

Review

BIOMEDICAL POTENTIAL OF MARINE CYANOBACTERIA

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ABSTRACT

Cyanobacteria, in particular those found living in the ocean, are emerging as an important source of unique bioactive secondary metabolites. A plethora of natural products, mostly nitrogen-containing molecules, have been reported with majority belonging to the polyketide synthase (PKS) and/or non-ribosomal polypeptide synthetase (NRPS) structural class. Curacin A and the dolastatins are examples of important marine cyanobacterial metabolites possessing exquisite anticancer properties. Genetic studies on the biosynthetic capacity of these marine microalgae revealed many novel biochemical features pertaining to the enzymology of secondary metabolism. Biodiscovery of marine cyanobacteria for new therapeutic agents as well as harnessing its biosynthetic gene clusters represent an exciting and fruitful area of research in marine biotechnology.

Keywords: Biomedical, marine, cyanobacteria

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INTRODUCTION

Nature has been an important source of new pharmaceuticals in the treatment of various human diseases. For instance about 60% and 75% of current drugs used in cancer and infectious diseases, respectively, derived from natural origin (Newman et al, 1997). Natural products research has traditionally been dominated by terrestrial organisms, such as plants and microbes. This is not surprising due to the long history of human reliance on nature, especially plants for food and cures for human ailments. However, the rate of discovery of novel compounds as potential drugs from terrestrial organisms in recent years is decreasing. This is compounded by the urgent need for new therapeutics, especially antibiotics, driven largely by the emergence of multi-drug-resistant pathogens. Moreover, recent

innovations such as combinatorial chemistry have met with limited success with regards to the development of new drugs. Therefore, it is critical that new sources of novel pharmaceuticals be uncovered and one of the solutions is natural products from marine organisms.

The history of marine natural products started in the early 1950s and in spite of its short history, a number of notable biomolecules have been reported and they are currently in either preclinical or clinical testing (Newman and Cragg, 2004). These include ecteinascidin 743 (Yondelis™), bryostatin 1, and aplidine as potential anticancer drugs. Some of these marine natural products are also potential lead compounds for synthesis of more potent analogs. To date, more than 10,000 marine-

derived compounds have been isolated and this is coming from less than 1% of the total marine biodiversity. The range of marine organisms tapped for their natural products production includes sponges, tunicates, bryozoans, nudibranchs, and gorgonians. Of these marine organisms, one particular group that is emerging as a source of important bioactive compounds is the marine blue-green algae or cyanobacteria (Gerwick et al, 2001).

Considered to be an ancient group of microorganisms with fossil records dating back 3.5 billion years ago, these prokaryotic marine cyanobacteria are ubiquitous in nature and are found in wide ranging niches. Although they are microscopic, certain filamentous marine species are rather conspicuous occurring as extensive mats along tropical coastal areas. Some unicellular species can also be found living in symbiotic relationships with other marine invertebrates such as tunicates and sponges. It is well documented that certain strains e.g. *Lyngbya majuscula* and *Microcystis aeruginosa*, produced cyanotoxins that have implications in human health. These include the dermatotoxin, lyngbyatoxin A and the hepatotoxins, microcystins and nodularins (Carmichael, 1992). In addition of these cyanotoxins, a wide range of useful secondary metabolites have also been reported and this review highlights the chemistry, biology as well as the biosyntheses of some of these unique marine cyanobacterial natural products.

MARINE CYANOBACTERIAL NATURAL PRODUCTS

Two main research groups, Professor Richard Moore's research group at the University of Hawaii and Professor William Gerwick's research group at University of California at San Diego, contributed significantly to the knowledge of natural products chemistry of marine cyanobacteria (Gerwick et al, 2001; Moore, 1996). Majority of their cyanobacterial collections centered on reef systems in Hawaii, Guam, the Caribbean, Madagascar, and Papua New Guinea. From their extensive studies, it has been shown that cyanobacterial species, especially those belonging to the benthic genus *Lyngbya* are a prolific source of unique bioactive natural molecules (Gerwick et al, 2001). The circumtropical *Lyngbya* species are widespread in distribution and are considered to be one of the most abundant filamentous marine cyanobacteria. For instance, one particular species, *L. majuscula*, yielded over 30% of all marine cyanobacterial metabolites, reflecting its impressive biosynthetic capacity with regards to natural products production. Certain strains of *L. majuscula* are also deemed as "super" producer of secondary metabolites, represented by different structural classes (Gerwick et al, 2001). An example is *L. majuscula* strain 19L (Figure 1) collected from the Curaçao where at least six compounds, curacin A (antimitotic), barbamide (molluscicidal), malyngamide H (brine shrimp toxic), antillatoxin (neurotoxin and ichthyotoxic), carmabins A and B, have been reported, each with unique biological properties.



Figure 1. *Lyngbya majuscula* (19L)
(Picture provided by Dr. William Gerwick)

Currently, there are well over 200 secondary metabolites, mostly nitrogen-containing molecules, being reported from marine cyanobacteria. These natural products represent great structural diversity, belonging to the polyketide synthase (PKS), non-ribosomal polypeptide synthetase (NRPS), as well as hybrid PKS-NRPS structural classes (Gerwick et al, 2001). Certain signature structural motifs can also be observed amongst these diverse cyanobacterial molecules. There is a high preponderance of compounds containing amino or hydroxy acids (e.g. the wewakpeptins in Figure 3), pyrrolidone rings (e.g. jamaicamide A in Figure 3) and heterocyclic moieties such as thiazoline, thiazole, oxazoline, and oxazole flanked by amino or hydroxy acids (e.g. dolastatin 10 in Figure 2). In addition, majority of the PKS-derived amino or hydroxy acid moieties are found in cyclic peptides and they possess a methyl (e.g. hectochlorin in Figure 3) or dimethyl group (e.g. lyngbyabellin A and wewakpeptins in Figure 3) at the C position.

A number of important marine cyanobacterial molecules, including dolastatin 10, cryptophycins and curacin A, have been discovered and these were either

in preclinical or clinical testing as anticancer agents (Newman and Cragg, 2004). Marine cyanobacterial molecules with potent biological activities are also lead compounds for the development of synthetic analogs having increased potency and decreased toxicity (Figure 2). For instance, the potent antimitotic agent, curacin A (Figure 2), was initially isolated from a field collection of the marine cyanobacterium, *Lyngbya majuscula*, obtained from Curaçao (Gerwick et al, 1994). Since its discovery curacin A has served as a lead compound for the development of synthetic analogs which are more soluble (Wipf et al, 2004). Another important class of molecules is the dolastatins which were initially isolated from the sea hare *Dolabella auricularia*. However, recent studies have revealed the dietary origin of these potent molecules from marine cyanobacteria. Two synthetic analogs, cematodin (a water soluble analog of dolastatin 15) and TZT-1027 (dolastatin 10 analog) are currently being evaluated in phase II clinical trials as potential drugs for treatment of advanced solid tumors (Figure 2) (Newman and Cragg, 2004).

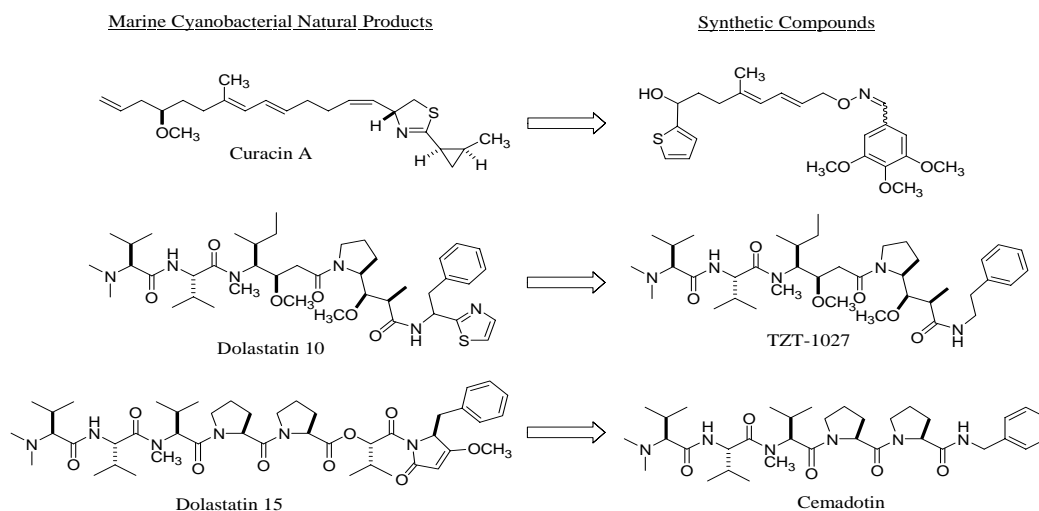


Figure 2. Marine cyanobacterial metabolites as lead compounds.

Recent literature on natural products from marine cyanobacteria indicated that these microorganisms continue to be impressive with reports of novel chemical structures along with useful biological properties. Despite wide ranging biological activities reported for these compounds, several main pharmacological trends are emerging. Some marine cyanobacterial molecules are found to target either the polymerization of actin (e.g. hectochlorin) or tubulin (e.g. curacin A and dolastatin 10). In addition, a number of neurotoxins act as either blockers (e.g. jamaicamides and kalkitoxin) or activators (antillatoxin) of the mammalian voltage-gated sodium channel (Figure 3) (Gerwick et al, 2001).

A recent example of a potent promoter of actin assembly is hectochlorin, originally isolated from *Lyngbya majuscula* procured from Hector Bay, Jamaica and Boca del Drago Beach, Panama (Figure 3) (Marquez et al, 2002). Structurally it is very similar to another group of marine cyanobacterial compounds the lyngbyabellins (Figure 3) and dolabellin, a seahare-derived molecule. The unique feature of this molecule is that the molecule is composed of carbonyl ester linkages unlike most other compounds which consist of a mixture of carbonyl amide and carbonyl

ester bonds. Hectochlorin was tested in the National Cancer Institute and found to have pronounced antiproliferative activities against colon, melanoma, ovarian, and renal panels with an average GI_{50} against the 60 cell lines of 5.1 μ M. In addition, hectochlorin possess significant antifungal activity against *Candida albicans* (Marquez et al, 2002).

The lipopeptide, kalkitoxin is a potent neurotoxin initially isolated from *Lyngbya majuscula* from the Caribbean (Wu et al, 2000). The neurotoxic nature of this important molecule was first detected from fish toxicity assay. Since its discovery, a number of research groups have successfully reported its total synthesis. Preliminary research revealed that this molecule is more potent than saxitoxin in blocking the voltage sensitive Na^+ channel in mouse neuro-2a cells with EC_{50} of 1 nM,. Due to availability of synthetic material, kalkitoxin was further evaluated for its interaction with voltage-sensitive sodium channels in cerebellar granule neurons (CGN) and was found to have significant antagonistic effect on veratridine-induced cytotoxicity and Ca^{2+} influx in CGN. These series of biological data on kalkitoxin suggest the importance of this molecule as possible lead compound for the development of analgesic as well as neuroprotection drugs (LePage et al, 2005).

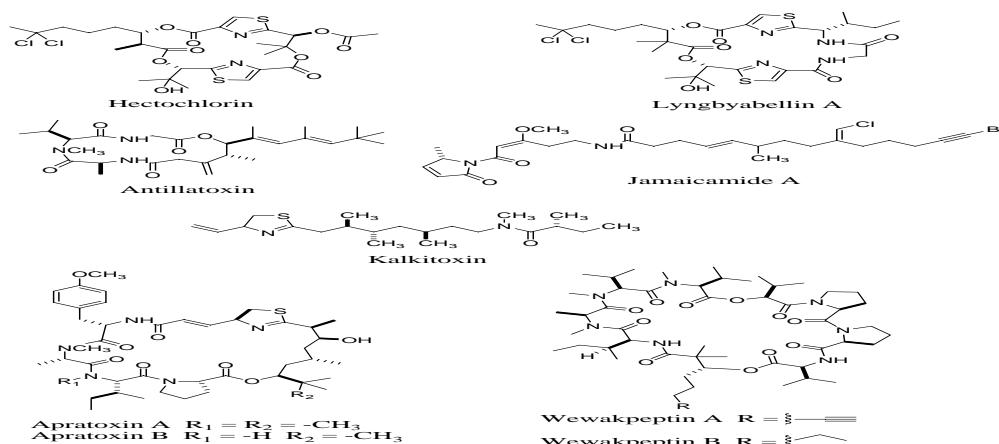


Figure 3. Selected examples of bioactive marine cyanobacterial molecules.

Other noteworthy marine cyanobacterial molecules reported recently in the literature having significant biological activities include the apratoxins (Luesch et al, 2001) (cytotoxic agents), the lyngbyabellins (Milligan et al, 2000) (cytotoxic and antifungal), and the wewakpeptins (Han et al, 2005) (Figure 3). Some of these molecules possess exquisite biological properties usually in the low micromolar or nanomolar range. For instance, apratoxin A, first isolated from *Lyngbya majuscula* found at Apra Harbor, Guam, is a potent cytotoxin with a unique carbon skeleton. It possesses an impressive biological profile in in vitro cytotoxicity against various human tumor cell lines with IC_{50} ranging from 0.36 to 0.52 nM. Its complete structure elucidation involved extensive NMR spectroscopy experiments as well as chemical manipulations. Total synthesis of apratoxin A has been accomplished leading the way to synthetic analogs for the purpose of new therapeutics (Chen and Forsyth, 2004).

BIOSYNTHESIS OF MARINE CYANOBACTERIAL NATURAL PRODUCTS

As evident from above examples, most of these marine cyanobacterial molecules are biosynthesized by large multi-modular enzymatic systems coded by polyketide synthase (PKS)-non-ribosomal polypeptide synthetase (NRPS) gene clusters. To date only three putative biosynthetic PKS-NRPS gene clusters of marine cyanobacterial molecules have been reported including barbamide, curacin A, and jamaicamides (Chang et al, 2002, 2004; Edwards et al, 2004). Despite just three examples, its genetic studies revealed high novelty and complexity of the gene clusters. The first complete biosynthetic gene cluster reported was barbamide, a molluscicidal compound containing a unique trichloroleucine unit (Figure 4) (Chang et al, 2002).

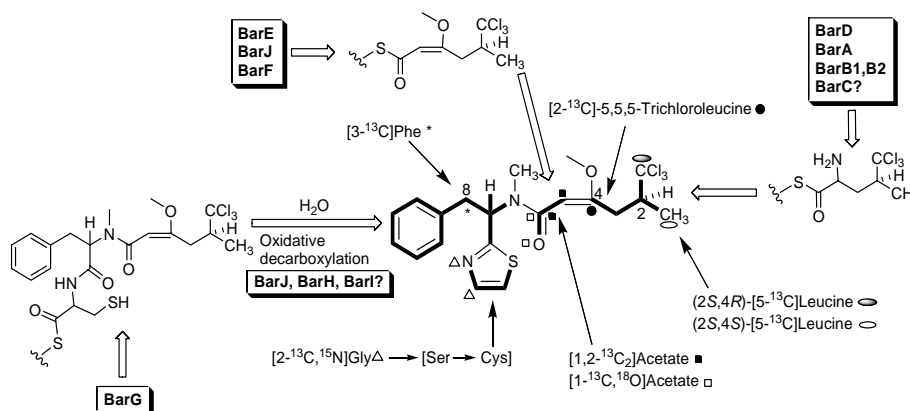


Figure 4. Biosynthetic studies and the involvement of *bar* gene clusters in the assembly of barbamide.

The putative identification of *bar* gene cluster was aided by extensive biosynthetic feeding experiments which suggested a mixed PKS-NRPS assembly of the molecule (Figure 4). A number of notable biosynthetic features include biochemical

mechanisms for chlorination of the starter unit, leucine, the formation of thiazole moiety through oxidative decarboxylation, and the formation of *E*-double bond in barbamide (Figure 4) (Chang et al, 2002). Genetic studies on other molecules such as curacin

a and the jamaicamides have also revealed some of these features are summarized in high degree of biochemical novelty and Table 1.

Table 1. Notable biochemical features of the biosynthetic gene clusters of marine cyanobacterial molecules.

Marine Cyanobacterial Molecules	Gene Size	Notable Biochemical Features
Barbamide (Figure 4)	26 kb	1. Unique chlorination of leucine 2. <i>E</i> -double bond formation between C4-C5 3. Carbon truncation during chain elongation 4. Thiazole ring formation
Curacin A (Figure 2)	64 kb	1. Involvement of HMG-CoA in formation of cyclopropyl ring 2. Unique chain termination involving product release and terminal dehydrative decarboxylation yielding a methylene group
Jamaicamides (Figure 3)	58 kb	1. Tight integration of mixed PKS/NRPS pathway 2. Involvement of HMG-CoA in formation of vinyl/vinyl chloride group 3. Incorporation of unique alkynyl starter unit 4. Unique chain termination yielding pyrrolinone ring

One interesting aspects of the biosynthetic gene clusters identified from marine cyanobacteria is the absence of self-resistance, regulatory, or transport genes. These are usually found in biosynthetic gene clusters of actinomycetes (Edwards et al, 2004). In addition to the mixed PKS-NRPS structural types, recent chemical studies have shown that marine cyanobacteria are capable of producing a wide range of other non PKS-NRPS molecules. This is indicated by the recent report of swinholide A, a macrolide, from *Symploca* cf. sp. and *Geitlerinema* sp. collected from the Fiji Islands and Nosy Mitso-ankaraha Island, Madagascar, respectively (Andrianasolo et al, 2005).

CONCLUDING REMARKS

Marine cyanobacteria would probably ranked along side the actinomycetes and myxobacteria as a prolific producer of unique natural products. Natural products research on these ancient microorganisms is still in its infancy despite the seemingly

numerous publications on its chemistry. Majority of the research papers is dominated by cyanobacterial collections from reef systems in Hawaii, the Caribbean, Madagascar, and Papua New Guinea. However, nothing is known on the chemistry of marine cyanobacteria from other parts of the world, such as South East Asia where biodiversity is high. For instance, preliminary field surveys at just two southern islands, St. John's Island and Pulau Hantu in Singapore, indicated presence of abundant *Lyngbya* species (author's unpublished data). Field collections of these microorganisms were initiated and their organic extracts indicated significant biological activity based on brine shrimp (*Artemia salina*) lethality assay (author's unpublished data). Thus it is believed that many more unique marine cyanobacterial biomolecules will be uncovered from other geographically unexplored areas.

Results from marine cyanobacterial biosynthetic gene clusters studies provided important insights to understanding the molecular mechanisms of secondary metabolism in these microorganisms. A

number of biochemical novelties have been revealed, including genes coding for unique tailoring enzymes for the final production of natural products. Identification of putative modular gene cluster systems set the stage for future work in combinatorial biosynthesis in producing “unnatural” natural products through modular swapping as well as heterologous expression of important cyanobacterial biomolecules using *Escherichia coli* or *Streptomyces lividens* model. The latter approach would be useful especially from marine cyanobacterial strains that are difficult to culture or slow growing. In conclusion natural products research coupled with genetic studies on the biosynthesis of unique metabolites in marine cyanobacteria represent an exciting and fruitful enterprise in the area of marine biotechnology.

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