Study on biological compounds from the cyanobacterium Lyngbya cf. majuscula

Summary

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[Introduction]

Cyanobacteria, which are often called “blue-green algae”, have been found to cause hepatic, neurologic, and dermal system damage secondary to the toxins they produce, “cyanotoxins”. One of the most toxic genera of filamentous marine cyanobacteria, *Moorea producens* (*M. producens*) is a rich source of diverse compounds which possess a variety of biological activities. *M. producens* was reported to be the causative organism of food poisoning as well as seaweed dermatitis known as “swimmer’s itch”. Aplysiatoxin, debromoaplysiatoxin and lyngbyatoxin A, which are produced by *M. producens*, have been reported to be the causative agents of this health problem. Fatal intoxication due to ingestion of lyngbyatoxin A contaminated flesh of the turtle *Chelonia mydas* has been reported. In addition, aplysiatoxin and related toxins were revealed to be the causative agents of food poisoning by the red alga *Gracilaria coronopifolia*. Later, the true producer of these toxins involved in these poisoning cases was deduced to be *M. producens*. Furthermore, these three toxins have also been shown to possess potent tumor-promoting activity originating from the activation of protein kinase C(PKC) by the toxins. Therefore, the study of toxins produced by *M. producens*is very important from an ecotoxicological point of view. Additionally, *M. producens*, a rich source of unique compounds, has led to the extensive study of its bioactive compounds**,** which may lead to the discovery of novel therapeutics agents. In this study, the toxic components in the extracts of *M. producens* were examined.

Moreover, marine biofouling is a major problem in marine related industries, which can lead to significant operational stress and economic damages on marine infrastructures. Currently, the use of biocidal products in surface coatings becomes the most common way to avoid marine

biofouling. However, because of the recent global prohibition of harmful antifoulants including tributyltin, the need for environmentally friendly antibiofouling compounds has increased rapidly. Moreover, periphytic diatoms have been shown to contribute significantly to biofilms, which play an important role in biofouling. Therefore, inhibiting the proliferation of fouling diatoms becomes a very important step in the prevention of marine biofouling. It was observed that the growth of diatom cells was inhibited by an all elopathic effect of cyanobacteria living in the same lake. In this study, a new established growth inhibition assay with the XTT colorimetric reaction against the diatom, *Nitzschia amabilis* cells, was used to screen allelopathic compounds from *M. producens*.

[Experimental Section]

For the freeze dried sample of *M. producens* collected from Hawaii, it was prepared with organic solvents in sequence: ethanol, methanol and acetone. The three extracts were combined and evaporated. The residue was then dissolved in 80% methanol and partitioned between hexane. After the 80% methanol layer was evaporated, the dried residue was re-suspended in distilled water and extracted with ethyl acetate. The fraction of distilled water was then dissolved with butanol and separated into two extracts. Since the fraction of ethyl acetate was measured to have the most potent cytotoxic activities. The ethyl acetate fraction was then subjected to an open glass column 20×300 mm packed with ODS resin to obtain 50%, 70%, 90% and 100% methanol layers. The cytotoxic components in70% and 90% methanol layers from ethyl acetate fraction were then investigated using reverse-phase HPLC and recycling HPLC for further isolation and purification.

For the freeze dried sample of *M. producens*, which was collected from Okinawa, it was soaked overnight in 7 L of ethanol at room temperature. After filtering the ethanol extract, the sample was then extracted 3times with 65 L of MeOH and once with 7 L of acetone. Three extracts were then combined and the solvent was evaporated. The residue was dissolved in 80% MeOH and partitioned using hexane. After the solvent of 80% MeOH layer was removed, the remaining sample was then partitioned between distilled water and ethyl acetate. The distilled water layer was then dissolved in butanol and separated into two extracts. The butanol layer was finally evaporated to dryness. Since there was few study about butanol extracts of *M. producens* up till now, it was then separated by a 40×170 mm open glass column filled with ODS resin with a stepwise increase of 30%, 50%, 70%, 85%, and 100% MeOH. The compounds in 85%-1methanol layer were finally purified and isolated by using reverse-phase HPLC as well as recycling HPLC.

[Results and discussion]

As the results of isolation and purification of M. *producens* collected from Hawaii, three new toxic compounds as well as lyngbyatoxin A (2), which is as a main toxic compound were achieved successfully from 70% and 90% methanol layers, respectively. Their structures were elucidated by analyses of HR-ESI-MS and NMR spectroscopies as well as optical rotations and CD spectra, indicating these new compounds to be 12-*epi*-lyngbyatoxin A (1). 2-oxo-3(*R*)-hydroxy-lyngbyatoxin A (3), as well as 2-oxo-3(*R*)-hydroxy-13-*N*-desmethyl-lyngbyatoxin A (4). Their cytotoxicity, lethal activities as well as protein kinase C δ(PKCδ) binding abilities using the PKC-C1B peptide were examined in this study. Lyngbyatoxin A (2) and its related compounds have been reported to be potent tumor promoters and have the ability to strongly activate PKC isozymes. As the results of bioactive activities in our study, while compounds 1and 2showed comparable cytotoxic and crustacean lethal activities, compound 1had more than 100 times lower binding affinity for PKC compared to lyngbyatoxin A (2). Moreover, the cytotoxicity and lethal activities of compounds 3 and 4 were approximately 10 to 150 times less potent than compound 2. However, the PKCδ binding activities of 3 and 4 possessed 10,000 times lower affinity when compared to lyngbyatoxin A(2). These results indicated that the acute toxicities such as cytotoxicity and lethality activity of these lyngbyatoxin derivatives might be mediated through some non-PKC activation pathways. Other targets that offer alternatives to PKC isozymes might exist for the expression of toxic activities by lyngbyatoxin-type compounds.

As the results of isolation and purification of M. *producens* collected from Okinawa, two new substances, compounds 5 and 6, were isolated from fraction 3-19 and fraction 3-18, respectively. Their structures were elucidated by analyses of HR-ESI-MS and NMR spectroscopies. As a result, these compounds were indicated to be dimer (5) and trimer (6) with the repetitive same portion partial structures. In this study, the cytotoxic assay, as well as growth inhibition assay against the diatom cells was carried out every time the HPLC separation of the extracts finished. Moreover, for the two newly isolated substances, compounds 5 and 6, their cytotoxicity, growth inhibition, as well as antibacterial assay against E. coli were also examined in this study. As the results, although no bioactivities of these two newly isolated compounds were detected, the newly established growth inhibition assay against the diatom cells was shown to be enabled to utilize as a screening program.