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# 主論文の要約

博士の専攻分野の名称：博士（水産科学）      氏名：李 成澤（Chengze Li）

## 学 位 論 文 題 目

Phylogenetic and molecular biological study of nitrogen transporters in Bangiales  
(Rhodophyta)

(原始紅藻類における窒素トランスポーター系統のおよび分子生物学的研究)

Nitrogen is macronutrients indispensable for plant growth in most terrestrial and aquatic ecosystems. It exists as organic forms like urea, and as inorganic forms of ion nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ) and ammonium ( $\text{NH}_4^+$ ), which are main nitrogen sources in soil and seawater. Influx of extracellular  $\text{NH}_4^+$  into cells is performed by ammonium transporters (AMTs), while urea is up taken into cell by urea transporter (DUR3s). In addition,  $\text{NO}_3^-$  is specifically imported into cells by nitrate transporter (NRTs). In Bangiales, nitrogen deficiency induces discoloration (“iro-ochi”) in thalli of *P. yezoensis*, which decreases quality of algae as a food. However, the discolored thalli can be recovered by increasing nitrogen sources in the medium. During this process, nitrogen transporters play crucial roles, since expression of genes encoding AMT and DUR3 is strongly induced under nitrogen-deficient conditions in *P. yezoensis*. Based on these background, phylogenetic and molecular biological study of nitrogen transporters in Bangiales have been performed in this thesis.

In Chapter 2, identification and phylogenetic and expression analyses of multiple *AMT* gene family members were performed in *P. yezoensis*. It was found that algal  $\text{NH}_4^+$  transporters are divided into the AMT1 and Rh subfamilies. The AMT1 subfamily of *P. yezoensis* consists of three groups containing genes whose expression patterns differ temporally and nitrogen deficiency-dependently during the life cycle, providing novel information about red algal  $\text{NH}_4^+$  transporters.

In Chapter 3, identification and phylogenetic classification of nitrogen transporters

in the *Bangia* species were performed. The *Bangia* material previously called *B. fuscopurpurea* was classified into the '*Bangia*' 2 group and designated '*Bangia*' sp. ESS1. This species has a gene family of AMT consisting of five AMT1s diversified into three independent phylogenetic groups with corresponding AMT1s from other red algae and one *Rh*. In addition, '*Bangia*' sp. ESS1 has only single copy gene of *NRT2* as other red algae and a gene family of *DUR3*.

In the Chapter 4, identification of suitable reference genes and gene expression analysis of nitrogen transporter genes under nitrogen-deficient conditions were performed in the *Bangia* species. The results showed that the *60S rRNA* and *actin* genes were most suitable for gene expression analyses in response to nutrient deficiency, high salinity, and temperature stress, whereas normalization of desiccation-inducible expression required use of the *EF1* and *tubulin* genes. Similar with the expression patterns of *AMT1s* genes from *Pyropia*, *AMT1s* genes from *Bangia* also displayed nitrogen deficient-inducible expression pattern, suggesting the highly conservation of nitrogen deficiency-inducibility in the *AMT1* families of two genera. However, the expression of *Rh* genes was not observed in both *Pyropia* and *Bangia*. Unlike nitrogen deficiency-inducible expression of *NRTs* in land plants, expression of the sole *NRT2* gene in red algae *P. yezoensis* and '*Bangia*' sp. ESS1 is insensitive to nitrate deficiency, suggesting the differences in the regulation of the *NRT2* gene expression between red algae and land plants. Since expression of *DUR3s* is up-regulated when '*Bangia*' sp. ESS1 was exposed to nitrogen-deficient conditions as observed in *P. yezoensis*, *DUR3s* might play essential roles in the uptake of urea under nitrogen-deficient conditions in Bangiales.

These studies could provide important knowledge useful for taking measures to recover the reduced productivity caused by discoloration in seaweed mariculture. Future study should be focused on functional significance of divergence of the *AMT1* and *DUR3* gene families and the presence of the *Rh* gene in Bangiales.