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学位論文要旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

Study of biological nitrogen fixation in legume and non-legume plants
（マメ科植物と非マメ科植物の生物的窒素固定の研究）

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Excess synthetic fertilizer usage caused emission since it produced from fossil fuel with high economic costs which pollute environment. Nitrogen fertilizer which known as the biggest amount of synthetic fertilizer used could be reduced with increase of biological nitrogen fixation. Understanding the mechanisms of biological nitrogen (N_2) fixation in legume and non-legume expects to decrease the synthetic nitrogen fertilizer consumption.

In this study, we used three plants to study the symbiotic nitrogen fixation. Firstly, we used *Lotus japonicus* as a model plant for genome studies in legumes, particularly about rhizobia symbiosis. In this term, we focused of effect of ferritin, an iron storage protein in nodule. Since legume nodule require large amount of iron for complex of such as nitrogenase and leghemoglobin. In nodules, ferritin is expressed at the initial stages of nodule development and increases at the nodule senescence stage. We investigated the effects of overexpressing and suppressing ferritin on nitrogen fixation in *L. japonicus*. Acetylene reduction activity revealed nitrogen fixation was higher by the overexpression of ferritin under the higher iron concentrations, ferritin-suppressed nodule was observed higher nitrogen fixation at lower iron concentration. Our data suggest that suppressing ferritin in the nodules is effective for higher nitrogen fixation under iron deficient conditions. Over accumulated ferritin in nodule is effective under the higher iron conditions.

In the second study, we focused on biological nitrogen fixation in non-legume plants. This study is related the potential of endophytic bacteria in supplying the biological nitrogen fixation. Sugarcane is promoted growth by the endophytic bacteria. We surveyed the diversity of bacteria in soil and root of

sugarcane, *Saccharums sinense*, which produce low concentration of sugar but is used for producing Wasanbon (a fine-grained Japanese sugar) in Kagawa, Japan. Bacterial community analysis suggested that the most abundant phyla from all samples was Proteobacteria and the most abundance classes were *α-Proteobacteria* and *β-Proteobacteria*. To know the characterization of nitrogen fixing bacteria of sugarcane we have isolated 11 rhizobia and sequences. Phylogenetic analysis of 16S rRNA and *nifH* gene sequencing showed one *Bradyrhizobium*. sp and two *Rhizobium* sps. named as Gm42, and Gm44 and Ps43, have been isolated. The soybean (*Glycine max*) and common bean (*Phaseolus vulgaris*) nodule shape infected with soybean were determinate type nodule, pea (*Pisum sativum*) was indeterminate type nodule. Inoculation test showed that all isolates formed nodules in soybean, common bean and pea. However, the nodules of soybean and pea were Fix^+ (fix nitrogen fixation), however, they produced Fix^- nodules in common bean. This might be the effect of Nod factor independent manner.

To know the function of Nod factor independent plants and bacteria interaction in biological, we also conducting research related T3SS dependent nodulation. In this study we aimed to understands the effect of T3SS on *Clitoria ternatea*-isolated bacteria. Our finding indicated that *C. ternatea* produced nodules with infection of *Bradyrhizobium elkanii* USDA 61, however did not with T3SS mutants. We also isolated rhizobia from *C.ternatea* and produced T3SS mutants. As a result, these isolates produced Fix^+ nodules however, T3SS mutant produced Fix^- nodules.