



Marine Biotechnology in Brazil: Recent Developments and Its Potential for Innovation

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Marine biotechnology is an emerging field in Brazil and includes the exploration of marine microbial products, aquaculture, omics, isolation of biologically active compounds, identification of biosynthetic gene clusters from symbiotic microorganisms, investigation of invertebrate diseases caused by potentially pathogenic marine microbes, and development of antifouling compounds. Furthermore, the field also encompasses description of new biological niches, current threats, preservation strategies as well as its biotechnological potential. Finally, it is important to depict some of the major approaches and tools being employed to such end. To address the challenges of marine biotechnology, the Brazilian government, through the Ministry of Science, Technology, Innovation, and Communication, has established the National Research Network in Marine Biotechnology (BiotecMar) (www.biotecmar.sage.coppe.ufrj.br). Its main objective is to harness marine biodiversity and develop the marine bioeconomy through innovative research.

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INTRODUCTION

Throughout history, biotechnology has had an undeniable impact on all aspects of human life, from food and energy production, healing organisms, and ecosystems. With the same aims, marine biotechnology has emerged as a new area of biotechnological discovery. For developing countries, its potential rewards are attractive in the face of global economic challenges, but it can be difficult to overcome inherent limitations (Thompson et al., 2017). Thus, developing countries should carefully evaluate their capabilities and challenges before investing in marine biotechnology.

Marine biotechnology in Brazil began in the early 1970s with studies of shrimp farming in the State of Rio Grande do Norte. In the 1980s, marine biotechnology expanded to study

(i) secondary metabolites of sponges, seaweeds, and corals (Kelecom et al., 1980; Solé-Cava et al., 1981; Teixeira et al., 1985), (ii) sulfated glycans in *Ascidia* (Albano and Mourão, 1986), (iii) algae farming (Pace et al., 1986; Yoneshigue-Valentin and Oliveira, 1987), and (iv) proteins from marine bacteria (Colepicolo et al., 1989). In the 1990 and 2000s, marine biotechnology in Brazil also included metabolite isolation from marine invertebrates and elucidation of the chemical structures of bioactive compounds (e.g., guanidine alkaloids) from marine organisms (Berlinck et al., 1996; Costa et al., 1996; Chegade et al., 1997), and mariculture (Thompson et al., 1999; Wasielewski et al., 2006; da Silva et al., 2013; Ferreira et al., 2016). In the twenty-first century, Brazilian researchers have explored marine microbial products, including the isolation of biologically active compounds, identification of biosynthetic gene clusters from symbiotic microorganisms, developed diagnostic tools to investigate invertebrate diseases caused by potentially pathogenic marine microbes (Hernandez et al., 2000, 2004; Pimenta et al., 2010; Romminger et al., 2012; Trindade-Silva et al., 2012; Ferreira et al., 2016; Nicacio et al., 2017), and evaluated antifouling compounds derived from seaweed (Da Gama et al., 2002), and anti-HIV molecules derived from marine brown alga (Stephens et al., 2017). Some relevant actions in Marine biotechnology in Brazil are summarized in **Figure 1** and **Table 1**.

BRAZILIAN MARINE BIODIVERSITY AND THE GREAT AMAZON REEF SYSTEM

Biodiversity is the basis for marine biotechnology and is a potential asset for the bioeconomy. Despite the rich endemic biodiversity of Brazil, a significant portion remains unexplored. Marine biomes range from deep sea, oceanic islands, and reefs, such as the new reef ecosystem that was recently described at the mouth of the Amazon River, with a size of $\sim 56,000$ Km², representing the largest reef system in Brazil (Moura et al., 2016; Francini-Filho et al., 2018). This striking finding was reported by more than 400 EU, Asian, and US newspapers/media outlets, and an overview of the project expedition can be found¹.

Prior to the BiotecMar marine biodiversity studies, the Parcel do Manuel Luís Marine State Park (off the state of Maranhão) was thought to be the northern limit for shallow-water coral, with 20 coral species identified. Since then, Cordeiro et al. (2015) recorded a total of 38 coral species that are sources of interesting bioactive compounds near the mouth of the Amazon River. In addition, a recent study demonstrated the presence of a new clade of Brazilian *Arenosciera*-like Haplosclerida sponges with high biotechnological potential and proposed the new genus *Arenospicula* (Niphatidae) in the new Amazon reef biome (Leal et al., 2017). Carbonate organisms inhabiting these regions have been targeted as nutrient sources for soil fertilizers (Cavalcanti et al., 2014), and organisms such as sponges, tunicates, and molluscs are being evaluated for bone tissue

¹<http://www.cbsnews.com/pictures/amazon-coral-reef/5/>;
http://www.lemonde.fr/biodiversite/video/2017/02/03/plongee-sur-un-reef-coralien-au-large-du-bresil_5074389_1652692.html;
<https://youtu.be/vjA3fm4jFV0>

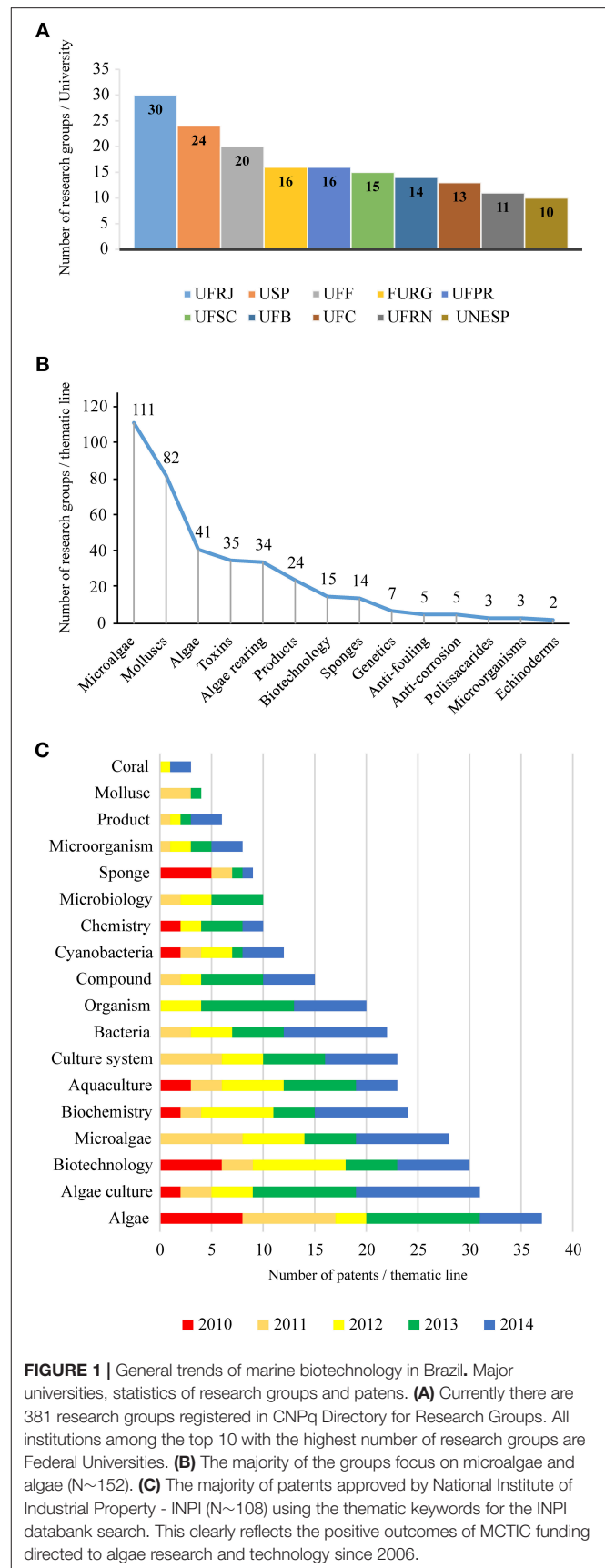


FIGURE 1 | General trends of marine biotechnology in Brazil. Major universities, statistics of research groups and patents. **(A)** Currently there are 381 research groups registered in CNPq Directory for Research Groups. All institutions among the top 10 with the highest number of research groups are Federal Universities. **(B)** The majority of the groups focus on microalgae and algae ($N \sim 152$). **(C)** The majority of patents approved by National Institute of Industrial Property - INPI ($N \sim 108$) using the thematic keywords for the INPI databank search. This clearly reflects the positive outcomes of MCTIC funding directed to algae research and technology since 2006.

TABLE 1 | Recent highlights of marine biotechnology in Brazil.

Target	Contribution	Reference
Microbial dark matter	Viromes	Gregoracci et al., 2015; Coutinho et al., 2017
Regulatory lncRNAs	Coral bleaching and gene expression	Huang et al., 2017
Bioactive peptides and proteins	Pharmacological compounds and conservation	Huang et al., 2016
Genes with biotechnological application	Oxidosqualene/terpene cyclases	Oliveira et al., 2015
Molecules with biotechnological application	Cycloartenol, elatol	Oliveira et al., 2015
New insights about microorganisms	<i>Endozoicomonas</i> spp. <i>Synechococcus</i> spp.	Moreira et al., 2014; Appolinario et al., 2016; Coutinho et al., 2016
Newly discovered Biomes	Great Amazon Reef	Cordeiro et al., 2015; Moura et al., 2016
Theories postulated	Piggyback-the-winner	Knowles et al., 2016
Cyberinfrastructure	BaMBa	Meirelles et al., 2015a
Graduate programs	Masters and PhD programs in Marine Biotechnology	
Patents registered	Antifouling (elatol)/synthesis of glycerophospholipids, antimalarial, method of extraction of heparin from molluscs Toxin production from fermentation process for marine strain <i>Bacillus thuringiensis</i> BCLTRH-01 with potential larvicide against <i>Aedes aegypti</i> (dengue vector) Dermatan sulfate from <i>Ascidia</i> with potent anticoagulant action	Bastos et al., 2011; PI 1101139-4 A2 Melo, 2017; BR 10 2017 018249 5 Mourão et al., 1998; PI9805128
Aquaculture systems	Bioflocs, biofilms, integrated systems	Abreu et al., 2007; Krummenauer et al., 2011; Marques et al., 2016b

engineering (Granito et al., 2017), polybrominated diphenyl ether biosynthesis (Agarwal et al., 2017), anti-cancer (Jimenez et al., 2012), and even heparin production (Gomes et al., 2015; Tovar et al., 2016). Sponges are also a rich source of novel microorganisms (Hardoim et al., 2009). Taken together, these studies show that reef organisms represent a rich source of new molecules (Supplementary Figure 1).

Analysis of microbial dark matter of the Amazon continuum dataset demonstrates that viral and microbial communities contribute to a globally relevant carbon sink system in the Amazon River plume (Silva et al., 2017). A recent study that aimed to characterize the diversity of viruses in the river and ocean identified 82,546 viral proteins, with 15 complete new viral genomes, and revealed a predominance of cyanophages in the plume, making an important contribution to current knowledge of the global ocean virome (Coutinho et al., 2017).

Despite its importance for biodiversity, the entire region is under pressure of development by large international oil and gas companies. Mesophotic reefs worldwide are new frontiers for oil and mining exploration (Clark et al., 2012), which threaten marine biodiversity and the environment as a whole: oil spills can destroy entire ecosystems and modify its associated microbiota (Gomes et al., 2010; Santos et al., 2011), and greenhouse gas emissions lead to global warming and ocean acidification. For example, in 2010, 3.19 million barrels of oil were released from the Macondo well in the Gulf of Mexico. The undeniable impact of industrial activities on environmental goods and services must be considered by developing countries that wish to develop capabilities in marine biotechnology (<http://www.marinebiotech.eu/sites/marinebiotech.eu/files/public/library/MBT%20publications/2010%20ESF%20Position%20Paper.pdf>). Thus, the Amazon reef system region needs a national spatial plan for the conservation and use of biological and mineral resources.

Efforts of the Brazilian government to consolidate knowledge of Brazil's biodiversity, include (i) joining the Global Biodiversity Information Facility (GBIF) as an associate member to improve access to biodiversity data; (ii) creating a national database of biodiversity, carried out by the Brazilian Ministry of Science, Technology Innovation, and communications, named "Sistema de Informação sobre a Biodiversidade Brasileira" (Information System of Brazilian Biodiversity; <http://www.sibbr.gov.br/>), and (iii) establishing a dedicated database for marine environmental datasets (BaMBa, <https://marinebiodiversity.lncc.br/bamba/>) (Meirelles et al., 2015a). However, despite its importance, Brazilian marine diversity is not yet thoroughly known, and the recent discovery of the Great Amazon Reef is a great example.

RECENT SCIENTIFIC DEVELOPMENTS IN BIOPROSPECTING

Numerous academic and technological developments have been achieved in the last decade (Table 1). Notable examples include the development of antithrombotic and antimetastatic drugs from marine organisms (Kozłowski et al., 2011; Gomes et al., 2015; Mourão, 2015; Tovar et al., 2016), elucidation of the antimalarial effect of marine sulfated polysaccharides (Marques et al., 2016a), enzymatic (proteolytic and phospholipase A2), inhibitory (metallo, cysteine and serine proteases), and hemagglutinating activities were determined from zoanthids (Guarnieri et al., 2018) and isolation of an antibiofilm dipeptide from marine fungi (Scopel et al., 2013). Microbial isolates from sponges having antimicrobial activity against important human pathogens have also been disclosed (Rua et al., 2014; Appolinario et al., 2016; Laport et al., 2017). Brazilian industry is becoming aware of the great potential of marine biodiversity to yield useful products.

BIOTECHNOLOGY TOOLS FOR REEF SYSTEM MODELING AND CONSERVATION

One relevant aspect of marine biotechnology relates to system management, which includes modeling and conservation. In Abrolhos reefs, anthropogenic disturbances cause phase shifts within dominant communities of fleshy organisms (Silveira et al., 2017). Metagenomics approaches provide the means to investigate how microbes and interactions within the benthic holobionts (e.g., corals, sponges, algae) contribute to carbon flow in reefs and how ecosystem-level microbial features respond to benthic species during phase shifts (i.e., community composition, biomass, metabolism, and viral predation). Shifts from coral to algal dominance correlate with fish biomass loss and increased microbial metabolism in coral reefs. Understanding the mechanisms underlying these shifts is key to preserving biodiversity and moving toward its sustainable use.

OMICS TECHNOLOGIES ARE POWERFUL TOOLS FOR HARNESSING MARINE BIODIVERSITY

The coral genus *Mussismilia* is a major reef builder of the Abrolhos Bank; thus, preserving *Mussismilia* corals is essential to maintaining the coral seed bank in Abrolhos. A metaproteomic study evaluating biomarkers of healthy and diseased coral showed that healthy *Mussismilia* corals possess a set of proteins that may serve as markers for holobiont homeostasis (e.g., tubulin, histone, ribosomal proteins). Cnidaria proteins found in healthy *M. braziliensis* are associated with cnidarian-*Symbiodinium* endosymbiosis and include chaperones, structural and membrane modeling proteins (e.g., actin), and proteins related to intracellular vesicular traffic (Rab7 and ADP-ribosylation factor 1) and signal transduction (14-3-3 protein and calmodulin). *Mussismilia* corals with white plague syndrome are associated with facultative/anaerobic sulfate-reducing bacteria (i.e., Enterobacteriales, Alteromonadales, Clostridiales, and Bacteroidetes), whereas healthy corals are associated with aerobic nitrogen-fixing bacteria (i.e., Rhizobiales, Sphingomonadales and Actinomycetales). Hsp60, hsp90, and adenosylhomocysteinase proteins are produced mainly by cyanobacteria in corals with black band disease, which is consistent with the elevated oxidative stress observed in hydrogen sulfide- and cyanotoxin-rich environments.

In the coral genus *Palythoa*, results of 16S rRNA pyrosequencing demonstrated lower bacterial diversity in disturbed Ponta Verde coral reefs compared to Sereia reefs (both located in the waters off the Brazilian state of Maceió). Differential expression of long non-coding (lnc) RNAs in two species of these zoanthids (*Palythoa caribaeorum* and *Protospalythoa variabilis*) was observed in response to bleaching (Huang et al., 2017). Further analysis of lncRNA expression in *Palythoa* undergoing bleaching and *Palythoa* from healthy colonies implicated specific lncRNA sequences in the posttranscriptional regulation of Ras-mediated signal transduction, which is involved in cell adhesion, as well as

components of the innate immune system (microbial recognition and defense) (Huang et al., 2017). Transcriptome analysis of *Protospalythoa* has revealed sequences encoding bioactive peptides, including precursors of proteins from different pharmacological classes such as neuropeptides, hemostatic and hemorrhagic proteins, membrane-active (pore-forming) proteins, and protease inhibitors (Huang et al., 2016). These studies demonstrate the value of -omics technologies to assess the health status and biotechnological potential of threatened reef systems.

Turf algae have also been shown to be involved in phase-shifting Abrolhos reefs (Francini-Filho et al., 2013). Reefs with higher turf cover have lower coral cover, indicating a negative effect of turf on corals (e.g., via toxin and H₂S production). However, the genomic repertoire of turfs is poorly understood. In a recent metagenomics study, it was shown that the Abrolhos turf microbiome consists primarily of Proteobacteria (~40%), Cyanobacteria (~35%), and Bacteroidetes (~10%) (Walter et al., 2016). In addition, turf microbes are a rich source of secondary metabolites. Ongoing genomic studies of cyanobacteria isolated from turfs have identified diverse gene clusters responsible for secondary metabolite production.

METABOLOMICS APPLIED TO MARINE BIOTECHNOLOGY

Metabolomics refers to the systematic study of the global metabolite profile of a whole organism or a biological system (e.g., cells, tissues, biofluids). Metabolomics aims to identify and quantify endogenous and exogenous low-molecular-weight metabolites (<1,000 Da) and to understand metabolite fluxes into the cell in response to certain environmental conditions. For example, the seaweed *Laurencia* produces a variety of secondary metabolites that are of interest in biotechnology. Transcriptomic analysis of *Laurencia* has revealed a repertoire of genes related to the production of diverse terpenes (Oliveira et al., 2012, 2015), including elatol. Although the size of this seaweed and its slow growth have been barriers to large-scale elatol production, heterologous production offers an avenue for industrial production, as demonstrated by the production of cycloartenol from *Laurencia* using a simple yeast fermentation method (Calegario et al., 2016). Metabolomics is also useful to understand the effects of pollution and xenobiotics, such as natural and synthetic estrogens, which can act as endocrine disruptors and are widespread in sewage discharges, rivers, lakes, and coastal seawater. This approach may be used to assess the health status of holobionts and entire marine systems, such as reef systems.

TOWARD A SUSTAINABLE AQUACULTURE

Aquaculture production has surpassed fishery production around the world, with an annual growth of 5.9% in the last decade (FAO, 2016a). In this scenario, Brazil aquaculture, with an average annual growth of 8.6% (FAO, 2017), has

been a major highlight, being in the top 15-world aquaculture producers. Between 2000 and 2015 Brazil has produced more than 200 thousand tones of marine and freshwater organisms annually, generating 73 thousand direct and indirect jobs (FAO, 2016b, 2017). However, most of this aquaculture activity is developed in extensive or semi-intensive production systems that occupy large areas and produce nutrient-rich effluents, which promote eutrophication being, therefore, highly criticized. Recently, these impacts have been lessened by biotechnological processes based on microorganisms present in biofilm (Abreu et al., 2007) and bioflocs (Krummenauer et al., 2011), as well through the use of integrated aquaculture systems (Marques et al., 2016b). In Brazil, improved biofloc technology allows the super-intensive production of shrimp (>300/m²) with little or no water renewal. This is possible because microbes (bacteria and protozoa) are used to recycle nutrients and provide an important complementary food source for the shrimp (Waselesky et al., 2006). Further development of biofilm and biofloc technology is most dependent of a proper identification of bacteria using molecular biology techniques (Del'Duca et al., 2013) and a better understanding of the environmental factors that affect biofloc and biofilm formation, especially the water turbulence (Lara et al., 2017).

Aquaculture can also serve as a reliable and sustainable source of bioproducts. For instance, large-scale microalgae production may represent an important feedstock for biodiesel production, and new microalgae culture systems (Roselet et al., 2013) and biodiesel production methods based on transesterification of microalgae lipids (Lemoes et al., 2016) have recently been developed in Brazil. Large-scale microalgae culture can also be used for effluent cleaning. Arriada and Abreu (2014) demonstrated that the marine microalga *Nannochloropsis oculata* can be cultivated in produced water, which consists of effluent extracted along with petroleum that contains inorganic salts and aliphatic and aromatic hydrocarbons. Besides cleaning the effluent, the produced biomass can be used as feedstock for bioproducts such as lipids, carbohydrates, pigments, and protein.

BIOMEDICAL DRUGS INNOVATION AND THE (LENGTHY) PATH TO THE CLINIC

Putting a new drug on the market is a lengthy endeavor. In general, it takes 2–4 decades for a new marine compound to enter the market. Therefore, Brazil has established a government program to promote marine biomedicine, which will be based on multidisciplinary collaborations and multi-institutional partnerships. Drug discovery research groups must collaborate with biomedical researchers to strengthen *in vitro* studies, validate target discoveries, and establish proof of principle. Academic research studies or by specialized research centers (e.g., <http://ciemp.org.br/en/home/>; http://www.butantan.gov.br/Paginas/default_en.aspx; <http://cevap.org.br/>) must generate sufficient quantities of the candidate drug under good laboratory practice conditions before translating biomedical research into clinical advances. Subsequently, nonclinical studies are needed to elucidate the metabolism, pharmacokinetics, safety, and dosage

of the new drug. The components of this complex preclinical pathway have not yet been consolidated in Brazil, which is the main reason most biomedical research conducted in the country does not enter the initial clinical phases.

THE BIOTECMAR NETWORK

Funding for the development of marine biotechnology in Brazil has been provided by federal and state governments. The Ministry of Science, Technology, Innovation and Communication (MCTIC), Ministry of Health (MS), and the National Council for Scientific and Technological Development (CNPq) have supported marine biotechnology by means of public research calls in the last decade. To address the challenges described above, the Brazilian government, through MCTIC, has established the National Research Network in Marine Biotechnology (BiotecMar) to foster the Brazilian bioeconomy (www.biotecmar.sage.coppe.ufrj.br). This national network was foreseen in the IX Sectoral Plan for Resources of the Sea of the Interministerial Commission for the Resources of the Sea (<https://www.marinha.mil.br/secirm/psrm>) and was a natural development to address local and global challenges in marine bioeconomy. Its main objective is to promote innovative research in the areas of biodiversity, microbiology, bioprospecting, genomics, post-genomics (-omics), structural elucidation, large-scale production, sustainability analysis, technical and economic feasibility, and transfer to the production sector. The network's mission is to move Brazil closer to developed nations in terms of research, marine technology, and marine bioeconomy over a 10-year horizon.

Networking is of paramount importance to (i) establish multidisciplinary specialized teams, (ii) integrate laboratories with complementary skills; (iii) carry out extensive geographic surveys in the yet poorly understood Brazilian marine environment (4.5 million km²); (iv) promote more harmonious and broader development of all geographical regions of the country through research, development, and innovation, and (v) build agreements and cooperation with the production sector and governmental regulatory agencies (e.g., ministries) to accelerate concerted action to expand the marine bioeconomy. To achieve this mission, the activities of the BiotecMar network include (i) research, development and innovation, (ii) development of state-of-the-art technology and quality services, (iii) human resources training, (iv) subsidies to government agencies for the elaboration of public policies and research funding, and (v) assistance in the elaboration and execution of international collaboration programs. The current activities of the BiotecMar Network are carried out in strategic areas that bring together the researchers and infrastructure of nine laboratories (Biodiversity, Omics and Bioinformatics, Prospecting of Drugs and Nutraceuticals, Anti-fouling, Aquaculture Production, Renewable Energy, Generation of Bioprocesses, Sustainability, and Bioassays) in different regions of Brazil (<http://www.biotecmar.sage.coppe.ufrj.br>). Establishing a world-class network requires sustained efforts to map the skills and infrastructure of laboratories and equipment

across the country and abroad, identify gaps in the infrastructure and competencies of the various laboratories, and integrate them into the network.

In an ever-growing complex market, it is important to identify the demands of users in the public and private sectors and identify ways to meet these demands through the best technologies and channels of communication with entrepreneurs. One example is the establishment of biobanks of molecules, extracts, and microbes for use by industry. Another recent development was the establishment of the first graduate program in Marine Biotechnology (Master and Ph.D.) in 2016 with the participation of scientists from the Instituto de Estudos do Mar Almirante Paulo Moreira (IEAPM), Federal Fluminense University (UFF), and Federal University of Rio de Janeiro (UFRJ). Most members of this multi-institutional and multidisciplinary program are also BiotecMar network members.

CONCLUDING REMARKS

It is clear that concerted efforts are required to develop marine biotechnology in Brazil; they should include the efforts of highly equipped and well-trained international teams that bring together academia, industry, and government. This integrative approach requires a joint plan and strategy to meet the expectations and challenges of developing a bioeconomy in this century. In addition to funding, government will likely play a key role in the development of marine biotechnology through unifying academic institutions toward common goals, promoting interaction between academia and private sector counterparts, passing legislation for access to sites, and exploring marine biological diversity.

BRAZILIAN MARINE BIOTECHNOLOGY NETWORK

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SUPPLEMENTARY MATERIAL

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