Journal of Marine Science and Technology

Volume 21
Issue 7 *Vol 22, Supplemental*

Article 22

GENETIC DIVERSITY OF FILAMENTOUS CYANOBACTERIA FROM SHORE REGIONS OF OKINAWA

Shoichiro Suda
Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus, Okinawa, Japan., sudas@sci.u-ryukyu.ac.jp

Rui Moriya
Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus, Okinawa, Japan.

Shinpei Sumimoto
Graduate School of Engineering and Science, Faculty of Science, University of the Ryukyus, Okinawa, Japan.

Osamu Ohno
Department of Chemistry, Faculty of Science and Technology, Keio University, Yokohama, Japan.

Kiyotake Suenaga
Department of Chemistry, Faculty of Science and Technology, Keio University, Yokohama, Japan.

GENETIC DIVERSITY OF FILAMENTOUS CYANOBACTERIA FROM SHORE REGIONS OF OKINAWA

Acknowledgements

Part of this study was supported by Okinawa Intellectual Cluster Program and International Research Hub Project for Climate Change and Coral Reef/Island Dynamics of the University of the Ryukyus.

Journal of Marine Science and Technology, Vol. 21, Suppl., pp. 175-180 (2013)
DOI: 10.6119/JMST-013-1220-7

GENETIC DIVERSITY OF FILAMENTOUS CYANOBACTERIA FROM SHORE REGIONS OF OKINAWA

Shoichiro Suda[1], Rui Moriya[1], Shinpei Sumimoto[2], Osamu Ohno[3], and Kiyotake Suenaga[3]

Key words: biological active substances, genetic diversity, marine filamentous cyanobacteria, molecular phylogeny.

ABSTRACT

Benthic marine cyanobacteria are ubiquitous oxygenic photosynthetic bacteria and they form major components of various communities, including epiphytic, epilithic and microbial mats, in coral reef environments. Filamentous groups may form almost monospecific macroscopic colonies, and sometimes make potentially harmful algal blooms (HABs). HABs bring serious problems for marine tourism but marine filamentous cyanobacteria are good resource for drug discovery. However, cultivation and identification of such filamentous cyanobacteria are difficult and there are taxonomic confusions. In this study, we investigate 17 samples from coral reef area of 10 localities in Okinawa. Macroscopic mats or cushions of filamentous cyanobacteria were collected. The samples were divided into three parts: (1) for morphological observation, (2) for molecular work, and (3) cultivation for further study. Dominant cyanobacteira of each sample identified morphologically as *Anabaena*, *Hydrocoleum*, *Lyngbya*, *Oscillatoria*, *Phormidium*, *Schizothrix* and *Symploca*. Molecular results of partial 16S rDNA sequences revealed six groups that composed of heterocystous group, *Leptolyngbya* group, *Symploca* group, *Moorea* group, *Oscillatoria* group and unknown group, respectively. Some samples corroborated between morphology and molecular results but some were not. In addition, four out of six groups closely related with natural product producers. These results suggest that there are high biodiversity of marine benthic filamentous cyanobacteria in Okinawa that may become seeds of HABs as well as providing promising resources for drug discovery.

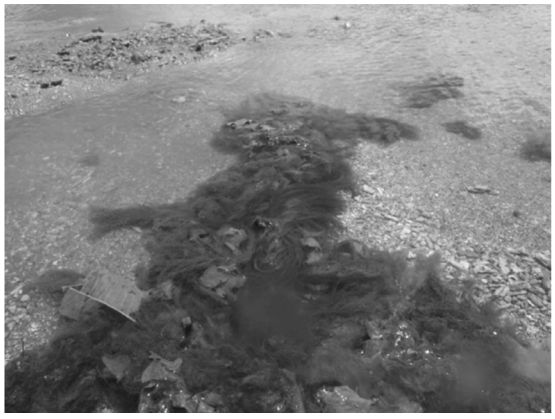


Fig. 1. Potentially harmful algal bloom in Kuba coast, Nakagusuku, Okinawa in June to July, 2010. The coast was covered by the filamentous cyanobacterium.

I. INTRODUCTION

The intensive human activities increase runoff of nutrients into coastal areas, encouraging harmful algal bloom in worldwide [3]. Filamentous harmful algal bloom of *Lyngbya majuscula* and relatives were reported in many places [1, 4, 5, 15, 20, 21, 23, 26].

Okinawa is located in the most south-western part of Japan and influenced by Kuroshio warm current. Many tourists from in- and outside Japan seek for the beautiful cobalt-blue shore, white sandy beach, and coral reefs decorated with colorful tropical fishes. Seaweed farming of "Mozuku" (*Cladosiphon okamuranus*) is also one of the abundant aquaculture in Okinawa that uses shallow sandy and seagrass beds area. Such activity might be damaged by harmful algal bloom of filamentous cyanobacteria. In fact, harmful algal bloom of filamentous cyanobacteria occurred in June to July 2010 along the Kuba coasts in Nakagusuku, Okinawa (Fig. 1). The lyngbya

Paper submitted: 10/31/13; revised 12/15/13; accepted 12/20/13. Author for correspondence: Shoichiro Suda (e-mail: sudas@sci.u-ryukyu.ac.jp).
[1] *Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus, Okinawa, Japan.*
[2] *Graduate School of Engineering and Science, Faculty of Science, University of the Ryukyus, Okinawa, Japan.*
[3] *Department of Chemistry, Faculty of Science and Technology, Keio University, Yokohama, Japan.*

Table 1. Sampling sites.

Locality	Abbreviation	Longitude	Latitude	Date
Serikyaku, Izena, Okinawa	iz-	26.924N	127.920E	25, Oct, 2010
Cape Maeda, Onna, Okinawa	mae-	26.445N	127.772E	11, Sep. 2010
Cape Maeda, Onna, Okinawa	Maeda-	26.440N	127.777E	04, Sep, 2013
Zanpa, Yomitan, Okinawa	zan-	26.439N	127.713E	02, Dec. 2010
Odo, Itoman, Okinawa	odo-	26.089N	127.710E	14, Oct. 2010
Akajima, Okinawa	aka-	26.193N	127.287E	10, Jul. 2010
Kushibaru, Akajima, Okinawa	kushi-	26.201N	127.268E	11, Jul. 2010
Tomori, Miyako, Okinawa	miya1-	24.724N	125.358E	27, Mar. 2010
Kabira, Ishigaki, Okinawa	ishi-	24.461N	124.146E	25, Jul. 2010
Sunabe, Chatan, Okinawa	suna-	26.332N	127.743E	2010

The longitude and latitude were from Google Map.

toxin and relatives were detected from the sample [12]. If such HABs frequently occur in future, marine tourism and seaweed farming in Okinawa will be seriously impaired. Hence, it is important to study the classification and prolific growth mechanism of macroscopic colonies of marine filamentous cyanobacteria in Okinawa.

On the other hand, numerous biological active substances including pharmaceutically potent molecules have been discovered from marine filamentous cyanobacteria from coral reef area [7, 10, 17, 24, 27]. Molecular phylogenetic researches based on 16S rRNA sequences of cyanobacteria revealed that there are large discrepancies between molecular and morphological taxonomy [6, 10, 13, 28]. Recently there are correlations between chemical grouping of natural products and clades of molecular phylogenies in filamentous cyanobacteria [10, 23]. This paper presents molecular diversity of macroscopic colonies of filamentous cyanobacteria from several shore regions in Okinawa. These cyanobacteria have the possibility to become seeds of HABs and may also provide potential resources of secondary metabolites for drug discovery.

II. MATERIALS AND METHODS

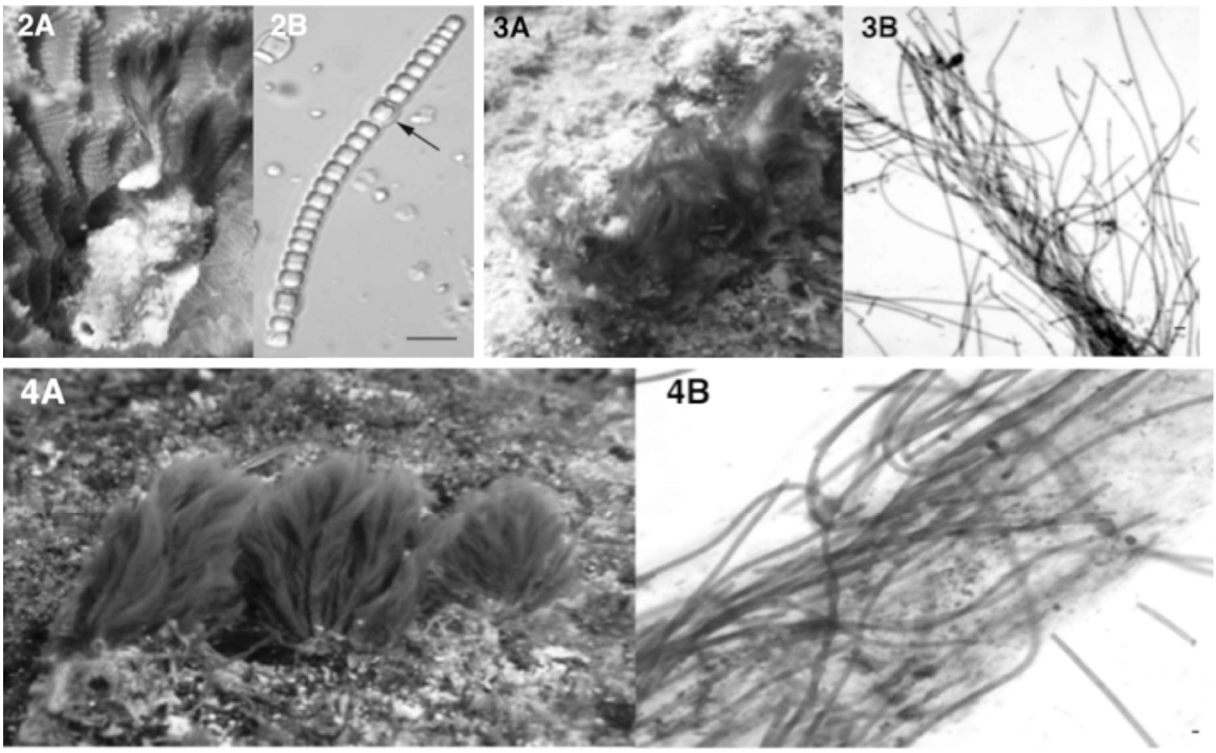
Macroscopic colonies, mats or cushions of filamentous cyanobacteria were collected from 10 coral reef localities in Okinawa during low tide, either by foot or skin diving (Table 1). The *in situ* samples were recorded using two digital cameras (Lumix DMC-FX07 and DMC-FT2, Panasonic, Co. Tokyo, Japan). The samples were purified under microscope by pipette washing method and divided into three parts, one part was fixed using 2.5% glutaraldehyde solution for morphological observation, the other part was fixed using 100% ethanol for molecular work, and the rests were attempted to be cultured for further study. Morphological identification of the samples was referred to Umezaki [30].

Ethanol-fixed samples or fresh samples were purified using pipette washing method, transferred onto agar plates to eliminate other attached cyanobacteria, and subsequently used for DNA extraction. Filaments were finely grinded using mortar and pestle. DNA was extracted and purified by DNesy Plant mini kit (Qiagen, Japan). The fragments of the 16S rDNA region were amplified using primers CYA106F [19] -CYA1371R (1+2+3) [18]. PCR cycles were set to 94°C for 5 min, following 30 cycles of 94°C denature for 1 min, 64°C annealing for 1 min, and 72°C extension for 2 min, additional 72°C extension for 10 min, and ending hold at 4°C. The PCR

Table 2. Partial 16S rRNA gene sequences from macroscopic filamentous cyanobacteria strains.

strain	length (bp)	Accession number
aka2mo28	1196	AB863113
aka2mo30	1197	AB863114
ishi2mo26	1212	AB863115
ishi2mo27	1198	AB863116
ishi2mo36	1179	AB863117
iz9mo10	1187	AB863118
iz19mo58	926	AB863119
kushi1mo20	1201	AB863120
kushi1mo21	1190	AB863121
kushi2mo23	1187	AB863122
Maeda130904A*	1873	AB857842
Maeda130904B	1169	AB863123
MaedaD5*	1895	AB863124
miya1-1mo9	1196	AB863125
miya1-2mo41	1191	AB863126
miya1-4mo35	1201	AB863127
odo1mo59	1211	AB863128
odo3mo16	1182	AB863129
odo4mo64	1203	AB863130
odo5mo67	1210	AB863131
odo6mo69	1180	AB863132
suna1mo33	1183	AB863133
zan1mo51	1208	AB863134
zan4mo54	1194	AB863135

*The sequence is composed of 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, tRNA-Ile and tRNA-Ala genes, complete sequence; and 23S ribosomal RNA gene, partial sequence.



Figs. 2-4. Macroscopic colony and its microscopic view. Scale bars = 10 µm. Fig. 2. Strain iz19mo58. 2A, A macroscopic colony attached to massive coral. 2B, Dominant filament has a heterocyst (arrow). Fig. 3. Strain zan4mo54. 3A, A macroscopic colony attached to the dead coral. 3B, Dominant filaments were tangled each other. Fig. 4. Strain odo5mo67. 4A, Macroscopic colonies attached to the dead coral. 4B, Dominant filaments were tangled and had thin hyaline sheath.

products were sent to Macrogen Japan, Co. for sequencing. For phylogenetic analyses, sequences of approximately 1200 base pairs of 16S rDNA from 20 strains were used. In addition to the newly acquired 20 strains' sequences, 54 sequences from Engine *et al*. [10] were obtained from NCBI GenBank and used to construct the phylogenetic tree. All sequences were aligned using CLUSTAL X v2.1 [15]. A maximum likelihood (ML) tree was constructed with RaxML [23]. The gamma-distributed model (GTR+G+I) was used and the outgroup selected as *Gloeobacter violaceus* PCC7421. Bootstrap values were tested 1000 times using the rapid bootstrap option. Bayesian trees were made by Mr. Bayes 3.1.2 [22] under GTR+G+I. One cold and three heated Markov chains Monte Carlo (MCMC) with default-chain temperatures were run for 1 million generations, sampling loglikelihoods (InLs), and trees at 1000-generation intervals (1000 InLs and trees were saved during MCMC). The likelihood plots for the dataset suggested that MCMC reached the stationary phase after the first 250 generations (standard deviation of split frequencies = 0.008085). Thus, the remaining 750 trees were used to obtain clade probabilities and branch-length estimates.

III. RESULTS

From 10 localities (Table 1), 17 samples were obtained. The major parts of samples could be characterized by morphologically and eventually seven genera were identified, namely *Anabaena*, *Hydrocoleum*, *Lyngbya*, *Oscillatoria*, *Phormidium*, *Schizothrix* and *Symploca*. Unfortunately, we could not establish any cultures from the samples. The 24 sequences (Table 2) from the 17 samples were obtained and nucleotide Blast search [2] was conducted to compare with the closest cyanobacteria data available in GenBank.

Several representatives of the strains were shown as follows. Strain iz19mo58 was sampled from Izena Island. The colony was green and attached on a massive coral (Fig. 2A). Under microscopic observation, most of the filaments were heterocystous (Fig. 2B) and could be classified into *Anabaena*, *Nodularia* or related cyanobacteria. Result of molecular phylogeny agreed with morphological classification. The closest relative from GenBank was *Anabaena* sp. PCC9109 (AY768408) with 9 bp substitutions Phylogenetic analysis revealed that the strain iz19mo58 was almost the same position with strain odo2mo14 and closely related with *Nodularia* sp. M8 (JQ247694) using 367 bp of 16S rDNA (data not shown).

Strain zan4mo54 was sampled from cape Zanpa, Yomitan, Okinawajima Island. The colony was brown and attached on a dead coral (Fig. 3A). Under microscopic observation, the filaments tangled with thin hyaline sheath (Fig. 3B). Cell

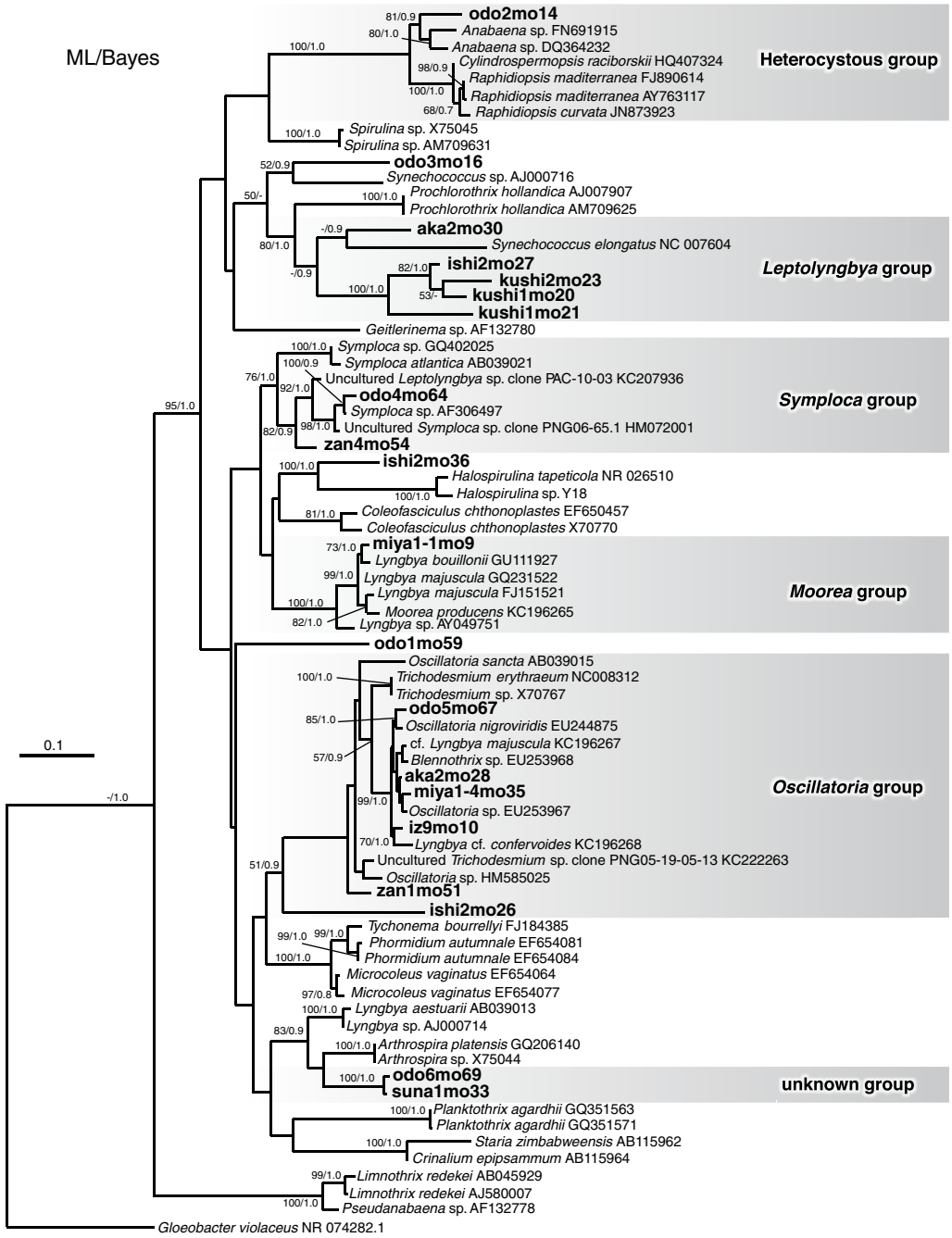


Fig. 5. A Maximum likelihood (ML) tree inferred from 16S rRNA gene sequences (1195 bp) using the GTR+G+I model with *Gloeobacter violaceus* PCC7421 (NR 074282.1). The numbers at the nodes of the branches indicated the ML bootstrap (left) and Bayesian posterior probability (right) values; values over 50% or 0.5 were shown. Six main groups were indicated.

length and width were 5.7 μm and 5.8 μm, respectively. Based on these characters, strain zan4 was identified as *Symploca* or related cyanobacteria. Results of molecular phylogeny agreed with *Symploca* sp. BP642b (AY032933) and 12 bp substitutions were observed.

Strain odo5mo67 was sampled from Odo, Itoman, Okinawajima Island. The colony was dark blue and attached on a dead coral (Fig. 4A). Under the microscopic observation, the filaments tangled with thin hyaline sheath (Fig. 4B). Cell length and width were 3.1 μm and 11.6 μm, respectively. These morphologies indicated that the strain odo5 was *Oscillatoria*, *Schizothrix* or related cyanobacteria. Result of molecular phylogeny agreed with *Oscillatoria margaritifera* NAC8-55 (GU724208) and 5 bp substitutions were observed.

Strains Maeda 130904A and Maeda D5 were closely related with *Oscillatoria miniata* NAC8-50 (GU724203), and strain Maeda 130904B was grouped with *O. margaritifera* NAC8-55 (GU724208) with strains odo5mo67, miya1-4mo35,

aka2mo28 and iz9mo10 in a phylogenetic tree inferred from 959 bp of 16S rDNA (data not shown).

Phylogenetic tree inferred from1195 bp of 16S rDNA sequences of 20 strains with 54 data were shown in Fig. 5. Strain odo2mo14 was grouped in the Heterocystous group, strains aka2mo30, isi2mo27, kushi2mo23, kushi1mo20 and kushi1mo21 were grouped in the *Leptolygbya* group, strains odo4mo64 and zan4mo54 were grouped in the *Symploca* group, strain miya1-1mo9 was grouped in the *Moorea* group, strains odo5mo67, aka2mo28, miya1-4mo35, iz9mo10, zan1mo51 and ishi2mo26 were grouped in the *Oscillatoria* group, respectively. In contrast, the strains odo3mo16, ishi2mo36, odo1mo59, and group of odo6mo69 and suna1mo33 were no closely related data and were likely represent independent cyanobacteria.

IV. DISCUSSION

From the results, many strains were corresponded well between morphology and molecular phylogeny. However, the same morphotype of cyanobacteria were genetically divided into several independent groups. In fact, the morphotype *Lyngbya* were distributed in several parts in the phylogenetic tree [8, 29]. Hence, each genetically independent group was classified into new taxon, recently. For instance, the genera *Moorea* [9] and *Limnoraphis* [14] were recently established. From the phylogenetic tree, macroscopic filamentous cyanobacteria were genetically diverse and each independent group could be established as independent taxon after revising the type species of each cyanobacterial taxon.

Recent papers suggested that the secondary metabolites and genetic groups were correlated to each other [10, 23]. Several strains were also closely related with natural product producers. For instance, the strain miya1-1mo9 was grouped in the *Moorea* group (Fig. 5). Except for strain miya1-1mo9, all members of the group were classified into clade I that composed of producers of 37 kinds of natural products [10]. The strains aka2mo28 and miya1-4mo35 were form a clade with strains cf. *Lyngbya majuscula* (KC19267), *Blenothrix* sp. (EU253968) and *Oscillatoria* sp. (EU253967) (Fig. 5). The members of the clade were classified into clade II that composed of producers of 56 kinds of natural products [10]. Instead of possible closely related natural product producers existed, several strains were no close relatives in GenBank data (Fig. 5). These results suggest there were high biodiversity of marine benthic filamentous cyanobacteria in Okinawa that are possible to become seeds of HABs and provide promising potential to be used in drag discovery. However, macroscopic filamentous cyanobacterial colonies are greatly diverse, and therefore, further studies are needed.

ACKNOWLEDGMENTS

Part of this study was supported by Okinawa Intellectual Cluster Program and International Research Hub Project for Climate Change and Coral Reef/Island Dynamics of the University of the Ryukyus.

REFERENCES

1. Ahern, K. S., Ahern, C. R., and Udy, J. W., "In situ field experiment shows *Lyngbya majuscula* (cyanobacterium) growth stimulated by added iron, phosphorous and nitrogen," *Harmful Algae*, Vol. 7, No. 4, pp. 389-404 (2008).
2. Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J., "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs," *Nucleic Acids Research*, Vol. 25, No. 17, pp. 3389-3402 (1997).
3. Anderson, D. M., Glibert, P. M., and Burkholder, J. M., "Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences," *Estuaries and Coasts*, Vol. 25, No. 4, pp. 704-726 (2002).
4. Arthur, K. E., Limpus, C. J., Roelfsema, C. M., Udy, J. W., and Shaw, G. R., "A bloom of *Lyngbya majuscula* in Shoalwater Bay, Queensland, Australia: an important feeding ground for the green turtle (*Chelonia mydas*)," *Harmful Algae*, Vol. 5, No. 3, pp. 251-265 (2005).
5. Capper, A., Tibbetts, I. R., O'Neil, J. M., and Shaw, G. R., "Feeding preference and deterrence in fabbitfish *Siganus fuscesces* for the cyanobacterium *Lyngbya majuscula* in Moreton Bay, south-east Queensland, Australia," *Journal of Fish Biology*, Vol. 68, No. 5, pp. 1589-1609 (2006).
6. Casamatta, D. A., Johansen, J. R., Vis, M. L., and Braoadwater, S. T., "Molecular and morphological characterization of ten polar and nea-polar strains within the Oscillatoriales (Cyanobacteria)," *Journal of Phycology*, Vol. 41, No. 2, pp. 421-438 (2005).
7. Dixit, R. B. and Suseela, M. R., "Cyanobacteria: potential candidates for drug discovery," *Antonie van Leeuwenhoek*, Vol. 103, No. 5, pp. 947-961 (2013).
8. Engene, N., Coates, R. C., and Gerwick, W. H., "16S rRNA gene heterogeneity in the filamentous marine cyanobacterial genus *Lyngbya*," *Journal of Phycology*, Vol. 46, No. 3, pp. 591-601 (2010).
9. Engene, N., Rottacker, E. C., Kastovisky, J., Byrum, T., Choi, H., Ellisman M. H., Komárek, J., and Gerwich, W. H., "*Moorea producens* gen nov., sp. nov. and *Moorea bouillonii* comb. nov., tropical marine cyanbacteria rich in bioactive secondary metabolites," *International Journal of Systematics and Evolutionary Microbiology*, Vol. 62, No. Pt 5, pp. 1171-1178 (2012).
10. Engene, N., Gunasekera, S. P., Gerwick, W. H., and Paul, V. J., "Phylogenetic inferences reveal a large extent of novel biodiversity in chemically rich tropical marine cyanobacteria," *Applied and Environmental Microbiology*, Vol. 79, No. 6, pp. 1882-1888 (2013).
11. Huelsenbeck, J. P. and Ronquist, F., "MrBayes: Bayesian inference of phylogenetic trees phylogeny," *Bioinformatics*, Vol. 17, pp. 754-755 (2001).
12. Institute of Health and Environment, Okinawa Prefecture, "Caution the harmful algal bloom (in Japanese)," http://www.eikanken-okinawa.jp/seitaiG/yuudokumo/yuudokumo.htm (2010).
13. Ishida, T., Yokota, A., and Sugiyama, J., "Phylogenetic relationships of filamentous cyanobacterial taxa inferred from 16S rRNA sequence divergence," *Journal General and Applied Microbiology*, Vol. 42, No. 4, pp. 237-241 (1997).
14. Komárek, J., Zapomelová, E., Zmarda, J., Kopecky, J., Rejmánková, E., Woodhouse, J., Neilan B. A., and Komárková, J., "Polyphasic evaluation of *Limnoraphis robusta*, a water-bloom forming cyanobacteria from lake Atitlán, Guatemala, with a description of *Limnoraphis* gen nov.," *Fottea, Olomouc*, Vol. 13, No. 1, pp. 39-52 (2013).
15. Kuffner, I. B. and Paul, V. J., "Effects of the benthic cyanobacterium *Lyngbya majuscula* on larval recruitment of the reef corals *Acropora surculosa* and *Pocillopora damicornis*," *Coral Reefs*, Vol. 23, No. 4, pp. 455-458 (2004).
16. Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R.,

Thompson, J. D., Gibson, T. J., and Higgins, D. G., "Clustal W and Clustal X version 2.0," *Bioinformatics*, Vol. 23, pp. 2947-2948 (2007).
17. Liu, L. and Rein, K. S., "New peptides isolated from *Lyngbya* species: a review," *Marine Drugs*, Vol. 8, pp. 1817-1837 (2010).
18. Murakami, A., Miyashita, H., Iseki, M., Adachi, K., and Mimuro, M., "Chlorophyll *d* in an epiphytic cyanobacterium of red algae," *Science*, Vol. 303, No. 5664, p. 1633 (2004).
19. Nübel, U., Garcia-Pichel, F., and Nuyser, G., "PCR primers to amplify 16S rRNA genes from cyanobacteria," *Applied and Environmental Microbiology*, Vol. 63, No. 8, pp. 3327-3332 (1997).
20. Osborne, N. J. T., Webb, P. M., and Shaw, G. R., "The toxins of *Lyngbya majuscula* and their human and ecological health effects," *Environmental International*, Vol. 27, No. 5, pp. 381-392 (2001).
21. Paul, V. J., Thacker, R. W., Banks, K., and Golubic, S., "Benthic cyanobacterial bloom impacts the reefs of South Florida (Broward County, USA)," *Coral Reefs*, Vol. 24, No. 4, pp. 693-697 (2005).
22. Ronquist, F. and Juelsenbeck, J. P., "MrBayes 3: Bayesian phylogenetic inference under mixed models," *Bioinformatics*, Vol. 19, No. 12, pp. 1572-1574 (2003).
23. Sharp, K., Arthur, K. E., Gu, L., Ross, C., Harrison, G., Gunasekera, S. P., Meickle, T., Matthew, S., Luesch, H., Thacker, R. W., Sherman, D. H., and Paul, V. J., "Phylogenetic and chemical diversity of three chemotypes of bloom-forming *Lyngbya* species (Cyanobacteria: Oscillatoriales) from reefs of Southeastern Florida," *Applied and Environmental Microbiology*, Vol. 75, No. 9, pp. 2879-2888 (2009).
24. Singh, R. K., Tiwari, S. P., Rai, A. K., and Mohapatra, T. M., "Cyanobacteria: an emerging source for drug discovery," *Journal of Antibiotics*, Vol. 64, pp. 401-412 (2011).
25. Stamatakis, A., Ott, M., and Ludwig, T., "RaxML-OMP: an efficient program for phylogenetic inference on SMPs," *Lect Notes Computer Science*, Vol. 3606, pp. 288-302 (2005).
26. Stielow, S. and Ballantine, D. L., "Benthic cyanobacterial, *Microcoleus lyngbyaceus*, blooms in shollow, inshore Puerto Rican seagrass habitats, Caribbean sea," *Harmful Algae*, Vol. 2, No. 2, pp. 127-133 (2003).
27. Tan, L. T., "Filamentous tropical marine cyanobacteria: a rich source of natural products for anticancer drug discovery," *Journal of Applied Phycology*, Vol. 22, pp. 659-676 (2010).
28. Taton, A., Grubisic, S., Brambilla, E., De Wit, R., and Wilmotte, A., "Cyanobacterial diversity in natural and artificial microbial mats of lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach," *Applied and Environmental Microbiology*, Vol. 69, No. 9, pp. 5157-5169 (2003).
29. Thacker, R. W. and Paul, V. J., "Morphological, chemical, and genetic diversity of tropical marine cyanobacteria *Lyngbya* spp. and *Symploca* spp. (Oscillatoriales)," *Applied and Environmental Microbiology*, Vol. 70, No. 6, pp. 3305-3312 (2004).
30. Umezaki, I., "The marine blue-green algae of Japan," *Memoirs of the College of Agriculture, Kyoto University*, Vol. 83, Fisheries Series No. 8, pp. 1-149 (1961).