学 位 論 文 内 容 の 要 旨

Dissertation Abstract

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学 位 論 文 題 名

Title of dissertation

Isolation and structure elucidation of novel cyanobacterial secondary metabolites using OSMAC approach

（OSMAC法を用いた藍藻由来新規二次代謝産物の単離と構造決定）

Cyanobacteria are an ancient group of photosynthetic prokaryotes that have colonized diverse habitats. They have been successfully mined for natural product derived drug candidates and play a pivotal role in drug development and biotechnological applications. However, the redundancy in identifying novel compounds from traditional sources has presented a challenge. To address this issue, the One Strain Many Compounds (OSMAC) approach has emerged as a valuable strategy for exploring the biosynthetic potential of microorganisms. The aims of this study are to evaluate the production of metabolites in many cyanobacterial strains in response to various physico-chemical parameters using the OSMAC approach and the subsequent isolation and structure elucidation of novel cyanobacterial metabolites.

A preliminary screening method was used to cultivate cyanobacterial strains in small-scale cultures (500 mL) in triplicates and subjected them to different fermentation conditions such as media composition (addition of trace metals, nutrient depletion, nutrient limitation), pH, temperature, and salinity. Furthermore, coculture experiments were conducted to explore potential synergistic effects. After culturing in these different conditions, cells were extracted with methanol, chemical profile analyzed by liquid chromatography coupled to mass spectrometer. Based on the preliminary screening, five new secondary metabolites from cyanobacteria were selected for further analysis.

From the large-scale (100 L) laboratory culture of the cyanobacterium *Tychonema bourrellyi* NIES-846, a new linear depsipeptide was isolated. The planar structure of this new metabolite was established by mass spectrometric and NMR analyses and the configurations were determined by Marfey's analysis and Mosher's ester. The compound has an L-threonine, D-isoleucine residue, a terminal *S*-glutaric acid and an isoxazole unit which highlights the structural novelty of this compound. This compound displayed moderate antioxidant activity with an IC_{50} of 43 μM.

During the OSMAC screening, attention was drawn to the presence of three new compounds, argicyclamides A-C, produced by the cyanobacterium *Microcystis aeruginosa* NIES-88 across all culture conditions. The culture was upscaled to 80 L to target the isolation of these three metabolites. The structures of the isolated argicyclamides A-C were elucidated by careful analysis of their 1D and 2D NMR data. They were found to be octapeptides including 2 proline residues, 2 valine, isoleucine, leucine, phenylalanine, and arginine. Structural elucidation revealed that argicyclamide A was bis prenylated on its arginine residue, while argicyclamide B

was mono prenylated, and argicyclamide C lacked prenylation modifications. Stereochemistry of argicyclamides was determined by Marfey's analysis and they are composed of exclusively L-amino acids. Bioactivity assessments showed that argicyclamide A exhibited remarkable potency, with a minimum inhibitory concentration (MIC) of 6.25 μM against panel of Gram-positive bacteria, including the methicillin resistant *Staphylococcus aureus* strain which is responsible for the healthcare-associated infections worldwide and livestock infections. In contrast, it was not active against Gram-negative bacteria tested. Argicyclamide B and C displayed negligible activity under the tested conditions. The potent antimicrobial activity of argicyclamide A highlights its potential as a promising therapeutic candidate.

M. aeruginosa NIES-88 was found to produce a new compound, argicyclamide D in iron limited medium. The culture was upscaled to 400 L in iron limited BG-11 medium where the concentration of ferric ammonium citrate in the medium was modified to 0.6 mg/mL. On the basis of the ^{1}H NMR, MS/MS fragmentation pattern of argicyclamides and Marfey's analyses, the structure of argicyclamide D was proposed to comprise of the same macrocyclic structure as argicyclamide A with bis-prenylation on the arginine residue with an *N*-hydroxy group.

In the OSMAC experiments, argicyclamide C was upregulated when culture in high temperatures of 37 °C compared to the control grown in 25 °C. This prompted us to investigate more into the metabolism of the cyanobacterium using a targeted and untargeted metabolomics approach. The analysis revealed significant changes in the metabolomic profiles of the cyanobacteria under high-temperature conditions, with several metabolites being upregulated or downregulated in response to the stress. Production of microcystin LR, microcystin RR and micropeptins 88-C, 88-D and 88-E were downregulated by at least 6-fold. Production of argicyclamide A, argicyclamide B and kawaguchipeptin A seemed to vary throughout the experiment with argicyclamide B showing the least change between the control and heat shock treatments. Day 14 and 30 seemed to favor the production of argicyclamide A at 37 °C while on the other days there was a slight reduction in their production in the heat shock treatment. A 2-fold increase in kawaguchipeptin A was observed on day 14 and a decrease in its production at the end of the growth cycle. On the other hand, production of argicyclamide C was significantly upregulated with a highest 10-fold increase on day 14 in the heat shock treatments.

This study is the first report on the effect of high temperature on micropeptins 88-C – 88-E, kawaguchipeptin A and argicyclamides A-C. Iron limitation and high temperatures are known to induce oxidative stress in cyanobacterial cells. Since argicyclamides C and D were upregulated in high temperature and induced in iron limited medium, respectively, their siderophore and antioxidant activity was determined. However, argicyclamides showed negligible antioxidant or siderophore activity. Moreover, no synergistic or antagonistic antibacterial effect between argicyclamide A and argicyclamide D was found. Hence, it is proposed that the argicyclamides might have some protective or info-chemical functions. This study successfully reports the discovery of new compounds using the OSMAC approach in cyanobacteria and provides new targets to research on the interaction and mechanism of adaptation of cyanobacteria to stressful environmental conditions.