学 位 論 文 内 容 の 要 旨

博士（環境科学）　　氏 名 Jakia Jerin Mehjabin

学 位 論 文 題 名

Studies on new secondary metabolites from the marine cyanobacterium *Moorea bouillonii* collected in Sabah, Malaysia
（マレーシアサバ州で採集された海洋藍藻*Moorea bouillonii*から得られた新規二次代謝産物に関する研究）

Marine cyanobacteria are prolific producer of diverse secondary metabolites with intriguing bioactivities including cytotoxic, antiviral, antifungal, antifeedant, anti-inflammatory, antimicrobial, and antiproliferative activities. A large number of these nitrogen-containing metabolites with an enormously broad spectrum of pharmacological properties are mainly biosynthesized by nonribosomal peptide (NRP) or mixed polyketide-NRP enzymatic system. Having an increasing number of new secondary metabolites in recent decade, the marine cyanobacteria have become an attractive source for the isolation of novel compounds.

For this study, a total twenty one samples of marine cyanobacteria were collected by SCUBA from different locations of Northern Bornean coast, Sabah, Malaysia. Among them nine samples were successfully identified by 16S rRNA gene sequence analysis using cyanobacteria specific primers. Based on 16S rRNA gene sequencing and phylogenetic tree analysis M1621 was identified as *Symploca* or *Caldora* sp., M1623 was identified as *Okeania* sp., and other collected samples M1620, M1622, M1629, M1701, M1704 and M1705 were identified as *Moorea* sp. The cyanobacterial samples were extracted with MeOH and then partitioned with ethyl acetate (EtOAc), *n*-butanol (BuOH) and water (H_2O). The chemical profiling and dereplication of EtOAC fractions of *Moorea* sp. by LC-MS revealed the richness of secondary metabolites including known compounds apratoxins, wewakazole, lyngbyabellins, laingolides, madangolide, kanamienamide and unknown halogenated compounds. The cyanobacterial extracts were subjected to oil displacement assay for examining the surfactant properties of secondary metabolites. The EtOAc fractions of cyanobacterial extracts showed higher activity in terms of oil displacement area compared to other fractions and subjected to MTT assay against human MCF7 breast cancer cell lines for cytotoxicity. Among the extracts two *Moorea bouilllonii* samples M1629 and M1705 were chosen for the isolation of novel secondary metabolites because of their higher amount, rich chemical profile and activity.

Biosurfactant assay guided isolation of *M. bouillonii* (M1705) afforded three new acyl amides, columbamide F-H along with known cytotoxic apratoxin A and C, kanamienamide and columbamide D. The planar structure of columbamide F-H was established by mass spectrometric and NMR spectroscopic analyses. An *E* configuration of the double bond for columbamides was obtained by measuring the coupling constant ($^3J_{H,H}$ = 16-17 Hz) from the ^{13}C satellites observed by non-decoupled HSQC analysis. The absolute configuration of the *N*,*O*-dimethylserinol of columbamide F-H were determined by synthesis and Marfey's analysis of the synthetic (*R*)- and (*S*)-*N*,*O*-dimethylserinol and the hydrolysates of the columbamides.

The absolute configuration of chloromethine on the long alkyl chain of columbamide F was determined by chiral-phase HPLC analysis after conversion and esterification with Ohrui's acid, (1*S*,2*S*)-2-(anthracene-2,3-dicarboximido)

cyclohexanecarboxylic acid. Since, columbamide D and F have similar alkyl chain structure, columbamide D standards (10*S*,20*R*) and (10*R*,20*R*) were used as standards in the Ohrui reaction for columbamide F. Synthesized diastereomers of columbamide D and natural columbamide F were converted to diol by dihydroxylation and then cleaved oxidatively with sodium periodate ($NaIO_4$) to form two aldehydes. After separation, aldehyde that contained chloromethine was reduced with $NaBH_4$ to furnish linear alcohol which was esterified with (*S*,*S*)-2-(anthracene-2,3-dicarboximido)cyclohexanecarboxylic acid in the presence of EDCI and DMAP to afford Ohrui ester of synthetic standards and natural columbamide F. Chiral-phase HPLC analysis of synthetic 10*S* and 10*R* standards revealed that a coinjection experiment can separate both standards. Then, by a coelution experiment, columbamide F gave a single peak with the synthetic 10*R* isomer while two peaks were observed with the synthetic 10*S* isomer confirming the *R*–configuration for the chloromethine.

The biosurfactant properties of columbamide F was measured using oil displacement assay which is an indirect measurement of surface activity. The diameter of the clear zone was measured as ~ 90 mm for concentration of 10 mg/mL which is a slightly higher displacement area compared to SDS (~ 84 mm) and pluronic F-68 (~54 mm) for the same concentration. Due to the limited amount of sample it was not possible to determine the biosurfactant properties of columbamide G and H, however, the related fatty acid amides columbamide D and serinolamide C were checked for their biosurfactant properties. We measured the critical micelle concentration of these two fatty acid amides that showed biosurfactant properties in their initial screening results by oil displacement assay. The critical micelle concentrations (CMC) of columbamide D and serinolamide C were 0.34 mM, and 0.78 mM, respectively, that are lower than the CMC value of the synthetic surfactant SDS (8.2 mM) and comparable to pluronic F-68 (0.07-0.35) mM and triton X-100 (0.33 mM). Serinolamide C isolated from *Okeania* sp. showed potent antifouling activity (0.88 µg/mL) against *Amphibalanus amphitrite* larvae. The biosurfactant properties of serinolamide C could reduce the surface tension of seawater in the microplate well, and therefore the larvae cannot adhere easily. Furthermore, columbamides F-H were tested for the cytotoxicity against MCF-7 breast cancer cell line but were inactive (IC_{50} >10 µg/mL).

LC-MS guided separation of organic extract from *M. bouillonii* (M1629) collected in Udar Island, Sabah, Malaysia led to the isolation of new madangolide along with known compounds madangolide, laingolide, laingolide A and (1*R**,2*E*,4*R**,7*E*,10*S**,11*S**,12*R**)-10,18-diacetoxydolabella-2,7-dien-6-one which was originally isolated from the sponge *Dysidea* sp. The planar structure of new madangolide was established by combination of 1D and 2D NMR spectroscopic and mass analyses. The ^{13}C NMR peak revealed the presence of two carbonyls (δ 176.7, 175.5), four olefinic methines (δ 134.9, 122.7, 121.7, and 129.7), four methines; one is connected to oxygen atom (δ 76.2), one *N*-methyl (δ 35.4) which showed similarities of this compound with known madangolide except the replacement of isopropyl group instead of *tert*butyl group. The anti-allergic activity of crude extract and pure compounds laingolide A, madangolide and (1*R**,2*E*,4*R**,7*E*,10*S**,11*S**,12*R**)-10,18-diacetoxydolabella-2,7-dien-6-one were checked by β-Hexosaminidase release in rat basophil leukemia (RBL-2H3) cells. An allergic condition described a hypersensitivity disorder in which immune system response to a normally harmless environmental substance. The preliminary screening of the anti-allergic activity of crude extract as well as pure compound laingolide A and madangolide exhibited the reduction of β-Hexosaminidase, chemical mediator responsible for the allergic reactions. The details analysis of absolute configuration of new madangolide and the anti-allergic activities will be investigated in the future.

This study demonstrated the capabilities of marine cyanobacteria form Sabah, Malaysia to produce novel secondary metabolites. To date, only one paper reported the secondary metabolites from *M. bouillonii* collected in this region and very few reports revealed the biosurfactant and anti-allergic properties of secondary metabolites from marine cyanobacteria. The unique structure of new acyl amides columbamide F-H and new madangolide would be leading to the further future research about their bioactivities and isolation of novel compounds from the unexplored marine cyanobacteria from Sabah, Malaysia.