学 位 論 文 内 容 の 要 旨/Dissertation Abstract

博士（環境科学）　　氏　名/Applicant　Ling Zhengyi（凌正一）

学 位 論 文 題 名/Title of Dissertation

Enzyme catalysis for algal secondary metabolites: Studies on halogenated compounds from *Laurencia* spp. and cyanobacterial homotyrosine

（藻類の二次代謝における酵素反応：ソゾ類のハロゲン化合物および藍藻の
ホモチロシンに関する研究）

Algae play a vital role in the aquatic ecosystem, they are the primary producers, participating in the process in bioremediation, and they interact with the surroundings by releasing secondary metabolites. Algae can be broadly classified into macroalgae and microalgae. In these studies, the representatives of macroalgae is red algae, *Laurencia* spp. while microalgae is focused on cyanobacteria, as these two categories produce a wide range of compounds.

In Chapter 1, novel compounds such as anabaenopeptin 871, anabaenopeptin 885 and microginin 733C were isolated from *Microcystis aeruginosa* NIES-4285, in which contained a rare amino acid, doubly homologated tyrosine. Therefore, the first chapter was to study the doubly homologated tyrosine biosynthesis in *M. aeruginosa* N-4285. The gene cluster relative to nonribosomal peptide-synthetase (NRPS) as well as homologated amino acids were identified through uploading the genome to antiSMASH. Thereafter, four genes, including *MahphA* (isopropylmalate synthase), *MahphB* (isopropylmalate dehydrogenase), *MahphCD* (isopropylmalate dehydratase) and *MahphE* (aminotransferase) were postulated to produce homologated amino acids. Thereby, they were cloned and heterologously expressed in *E. coli* BL21 (DE3) for *in vivo* assay.

First of all, L-Tyr was administrated to the *E. coli* with *MahphABCDE* and incubated for 24 h, the supernatant was then subjected to LC-MS analysis. The products were compared with the standard, L-Tyr (*m/z* 182), L-hTyr (*m/z* 196), L-di-hTyr (*m/z* 210) and L-tri-hTyr (*m/z* 224). The LC-MS data suggested that hTyr and di-hTyr can be detected from the supernatant. When extended the incubation time to 72 h, tri-hTyr was also detected. To test whether *MahphABCDE* can accept hTyr as substrate, hTyr was incubated with the strain for *in vivo* assay. The LC-MS results were similar, in which di-hTyr was detected in 24 h, while tri-hTyr was detected in 72 h. Therefore, it demonstrated that *MahphABCDE* was able to convert Tyr into hTyr, di-hTyr and even to tri-hTyr. The promiscuity was then tested via administrating L-Phe to the strain, follwed by the same method. The results indicated that *MahphABCDE* was able to produce hPhe as well as di-hPhe from Phe. Hence, this is the first gene cluster that transforms two distinct amino acids into their doubly homologated amino acids.

In Chapter 2, as the mechanism of VBPO (vanadium-dependent bromoperoxidase) in biosynthesis of laurencin remained ambiguous, to address this problem, three standards, including deacetyllaurencin,

TMS-deacetyllaurencin and TMS-laurediol were prepared from laurencin which was extracted from *Laurencia nipponica*. Four VBPOs, *Ln*VBPO1 and *Ln*VBPO2 (from *L. nipponica*) as well as *Ls*VBPO1 and *Ls*VBPO2 (from *L. saitoi*) were constructed into pCold II, linked with SUMO tag and His_6 tag, followed by heterogolously expressing in *E. coli* BL21 (DE3). The four VBPOs were then purified through Ni-NTA column for *in vitro* assay. Through incubating four VBPOs with TMS-laurediol, respectively, two brominated compounds were detected by LC-MS from each sample, one of them showed the same retention time and mass pattern as TMS-deacetyllaurencin standards. After the large assay and HPLC purification, the NMR chemical shifts of the product matched well with the standards, leading to the confirmation of the pathway from laurediol to deacetyllaurencin. Further *in vitro* assay via incubating four VBPOs separately with deacetyllaurencin resulted in the detection of dibrominated compounds. After the large scale *in vitro* assay and silica gel chromatography, four dibrominated compounds were purified via HPLC. One of them was determined as laureoxanyne, a natural compound that was isolated from *L. nipponica*. And additional three dibrominated compounds were also analyzed by NMR for structure determination. Collectively, these results firmly established the pathway from laurediol to deacetyllaurencin and further to laureoxanyne. Moreover, the four VBPOs are of promiscuity as they can accept more than one substrate and multiproducts as they converted one precursor into more than one products.

In Chapter 3, the attention was shifted to *L. saitoi*, as this species produces polyether triterpenoids such as magireol-A, magireol-B and thyrsiferol. Although the precursor has been suspected to be squalene tetraepoxide, the role of VBPOs in converting epoxide into cyclic ether remains unclear. Thus, two simple compounds were organically synthesized as model substrates, monoepoxide 154 and monoepoxide 222. The endeavour of isolating the products from *Ls*VBPO1-monoepoxide 154 was failed due to the volatility of the product and no double bond within the structure, making it arduous to accumulate and purify. The attempt of obtaining it via MTPA-Cl esterification was also unsuccessful. Thereafter, monoepoxide 222 was employed for *in vitro* assay. With the same method, three brominated compounds were isolated, followed by NMR identification. Two of them were linear structure, while the third one was determined as a cyclic ether, it was hence demonstrated that VBPOs were able to produce cyclic ether from epoxide. Notably, the structure of linear compounds resembled halonerolidol, a bromo-chloro compound discovered from *L. composita*, making it worth testing whether the VBPOs were able to produce bromo-chloro compounds. Hence, (*E*)-nerolidol was chosen as substrate. By incubating *Ls*VBPO1 with (*E*)-nerolidol in the solution with Br^- and Cl^- for *in vitro* assay, five bromo-chloro compounds as well as seven brominated compounds were detected via LC-MS. Finally, several halogenated compounds were purified. Particularly, one of them showed the same NMR chemical shifts as those of halonerolidol, thus, halonerolidol is the first bromo-chloro compound from *Laurencia* spp. in which its biosynthesis is deciphered.

In this chapter, it illustrated that the four VBPOs exerted the catalysis of transforming epoxide into cyclic ether, which laid the foundation for the biosynthesis of polyether triterpenoids. Additionally, besides OH^-, other anions such as Cl^- can be incorporated into the products, this mechanism can further diversify the range of compounds produced by VBPOs.

Collectively, these studies contributed to the biosynthetic pathway of homologated tyrosine derivatives and halogenated compounds in *Laurencia* spp.