotechnological interest (Smit, 2004; Hellio & Yebra, 2009; Mayer et al., 2010). Examples of secondary metabolites produced by green seaweeds are halimedatriol (**1**) and caulerpenin (**2**); red seaweeds, lanosol (**4**), vidalol (**5**) and elatol (**6**); and brown seaweeds, dictyopterene C (**7**), pachydictyol A (**8**), fucodiphlorethol (**9**) and epitaondiol (**10**).

A quantitative analysis of the natural products of marine organisms demonstrated that more than 20000 compounds have been isolated and that the vast majority correspond to terpenoids and alkaloids from marine invertebrates (Hu et al., 2011; Blunt et al., 2011). Considering marine macroalgae, the number of isolated compounds corresponds to 10% of the total, mainly obtained from red and brown species. Halogenated compounds have received remarkable attention, mainly due to their strong bioactivity (Hu et al., 2011).

Brown seaweeds produce terpenoids, acetogenins, and terpenoid-aromatic compounds of mixed biosynthetic origin as their most common secondary metabolites (Blunt et al., 2011). In addition, these macroalgae also produce polyphenols (= phlorotannins), but in higher concentrations mainly in species from temperate (Toth & Pavia, 2006) and polar regions (Fairhead et al., 2006). Red species encompass more than 1.500 different compounds, belonging to all major classes of secondary metabolites (Maschek & Baker, 2008; Blunt et al., 2011). Among these chemicals, isoprenoid and acetogenin derivatives are recognized as the primary class of defensive metabolites in these algae (Harper et al., 2001). These red algae are also characterized by the production of an impressive array of halogenated compounds (Blunt et al., 2011). Green seaweeds, mainly the species of Bryopsidales are abundant and widely distributed in tropical seas and are known to produce sesquiterpenoid and diterpenoid compounds (Blunt et al., 2011).

Seaweeds are also a rich source of bioactive polysaccharides. As mentioned before, the main products of the seaweed industry are alginates, agar and carrageenans, which have the ability to increase the viscosity of aqueous solutions, to form gels of varying degrees of firmness, to form water-soluble films and to stabilize properties of water-based products (Draget et al., 2005). Throughout the years, these properties have also been explored by the biotechnology industry; tissue engineering and drug delivery are among the most promising applications for these polymers (Laurienzo, 2010). Additionally, sulfated polysaccharides from

algae have been associated with anticancer and antiviral activities (Smit, 2004; Pomin & Mourão, 2008; Vo & Kim, 2010; Jiao et al., 2011), increasing the possibilities of biomedical applications (Gomez D'Ayala et al., 2008; Laurienzo, 2010). These sulfated polysaccharides are structurally diverse and heterogeneous, which make their chemical characterization a real challenge. As an example, at least fifteen different carrageenan structures have been described (Lahaye, 2001; Jiao et al., 2011).

Potential of seaweeds-pharmaceuticals and antifouling agents

Prior to the 1950s, the pharmacological properties of chemicals from seaweeds were restricted to traditional and folk medicine (Lincoln et al., 1991). However, during the 1980s and 90s, secondary metabolites possessing biological or promising pharmacological activities were evidenced in several species of seaweeds (see Smit, 2004, for a review). Due to these important evidences, these algae were the source of about 35% of the newly discovered chemicals in the period 1977-1987, followed by marine invertebrates (Ireland et al., 1993). In recent years, marine invertebrates and microorganisms have been the most prolific source of secondary metabolites and have become the focus of great interest since they provide a large proportion of bioactive compounds exhibiting broad biological activities (Blunt et al., 2009). According to Imhoff et al. (2011), modern marine biotechnology has shifted its focus to marine microbes, using large-scale fermentation techniques to incorporate sustainability into the development of new pharmaceuticals. Moreover, a whole new realm of possibilities awaits, considering the barely known microbial biodiversity and the important role of secondary metabolites in mediating interactions between microorganisms and between microorganisms and their hosts (Hill & Fenical, 2010; Thomas et al., 2010; Waters et al., 2010).

There are several examples of pure compounds and crude extracts of seaweeds that exhibit biological activities. Here, we show examples of some of these activities, such as cytotoxicity, antiviral, antioxidant, antibacterial, neuromodulator, NAPDPH-dependent lipid peroxidation, antifungal, anthelmintic, anti-inflammatory, and anticancer activities (Table 1). Among the substances isolated from seaweeds that have received most attention from pharmaceutical companies for development of new drugs are the sulfated polysaccharides (anticoagulants, anticancer and antivirals) and the kahalalide F (anticancer and anti-aids compounds) (Smit, 2004; Vo & Kim, 2010; Jiao et al., 2011).

Table 1. Examples of bioactivity exhibited by chemicals produced by seaweeds.

Seaweed	Chemical	Activity	Reference
<i>Styopodium</i> sp.	stypoldione	cytotoxicity	Gerwick & Fenical, 1981
<i>Chondria</i> sp.	condriamide A	cytotoxicity	Palermo et al., 1992
<i>Chondria</i> sp.	condriamide A	against HSV type II	Palermo et al., 1992
<i>Eisenia bicyclis</i>	polyphenols	antioxidant	Shibata et al., 2002; Heo et al., 2005
<i>Gracilaria edulis</i>	crude extract	antibacterial	Vallinayagam et al., 2009
<i>Martensia fragilis</i>	alkaloids	antioxidant	Takamatsu et al., 2003
<i>Martensia fragilis</i>	alkaloids	NAPDPH-depending lipid peroxidation	Takahashi et al., 1997
<i>Rhodophyllis membranacea</i>	polyhalogenated indoles	antifungal	Brennan & Erickson, 1978
<i>Bryopsis</i> sp	kahalalide F	anticancer	Nuijen et al., 2000, Sparidans et al., 2001
<i>Chondria atropurpurea</i>	chondriamide B	anthelmintic	Davyt et al., 1998
<i>Fucus vesiculosus</i>	fucoïdan	anticoagulant	Bernardi & Springer, 1962
<i>Botryocladia occidentalis</i>	sulfated galactan	anticoagulant	Farias et al., 2000
<i>Sargassum vulgare</i>	alginate	anticancer	Sousa et al., 2007
<i>Kappaphycus striatum</i>	kappa-carrageenan	anticancer	Yuan et al., 2006
<i>Cladosiphon okamuranus</i>	fucan	antiviral	Hidari et al., 2008
<i>Schizymenia dubyi</i>	sulfated glucuronogalactan	antiviral	Bourgougnon et al., 1996
<i>Undaria pinnatifida</i>	crude extract	anti-inflammatory	Khan et al., 2008

Marine secondary metabolites are viewed as sources of antifouling technology due to their ability to inhibit attachment, settlement, or growth of several marine organisms (Hellio et al., 2001). For seaweeds, the antifouling potential of its chemicals is due to antibacterial or antifungal activity, to broad inhibition of the settlement of marine organisms, verified in field experiments, or to specific actions against the settlement of benthic organisms, such as the mussel *Perna perna*, the polychaete *Hydroides elegans*, the bryozoan *Bugula neritina*, and the crustacean *Balanus amphitrite* (Table 2). Due to these evidences, seaweed secondary metabolites and polysaccharides are viewed as the most environmentally friendly antifouling strategy, based on the characterization and development of chemicals used by marine organisms to maintain their body surfaces free of encrusting organisms (Fusetani, 2004; Hellio & Yebra, 2009; Qian et al., 2010).

The lists of chemicals exhibiting pharmaceutical (Table 1) and antifouling (Table 2) properties are no means exhaustive, but reveal that seaweed secondary metabolites possess noteworthy biological activities, with potential for application outside marine ecosystems as antifouling technology and in clinical use. Due to these few examples of broad biological activities, there is an interest among scientists and companies in the research and development of new bioactive agents from seaweeds. However, it is important to emphasize that drug or antifouling compound development is complicated, lengthy, and expensive.

Drug and antifouling discoveries

Both drug and antifouling discovery and development based on marine secondary metabolites require a very similar multidisciplinary approach. For example, both processes involve bioprospecting as the initial step, encompassing collection of samples of marine organisms. Of course, the key function of this first step is to provide some of the thousands of compounds that have interesting molecular structures and bioactivity (Kursar et al., 2007). In the next step, extracts from these organisms are prepared and subsequently tested for activities against various target diseases or micro- and/or macroorganism components of the fouling communities.

Usually, only a small number of extracts (*hits*) are registered for bioactivity. To develop a *hit* into a lead, a possible candidate for drug or antifouling, the *hit* extract needs to be purified to find the bioactive principle and to know its chemical or molecular structure. For example, 50000-100000 active compounds are necessary to generate a single *lead* (Hunt & Vincent, 2006). The lead compound will be subjected to a series of additional trials that represent a need for supply of sample material. For example, in the preclinical phase of drug discovery, at first efficacy and toxicity tests of the *lead* are established *in vivo* in animals, before tests proceed with humans. On the other hand, for antifoulants tests in the field would be necessary to evaluate the efficacy against several benthic organisms in the natural environment, incorporation in paints and toxicity tests. In the drug context, only one in about fifty preclinical leads will result in a marketable drug (Hunt & Vincent, 2006).

For drug discovery, approximately 15 years

Table 2. Examples of antifouling activities exhibited by chemicals produced by seaweeds.

Seaweed	Chemical	Activity	Reference
<i>Lobophora variegata</i>	lobophorolide	antifungal	Kubanek et al., 2003
<i>Canistrocarpus cervicornis</i>	diterpenes	against <i>Perna perna</i> settlement	Bianco et al., 2009
<i>Dictyota paffii</i>	diterpene	broad antifouling effect in the field	Barbosa et al., 2007
<i>Sargassum tenerrimum</i>	polyphenols	against <i>Hydroides elegans</i> settlement	Lau & Qian, 1997
<i>Plocamium brasiliense</i>	monoterpenes	against <i>Balanus amphitrite</i> settlement	Konig et al., 1999
<i>Laurencia obtusa</i>	crude extract	broad antifouling effect in the field	Da Gama et al., 2002
<i>Delisea pulchra</i>	furanones	against epibiosis	Dworjanyn et al., 1999
<i>Laurencia majuscula</i>	terpenoid	anti-bacteria	Vairappan et al., 2001
<i>Laurencia rigida</i>	elatol	against <i>Bugula neritina</i> settlement	Konig & Wright, 1997
<i>Bifuraria bifurcata</i>	diterpenes	broad antifouling effect	Hellio et al., 2001
<i>Ulva reticulata</i>	polysaccharides	against <i>Hydroides elegans</i> settlement	Harder et al., 2004

would be necessary, encompassing about thirteen years for research and clinical phases and two years for the administrative phase (Hunt & Vincent, 2006). In addition, the development of a new drug is expensive, costing US\$ 900 million (Hunt & Vincent, 2006). This estimative is not known for antifouling development, but it is possible to presume that there are similar aspects, both in terms of time and economic costs.

Supply problem and alternatives

Efforts at the discovery of drugs from the sea during the last forty years have yielded very few marine-derived compounds for the treatment of human diseases (Hill & Fenical, 2010), encompassing only vidarabine (Ara-A), cytarabine (Ara-C), both used clinically since 1970s, and, most recently, ziconitide (Prialt®), trabectedin (Yondelis®) and the halichondrin B synthetic derivative eribulin mesylate (Halaven™). Of course, only five drugs is disappointing given the high expectations for acquisition of drugs from the sea. The fact that very few marine natural products can be found on the shelves of pharmacies certainly is not due to a limited biological activity or low chemical diversity produced by marine organisms. For example, as stated above, seaweeds produce several secondary metabolites, many of them active as promising pharmaceuticals. In fact, the supply of material is a serious obstacle to the development of most marine secondary metabolites that are currently undergoing evaluation and trials (Munro et al., 1999; Waters et al., 2010). Since 1995, there has been a decrease in the interest in the search for new secondary metabolites from marine organisms, probably due to problems of supply.

The search for antifouling is only in the beginning when compared to pharmaceuticals, but it is possible to expect that supply is or will be the limitation for the development of an antifouling technology using

marine secondary metabolites.

In general, the supply problem has limited the investigation of bioactive marine secondary metabolites and providing a sufficient amount of these chemicals may be a difficult task (Sukarmi & Sabdono, 2008). Besides the small amount of compound found in the producing organism, or the limited quantity of the organism itself, the concentration of these chemicals can vary according to biotic and/or abiotic conditions (Sudatti et al., 2011). For example, in seaweeds, these compounds occur in relatively low concentrations, ranging from 0.2% to 2% of the algal dry mass, although compounds such as the polyphenols in brown seaweeds can occur at concentrations as high as 15% of the algal dry mass (Hay & Fenical, 1988; Steinberg, 1992), also including compounds possessing high bioactivity.

Concerning polysaccharides, the problem of supply is attenuated since most of these molecules present a considerable yield compared to secondary metabolites. Carrageenan, for example, can account for 13-40% of the seaweed dry weight (Reani et al., 1998; Ramalingam et al., 2003). However, the standardization of a commercial product based on algal polysaccharides is still a challenge due to their structural diversity and heterogeneity, as well as to the great variability among different species, or even in the same species collected at different sites or in different seasons (Jiao et al., 2011).

The problem of supply is better known for marine invertebrates. For example, many pharmacologically interesting secondary metabolite candidates for clinical trials, such as ecteinascidin 743 isolated from the ascidian *Ecteinascidia trubinata*, bryostatins from the bryozoan *Bugula neritina*, or the halichondrins from the sponge *Lissodendoryx*, can be isolated only in minute yields. To use halochondrins for the treatment of cancer, it has been estimated that the annual need of 1-5 kg per year would require that

about 3000-16000 metric tons of sponge biomass be collected and processed annually (Proksch et al., 2003). According to these perspectives, destruction of the environment and/or local extinction of species would or will be inevitable consequences.

Due to difficulty of gaining access to large tracts of biodiversity in natural environments, several alternatives or techniques have been developed to obtain secondary metabolites in non-natural ways. Besides offering an environmentally compatible and secure supply of naturally occurring metabolites for drugs or antifouling development, such technologies can be also used to produce more diverse chemicals. At first, both synthesis and culturing of marine organisms could reduce demand on wild sources and help to solve the problems of supply and impact on these natural resources. However, other alternatives or new experimental approaches, such as genetic recombinant techniques, chemoenzymatic synthesis, and *in vitro* tissue/cell culture have been mentioned as a possible solution to future supply problems (Cragg & Newman, 2001; Pomponi, 1999; Hunt & Vincent, 2006).

Synthetic production and/or manipulation are the most common alternative sources of bioactive compounds, including most of the marine drugs in clinical development today. Some compounds can be completely synthesized, whereas others may have their structure modified to increase their potency and/or reduce toxicity. For example, semisynthetic routes have been developed to obtain ET-743, based on the precursor safracin B from the bacteria *Pseudomonas fluorescens* (Cuevas et al., 2000), halichondrin B (Hart et al., 2000), and kalahide F (Lopez-Macia et al., 2001) to supply these anti-cancer compounds found in the ascidia *Ecteinascidia turbinata* (Rinehart et al., 1990), the sponge *Lissodendoryx* sp (Hart et al., 2000), and the green seaweed *Bryopsis* sp. (Newman & Cragg, 2004), respectively.

Synthesis can be also employed to supply bioactive chemicals from seaweeds, but this approach needs to be considered in the context of economic viability. For example, the synthesis of the known antifouling compound elatol, found in low amounts in species of the red seaweed *Laurencia* (e.g., Sudatti et al., 2006) was obtained after several steps (White et al., 2008). Other examples could be listed, but most of the synthesis of bioactive marine secondary metabolites involve several steps and are not worried about economic or commercial viability.

With advances in combinatorial chemistry techniques, marine natural products can become platforms for further drug development (Ganesan, 2001). From a basic structure (scaffold) or pharmacophoric unit of the natural product, it is possible to build a diverse compound library around it, with the hope of discovering more active

(and less toxic) analogs and derivatives (Cragg & Newman, 2001; Ganesan, 2001; Rouhi, 2003). Thus, for example, an analog of ET-743, called phthalascidin (PT650), that is more potent, structurally simpler, easier to synthesize, and more stable was produced in this way (Cuevas et al., 2000). The basic structure of the natural product psammaphlin A found in the marine sponge *Psammaphysilla* sp. was used to create a library of 3828 different compounds, six of which displayed antibacterial activity (Newman & Cragg, 2004). Synthesis of furanones, analogs to the molecules found in the red seaweed *Delisea pulchra* (e.g., De Nys et al., 1993), was employed to develop a library of compounds exhibiting antifouling (see De Nys et al., 2006, for a review) and pharmacological (Lattmann et al., 1999) properties. These and other libraries can improve synthetic routes to the original natural product (Ganesan, 2001) and/or structural variants of the synthetic version not previously possible (Rouhi, 2003).

Aquaculture trials have been carried out to supply bioactive compounds from marine invertebrates such as the bryozoan *Bugula neritina*, the ascidian *E. turbinata* and the sponges *Lissodendoryx* sp., and *Acanthella cavernosa*. Most of these have succeeded in producing organisms with economically viable concentrations of bioactive compounds (Hart et al., 2000; Mendola, 2000, 2003). For seaweeds, efforts were undertaken mainly to develop cultures of species that produce colloids, such as alginates, carrageen, and agar (Lüning & Pang, 2003), but very little is known about cultivation of seaweeds that produce secondary metabolites (but see Sudatti et al., 2011). However, the production of secondary metabolites in plants is generally a plastic trait that can be influenced by a number of biotic and abiotic extrinsic factors (e.g. Pavia & Brock, 2000), but this aspect is also very poorly explored for seaweeds (e.g. Sudatti et al., 2011).

Tissue cultures have been also used to obtain adequate quantities of commercially valuable chemicals, to produce compounds for screening (Stafford et al., 1998) or, by addition of chemicals, elicit the expression of different metabolic pathways (Gleba et al., 1999). However, these alternatives are better known for compounds from plants (see Bourgaud et al., 2001 for review). However, as known for seaweeds that are producers of colloids (see Hayashi et al., 2008), this technique could be a promising way to obtain bioactive secondary metabolites from seaweeds.

Another potential alternative for supply is to remove the appropriate genetic material that codes for bioactive secondary metabolites and incorporate it into a microorganism. For example, by introducing combinations of genes not found in nature into a productive strain of *Streptomyces*, a range of novel chemicals can be produced for screening (Katz & McDaniel, 1999; McDaniel et al., 1995). Heterologous expression of genes encoding bioactive compounds

may also be a promising alternative to the supply problem. For example, the development of bryostatins for biomedical and biotechnological applications included the cultivation of the bacterial symbiont and heterologous expression of the biosynthesis gene of this molecule (Trindade-Silva et al., 2010). Of course, these procedures could be useful for supplying bioactive compounds from seaweeds and other marine organisms.

Culturing of microorganisms (*e.g.*, bacteria) may have a considerable potential for producing a sustainable supply of bioactive marine natural products (Thomas et al., 2010), because many of the compounds found in marine invertebrates organisms may be of microbial origin (Jensen & Fenical, 2000). In fact, microbial symbionts are often suggested to be the real producers of the secondary metabolites found in marine invertebrates, usually based on the structural similarities to known microbial compounds (Trindade-Silva et al., 2010). For example, the structurally and biosynthetically complex bryostatins found in the bryozoan *B. nertitina* are typical bacterial secondary metabolites and are probably produced by the symbiotic bacterium 'Candidatus *Endobugula sertula*' (Lopaniuk et al., 2004; Davidson et al., 2001). To culture a sponge-associated symbiont remains very difficult because of the difficulty of isolating and maintaining a pure cultivation, but initial successes have been obtained (Li et al., 2007; Muscholl-Silberhorn et al., 2007).

Perspectives

On the basis of available information, seaweeds constitute a potential resource of bioactive secondary metabolites for bioprospecting. However, also according to available information, it is possible to verify that bioprospecting for bioactive secondary metabolites from seaweeds is a very complex process, including sample collection, activity detection, identification of leads, and development of preclinical and clinical trials in the context of pharmaceuticals. This type of marine bioprospecting also includes and/or is limited by the supply of molecules for both the clinical trials and commercial scale-up. However, today bioprospecting for these molecules is almost synonymous with screening, because most of the studies provide information on the biological activities of the compounds and, sometimes, on the mechanism of action for molecules that can be promising in the context of pharmaceuticals or antifouling. Despite the high potential of marine secondary metabolites as pharmaceuticals and antifouling agents, few sustainable production methods have been developed to supply these chemicals.

Given the concern related to the sustainability

of drug discovery based on marine natural products, current research trends include the search for reliable sources of molecules in associated microorganisms, encompassing endophytic fungi, as well as the use of a multidisciplinary approach linking molecular biology to natural products discovery (Tharkur et al., 2008; Hill & Fenical, 2010). While genome mining of gene clusters related to the biosynthesis of molecules increase the possibilities of finding new carbon skeletons, it also creates the knowledge to expedite the use of heterologous expression in natural and "unnatural" product biosynthesis (Lane & Moore, 2011).

Recently, the Brazilian government has included marine biotechnology as a strategic research field for the development of the country, aiming at the sustainable exploitation of the so-called "Blue Amazon", which includes the total area of 3.5 million km² of the Brazilian exclusive economic zone in the Atlantic Ocean with its unprecedented associated biodiversity. Brazilian bioprospecting is only in the beginning, but this is the crucial moment to think about better strategies. There are good reasons to believe that many species of Brazilian seaweeds have unique characteristics that can be exploited to create the basis for new products and processes, such as novel antifouling agents (*e.g.*, Da Gama et al., 2008) and pharmaceuticals (Veiga-Santos et al., 2010). However, as verified worldwide, supply is a serious obstacle today and a limiting factor for the development of pharmaceuticals using marine secondary metabolites. Indeed, supply problems have contributed to the disappointing fact that, after forty years, only few marine-derived compounds are in use for treatment of human diseases.

In general, Brazilian seaweeds are small and probably will fail to furnish large biomass for the sustainable development of antifouling compounds or pharmaceuticals. On the other hand, the risk of over-exploration grows if a seaweed bioactive compound is structurally complex, highly potent, and/or its mechanism of action is a novelty. After detection of a promising lead by screening, it would then be necessary to solve the supply problem in order to reduce the impact on the natural populations and the development of antifouling and pharmaceutical technologies. In fact, contemporary bioprospecting has multiple goals, encompassing conservation of biodiversity, the sustainable management of natural resources and economic development (Beattie et al., 2010).

A bioprospecting program based on the sustainable use of marine biodiversity has attracted much attention and this emerging approach is especially important to be developed in Brazil, not only to reap economic benefits but also to promote the protection and conservation of marine biodiversity. In other words, how can secondary metabolite bioprospecting

be implemented in such a way as to promote sustainable use and the protection and conservation of marine biodiversity, as well as contribute to economic development to Brazil?

Acknowledgments

The authors thank: Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro, Financiadora de Estudos e Projetos and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, who have long been sponsors of our work on marine chemical ecology and bioactivity of marine natural products.

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