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Review

CHEMICAL ECOLOGY OF MARINE CYANOBACTERIAL SECONDARY METABOLITES: A MINI-REVIEW

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ABSTRACT

More than 300 nitrogen-containing secondary metabolites have been reported from the procaryotic marine cyanobacteria. A majority of these compounds are of the polypeptide or mixed polyketide-polypeptide structural class and they are a potential source of novel pharmaceuticals. In spite of the chemical richness of marine cyanobacteria, not much is known regarding their ecological functions. To date only a handful of marine cyanobacterial compounds have been examined for their involvement in predator-prey interactions. This mini-review surveys the various chemical ecology studies conducted on marine cyanobacterial compounds. From these ecological studies, many marine cyanobacterial compounds are known to deter feeding by several species of marine predators. Such chemical defense may be crucial in maintaining the population of marine cyanobacterial bloom in nature. In addition, a series of ecological studies from our laboratory revealed the anti-settlement properties of a number of benthic marine cyanobacterial compounds. These studies suggested marine cyanobacteria as a potential source of natural antifoulants for the control of fouling organisms.

Key words**:** Marine cyanobacteria, marine chemical ecology, antifoulants, marine biofouling

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INTRODUCTION

The procaryotic marine cyanobacteria are known to be prolific producers of bioactive secondary metabolites (Gerwick *et. al.*, 2001; Tan, 2007). More than 300 nitrogen-containing natural products have been reported in the literature and a majority of these compounds belongs to either the polypeptide or the mixed polyketide-polypeptide structural class. Furthermore, many of these compounds possess important biological activities, ranging from antimitotic to antimicrobial, making them a good source of potential pharmaceutical agents. For instance the cytotoxic marine cyanobacterial compound, dolastatin 10, and its synthetic derivatives (e.g. TZT-1027) have gone through clinical testing as anticancer drugs. More recent examples of potent compounds include apratoxin A

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(Luesch *et al.*, 2001), largazole (Taori *et al.*, 2008), and hantupeptin A (Tripathi *et al.*, 2009).

In spite of the rich chemical diversity of marine cyanobacterial natural products, very little is known regarding their ecological functions. Certain benthic marine cyanobacterial strains are known to produce structurally diverse natural products in different concentrations. However, not much is known regarding their roles they play in maintaining the cyanobacterial population in nature. Early chemical ecology research on marine cyanobacterial compounds focused on their antifeedant properties against various reef predators such as herbivorous fishes. It was only recently that research is focusing on the ecological functions of compounds produced by marine cyanobacterial blooms.

This mini review serves to summarize the various chemical ecology studies conducted on marine cyanobacterial compounds. It is not meant to be comprehensive but to highlight important studies pertaining to the chemical ecology of marine cyanobacteria. We have recently initiated research on the chemical ecology of benthic marine cyanobacteria at our laboratory. In particular, we are interested in the anti-settlement properties of cyanobacterial molecules and preliminary data from such studies will be presented in this review article. Biofouling is considered to be a nuisance when they occur on submerged man-made structures such as ships' hulls, pipelines, and off-shore platforms. Significant economic losses can be caused by the build-up of fouling organisms resulting in reduction of fuel efficiency in maritime ships and clogging of submerged water pipes. Currently, the only effective way to control biofouling is the use of paint coating containing toxic chemicals such as organotin and copper. However, these compounds are undesirable due to their high toxicity to marine life and persistence in nature, resulting in the banning of their use in many countries (Sonak *et al.*, 2009). Research on chemical ecology often time could lead to potential applications and this review article will include a discussion regarding the use of marine cyanobacterial compounds as potential antifoulants in controlling marine biofouling.

Chemical ecology of marine cyanobacterial compounds

Antifeedant properties

Early chemical ecological studies focused on the palatability of marine cyanobacterial compounds on a variety of marine predators. These early investigations revealed the feeding deterrent properties of malyngolide (**Fig. 1**) to juvenile rabbitfishes *Siganus argenteus* and *Scarus spinus* and parrotfish *Scarus schlegeli*, malyngamides and majusculamides (**Fig. 1**) to various herbivorous fishes, and laxaphycin A (**Fig. 1**) to *S. schlegeli*, sea urchin *Diadema savignyi*, and the crab *Leptodius* sp. (Pennings *et al.*, 1997; Thacker *et al.*, 1997). The production of marine cyanobacterial natural products with antifeedant properties has been suggested to ensure the survival of these microalgae in reef environment.

Several studies have shown that the occurrence of marine cyanobacterial blooms could be mediated by the production of compounds as chemical defense against reef predators. A bioassay-guided fractionation of an organic extract prepared from the marine cyanobacterium, *Schizothrix calcicola*, provided the compound, ypaoamide (**Fig. 1**), as a feeding deterrent molecule (Nagle and Paul, 1998). Tested at natural concentrations, ypaoamide prevented feeding by two species of rabbitfishes, *Siganus spinus* and *S. argenteus*, the parrotfish, *S. schlegeli*, and the sea urchin, *Echinometra mathaei*.

From a persistent strain of the benthic marine cyanobacterium, *Lyngbya majuscula*, collected from Piti Bomb Holes in Guam, the major compound, pitipeptolide A (**Fig. 1**), was

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found to confer chemical defense against various generalized and specialized grazers (Cruz-Rivera and Paul, 2007). At natural concentrations, pitipeptolide A was deterrent to the sea urchin, *Echinometra mathaei*, the majid crab, *Menaethiusmonoceros* sp., and the amphipods, *Parhyalehawaiensis* sp. and *Cymadusa imbroglio*. However, the compound did not prevent feeding by the sea hare, *Stylocheilus striatus*, which is a specialist feeder on cyanobacteria.

A broader study investigating the chemical defense of the marine cyanobacterium, *Lyngbya majuscula*, from Moreton Bay, Australia, against sympatric and non-sympatric consumers were recently conducted by Capper *et al.* (2006). In this study, the palatability of the *L. majuscula* derived crude extract known to contain the toxins, lyngbyatoxin A and debromoaplysiatoxin (**Fig. 1**), were presented to various marine predators from Guam and Moreton Bay. The study revealed the extract prevented feeding of a variety of marine consumers from Guam and Moreton Bay. These consumers included the amphipods *Parhyale hawaiensis* and *Cymadusa imbroglio*, the majid crab *Menaethius monoceros*, and the sea urchin *Echinometra mathaei* from Guam and the cephalaspidean *Diniatys dentifer* and the rabbitfish *Siganus fuscescens* from Moreton Bay.

Larval recruitment

The effects of the benthic cyanobacterium, *Lyngbya majuscula*, on larval recruitment of two species of reef corals, *Acropora surculosa* and *Pocillopora damicornis*, were recently investigated by Kuffner and Paul (2004). Using cyanobacterial tufts, the results showed the microalga prevented larval recruitments of *A. surculosa* and *P. damicornis* in field tests. The unprecedented nature of this research suggested the influence of cyanobacterial blooms on coral larval recruitment. Such interactions could potentially be an important factor in the management of coral reef ecosystems.

Sequestration of marine cyanobacterial compounds by invertebrates

Certain marine invertebrates such as the opisthobranch sea hare, *Stylocheilus longicauda*, sequester marine cyanobacterial compounds for chemical defense against their predators. Studies have shown that dietary preference of sea hare could be affected by the different concentrations of certain chemicals produced by the marine cyanobacterium, *Lyngbya majuscula*. Marine cyanobacterial compounds, such as microcolin B (**Fig. 1**), ypaoamide, and malyngolide, can deter feeding by *S. longicauda* when tested at ecological concentrations (Nagle *et al.*, 1998). However, the molluscicide, barbamide (**Fig. 1**), was found to stimulate sea hare feeding (Nagle *et al.*, 1998).

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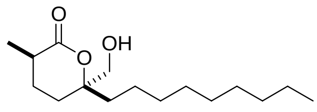
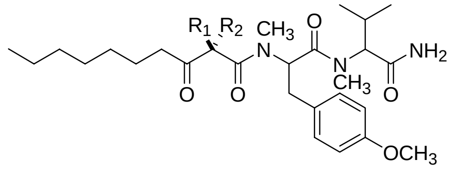
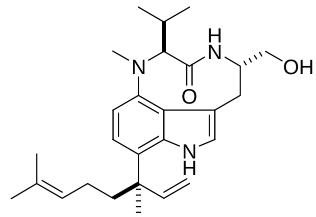


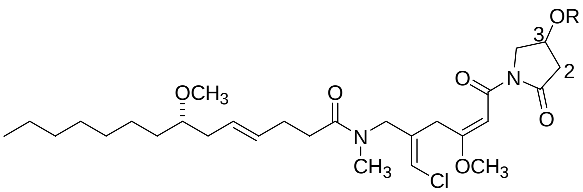
Malyngolide



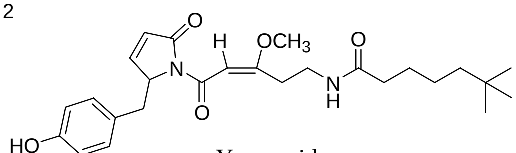
Majusculamide A: $R_1 = CH_3$, R_2 = H
Majusculamide B: R_1 = H, $R_2 = CH_3$



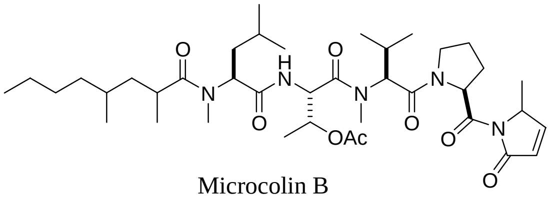
Lyngbyatoxin A



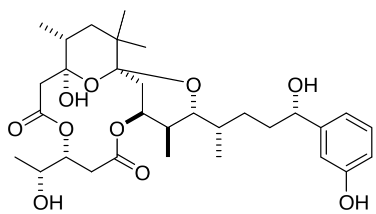
Malyngamide A: R = CH_3 (C_2-C_3 unsaturated)
Malyngamide B: R = H (C_2-C_3 saturated)



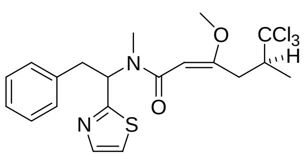
Ypaoamide



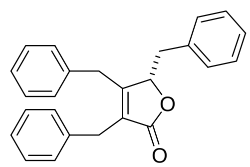
Microcolin B



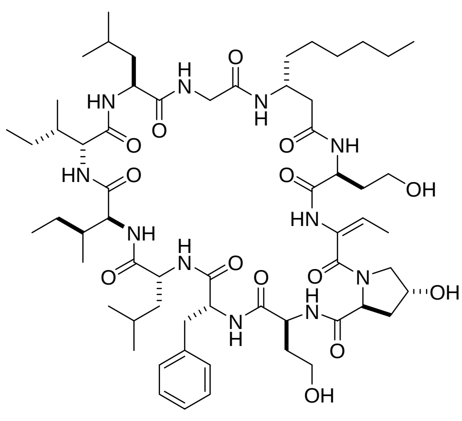
Debromoaplysiatoxin



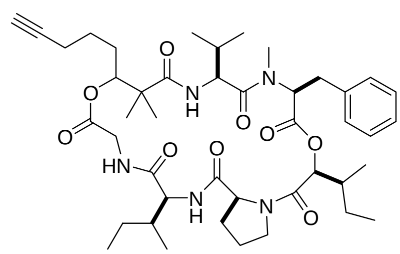
Barbamide



Maculalactone A



Laxaphycin A



Pitipeptolide A

Fig. 1. Chemical structures of selected marine cyanobacterial natural products where chemical ecological studies have been conducted on.

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Antifouling properties

Research on the antifouling nature of benthic marine cyanobacterial compounds is currently underexplored. To date only one marine cyanobacterial compound, maculalactone A (**Fig. 1**), was investigated for its antifouling property (Brown *et al.*, 2004). Maculalactone A belongs to the tribenzylbutyrolactone class of compounds and is produced by the marine cyanobacterium, *Kyrtuthrix maculans*. *Kyrtuthrix maculans*, a heterocystous cyanobacterium, occurs as a blackish-green layer of crust on exposed rocky shores in tropical to temperate regions. *In vivo* testing of maculalactone A revealed its toxicity against various species of barnacle larvae, such as *Tetraclita japonica*, *Balanus amphitrite*, and *Ibla cumingii* (Brown *et al.*, 2004). However, these studies did not investigate specifically the anti-settlement properties of maculalactone A. It might therefore be a misnomer to label the compound as having true antifouling property.

Anti-settlement properties of marine cyanobacterial compounds: Preliminary studies

Certain strains of benthic marine cyanobacteria are known to be prolific producers of natural products belonging primarily to the polyketide-polypeptide structural class. These compounds are found in different quantities and are usually analogs of each other differing only in the amino acid sequence or the carbon chain length of the polyketide moiety. The fact that such strains are able to produce a variety of similar compounds emphasizes the versatility of their biosynthetic gene clusters. In our quest for drug discovery from marine cyanobacteria in Singapore, we came across a persistent strain of the benthic marine cyanobacterium, *Lyngbya majuscula*, at the western lagoon of Pulau Hantu. To date we were able to purify and identify at least 14 natural products from this particular strain of marine cyanobacterium. A number of these compounds are highly cytotoxic, e.g. hantupeptins, and their structural elucidations have been published in the scientific literature (Tan *et al.*, 2008; Tripathi *et al.*, 2009).

One of the questions we like to address is why is this particular strain of marine cyanobacterium producing these compounds? To be more specific, what are their ecological functions and the roles they play in maintaining the marine cyanobacterial population in nature? This marine cyanobacterial strain also produces majusculamides which are known to possess antifeedant properties against herbivorous fishes from previous ecological studies. Perhaps other molecules might play different defensive roles in maintaining the marine cyanobacterial population. We were particularly interested in finding out if these compounds possess antifouling properties. This is due in part to the lack of research in exploring the anti-settlement properties of marine cyanobacterial extracts/compounds (Dahms *et al.*, 2006). To test this hypothesis, samples of this marine cyanobacterium, along with other marine organisms, were collected (six replicates of 35mL each for each marine species) and their organic extracts prepared using organic solvents. Using the field assay method originally developed by Henrikson and Pawlik (1995) and later modified by Pereira *et al.* (2002), these extracts were incorporated into Phytagel^{TM} in petri dishes and placed in the field for 4 weeks. Mean area coverage of biofilm, invertebrates, and algae were measured each week. In addition, the number of barnacles settling on the treated Phytagels^{TM} and control (without extracts) were counted over the same time period. Preliminary data suggested that the marine cyanobacterial extract possess anti-settlement activities (unpublished data). For instance, on

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week 4, extracts from *L. majuscula* had significantly less barnacle counts compared to control (**Fig. 2**).

Encouraged by the data from field assay, we continued to purify compounds from the marine cyanobacterium and tested them for anti-fouling activities based the anti-settlement assay using cyprids of the barnacle *Amphibalanus amphitrite*. Results from the anti-settlement assay revealed a number of natural products, including isomajusculamide (**Fig. 3**), majusculamide A (**Fig. 1**), and hantupeptin C (**Fig. 3**), having moderate anti-settlement activities (unpublished data) (**Table 1**). Currently, we are planning field testing of these compounds using the modified Phytagel™ method. To our knowledge these series of ecological studies conducted at our laboratory are unprecedented with regards to the antifouling nature of marine cyanobacterial compounds. These results indicated that compounds from marine cyanobacteria can have different ecological roles, such as antifouling and antifeedant, and they could contribute to the survival of benthic marine cyanobacteria in nature.

Table 1. Anti-settlement activities and toxicities of three compounds from *Lyngbya majuscula* from Pulau Hantu tested against cyprids of *Amphibalanus amphitrite*.

Compounds	EC_{50} (µg ml^{-1})	LC_{50} (µg ml^{-1})
Isomalyngamide A	4.2	167
Hantupeptin C	10.6	71

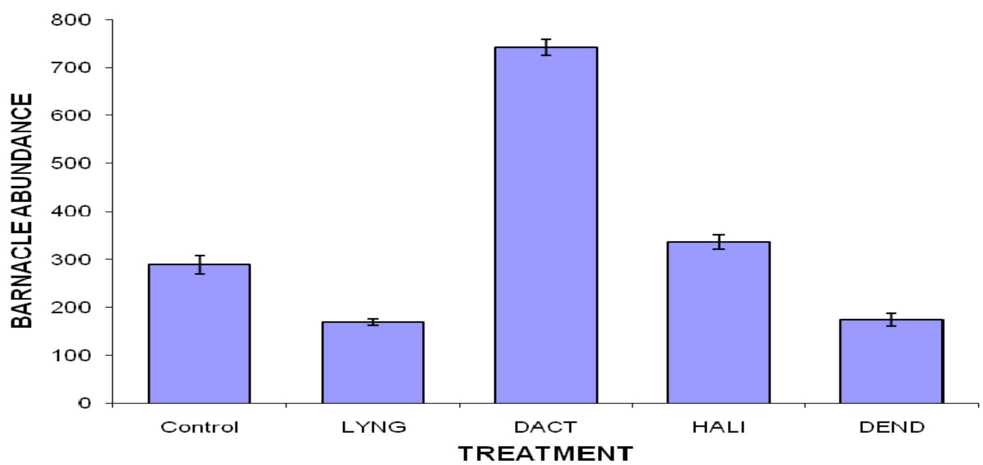

Fig. 2. Individual barnacle counts settling on Phytagels™ on week 4 (All bars are mean values of six replicates, vertical bars indicate standard error). Treatments are as follows: Control= Positive control gel, **LYNG** = *Lyngbya majuscula*, **DACT** = *Dactylospongia* sp., **HALI** = *Halimeda* sp., and **DEND** = *Dendronephthya* sp.

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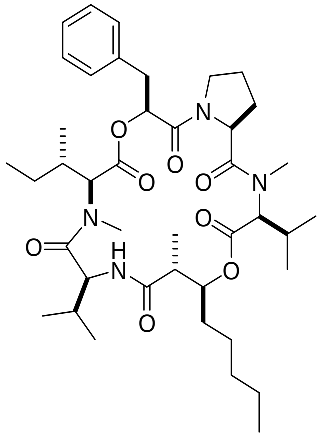
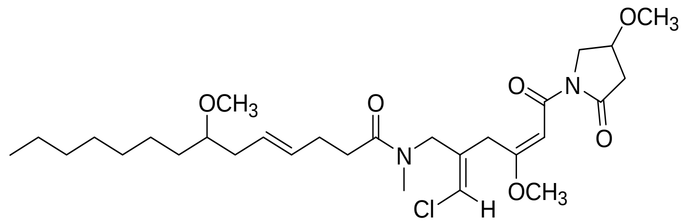


Hantupeptin C



Isomalyngamide A

Fig. 3. Antifouling compounds from the marine cyanobacterium, *Lyngbya majuscula*, collected from Pulau Hantu.

Marine cyanobacterial as potential source of antifoulants

Research on chemical ecology could sometimes lead to potential industrial applications. The discovery of marine cyanobacterial compounds with anti-settlement activities at our laboratory revealed these marine microbes as a potential source natural antifoulants in controlling biofouling. Scientists have been looking for alternative solutions to control biofouling and one such ways is the use of marine natural products (Fusetani, 2004). Marine benthic organisms, in particular, are constantly exposed to other larvae of fouling organisms as well as microbes. Over evolutionary time, they developed various strategies, such as the production of antifouling chemicals, to counter these fouling organisms. Many marine-derived antifoulants have since been identified from diverse marine organisms, such as marine algae, soft corals, and sponges (Fusetani, 2004). Promising natural antifoulants include a series of halogenated homoserine lactones produced by the red agla, *Delisea pulchra* (de Nys *et al.*, 2006). In spite of the diverse antifoulants from marine organisms, none has been identified from marine cyanobacteria (Dahms *et al.*, 2006). The unprecedented nature of such ecological studies from our laboratory emphasizes the importance of benthic marine cyanobacteria as a potential source of antifoulants.

CONCLUDING REMARKS

Diverse natural products have been isolated from marine cyanobacteria. A number of these molecules are highly potent and are potential source of novel pharmaceuticals. However, research on the ecological functions of these compounds is still in its infancy. This mini review showed that only a handful of marine cyanobacterial compounds have been investigated with regards to their ecological functions. This review also highlighted the antifouling properties of a number of marine cyanobacterial compounds based on a series of ecological research conducted at our laboratory. Research on chemical ecology of marine cyanobacterial compounds is important as it helps scientists understand their

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involvement in maintaining cyanobacterial population in nature. Furthermore, such research can lead to potential applications in marine biotechnology.

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REFERENCES

Brown, G.D., H.-F. Wong., N. Hutchinson., S.-C. Lee., B.K.K. Chan., and G.A. Williams. 2004. Chemistry and biology of maculalactone A from the marine cyanobacterium *Kyrtuthrix maculans*. *Phytochem. Rev.* 3:381-400.

Capper, A., E. Cruz-Rivera., V.J Paul., and I.R.Tibbetts. 2006. Chemical deterrence of a marine cyanobacterium against sympatric and non-sympatric consumers. *Hydrobiologia*. 553:319-326.

Cruz-Rivera, E. and V.J. Paul. 2007. Chemical deterrence of a cyanobacterial metabolite against generalized and specialized grazers. *J. Chem. Ecol.* 33:213-217.

Dahms, H.-U., X.Ying., and C. Pfeiffer. 2006. Antifouling potential of cyanobacteria: a mini-review. *Biofouling* 22:317-327.

De Nys, R., M. Givskov., N. Kumar, S. Kjelleberg., and P.D. Steinberg. 2006. Furanones. *Prog. Mol. Subcell. Biol.* 42:55-86.

Fusetani, N. 2004. Biofouling and antifouling. *Nat. Prod. Rep.* 21:94-104.

Henrikson, A.A. and J.R. Pawlik. 1995. A new antifouling assay method: results from field experiments using extracts of four marine organisms. *J. Exp. Mar. Biol. Ecol.* 194:157-165.

Kuffner, I.B. and V.J. Paul. 2004. Effects of the benthic cyanobacterium *Lyngbya majuscula* on larval recruitment of the reef corals *Acropora surculosa* and *Pocillopora damicornis*. *Coral. Reefs.* 23:455-458.

Luesch, H., W.Y. Yoshida., R.E. Moore., V.J. Paul., and T.H. Corbett. 2001. Total structure determination of apratoxin A, a potent novel cytotoxin from the marine cyanobacterium *Lyngbya majuscula*. *J. Am. Chem. Soc.* 123:5418-5423.

Nagle, D.G. and V.J Paul. 1998. Chemical defense of a marine cyanobacterial bloom. *J. Exp. Mar. Biol. Ecol.* 225:29-38.

Nagle, D.G., F.T. Camacho., and V.J. Paul. 1998. Dietary preference of the opisthobranch mollusk *Stylocheilus longicauda* for secondary metabolites produced by the tropical cyanobacterium *Lyngbya majuscula*. *Mar. Biol.* 132: 267-273.

Pennings, S.C., S.R. Pablo., and V.J. Paul. 1997. Chemical defenses of the tropical, benthic marine cyanobacterium *Hormothamnion enteromorphoides*: diverse consumers and synergisms. *J. Limnol. Oceanogr.* 42:911-917.

Pereira, R.C., A.G.V. Carvalho., B.A.P. Gama., and R. Coutinho. 2002. Field experimental evaluation of secondary

ISSN : 1410-5217
Accredited : 83/Dikti/Kep/2009

metabolites from marine invertebrates as antifoulants. *Braz. J. Biol.* 62:311-320.

Sonak, S., P. Pangam., A. Giriyan., and K. Hawaldar. 2009. Implications of the ban on organotins for protection of global coastal and marine ecology. *J. Environ. Manag. Suppl.* 1:S96-108.

Thacker, R.W., D.G. Nagle., and V.J. Paul. 1997. Effects of repeated exposures to marine cyanobacterial secondary metabolites on feeding by juvenile rabbitfish and parrotfish. *Mar. Ecol. Prog. Ser.* 714:21-29.

Tan, L.T. 2007. Bioactive natural products from marine cyanobacteria for drug discovery. *Phytochem.* 68:954-979.

Tan, L.T., Y.Y. Chang., and A. Tripathi. 2008. Besarhanamides A and B from the marine cyanobacterium *Lyngbya majuscula*. *Phytochem.* 69:2067-2069.

Taori, K., V.J. Paul., and H. Luesch. 2008. Structure and activity of largazole, a potent antiproliferative agent from the Floridian marine cyanobacterium *Symploca* sp.. *J. Am. Chem. Soc.* 130:1806-1807.

Tripathi, A., J. Puddick., M.R. Prinsep., P.P. Lee, and L.T. Tan. 2009. Hantupeptin A, a cytotoxic depsipeptide from a Singapore collection of *Lyngbya majuscula*. *J. Nat. Prod* 72:29-32.