(Format No. 3)

SUMMARY OF DOCTORAL THESIS

Name: NGUYEN THI MINH

Title: Diversity of rhizobia isolated from nodules on different soybean cultivars in a field and their multiple occupancy
(圃場で栽培した異なるダイズ品種から単離した根粒菌の多様性と多重感染)

Nodulating rhizobia infect roots of leguminous plants and establish a symbiotic relation because of fixing ability of nitrogen, which supports plant growth. Soybean is a major leguminous crop in many countries including Vietnam. Crop yields of soybean depend strongly on the infection and inoculation of nodulating rhizobia in unfertile soil or under nitrogen limitation. Accordingly, the inoculation of nodulating-rhizobia for leguminous crop plants has been very common in agriculture and has achieved success to some degree. An important objective in legume inoculation research is to select highly effective strains of rhizobia for a particular host plant. The multiple occupancy (distinct rhizobial bacteria based on phenotypes and genotypes occupy a soybean nodule) would give a chance for the all members to maintain their abundance in the field. A certain nodulating-bacterium which is expected high performance in view of plant growth but is less competitive could survive in nodules of double occupancies. Although the multiple occupancy of nodules has been observed, the agricultural and ecological importance of multiple occupancy formation, however, has not been elucidated. This study, therefore, was conducted focus on multiple occupancy in nodules of field-grown soybean cultivars and their effect on diversity of rhizobia association.

The diversity of infecting rhizobia assigned by phenotypic and genotypic characters and their effect on the formation of multiple occupancy nodules on field-grown plants were analyzed in detail by comparing the two soybean cultivars, Kyushu 151 and Sachiyutka (chapter 3). In Kyushu 151, single phenotypic and genotypic groups were isolated from every nodule examined. Most of them were slow-growing and alkali producing. In Sachiyutaka, plural strains belonging to distinct groups were isolated frequently from single nodules, indicating that multiple occupancy was established at high frequency. Fast-growing and acid producing isolates were predominant in Sachiyutaka nodules. There was no fixed combination existed among the phenotypic and genotypic groups forming multiple infection. Some nodules were inhabited by bacteria whose groups were isolated only from nodules with multiple occupancy although they were able to establish the single-infection of Sachiyutaka. An increase in the relative abundance of strains belonging to *Sinorhizobium fredii* (*Ensifer fredii*) occurred concomitantly with the increase in the proportion of nodules with multiple occupancy. Nearly 60% of the strains from Sachiyutaka belonged to *S. fredii*; 75% of them were obtained from nodules with multiple occupancy. These results suggest that Sachiyutaka (a Japanese soybean cultivar) selected *S. fredii* isolates that were able to infect it simultaneously with nodulating-rhizobia of different types.

In order to examine effects of inocula composition and host cultivars on the infection mode, the frequency of *S. fredii* and multiple occupancy formation was analyzed by focusing on the effect of the origin of their hosts and the host *Rj*-genotype

(chapter 4). The mixture of representative rhizobia isolated from Sachiyutaka or Kyushu 151 (K-mix or S-mix) and the known USDA strains (U-mix) were inoculated to both cultivars. The K-mix and S-mix were used in turn as inoculant in the first and second cultivation. The *Rj*-genotypes of host were determined as non-*Rj* for Sachiyutaka and Rj_4 for Kyushu 151, respectively. In the first cultivation, the promotion effects of either K-mix or U-mix inoculation to Kyushu151 were at a similar level and Sachiyutaka growth did not respond clearly on inoculation. In the second cultivation, the inoculation stimulated the growth of both plant cultivars in many respects, indicating that the plant growth was promoted simply by the inoculation of the rhizobial mixtures. The imbalance of the relative abundance of each genotype observed in the first cultivation series was somewhat recovered in a density-dependent manner. A considerable number of nodules were infected by two or three genotypes, irrespective of the host cultivar in the second cultivation. On average, a lower frequency was recorded more consistently for Kyushu 151 than Sachiyutaka with the two bacterial mixtures. *S. fredii* strains tended to infect over other genotypes in single nodules as well as they inhabited singly in nodules. Results showed the relative abundance of the *S. fredii* isolates correlated with the frequency of multiple occupancy nodules. In turn, the proportion of singly infecting *B. japonicum* isolates decreased with the increase in the share of *S. fredii*. The isolates in S-mix infected Sachiyutaka plants, in principle, depended on their relative abundance in the inoculum. Nearly half of the tested nodules were inhabited by two or more distinct genotypes of rhizobia (multiple occupancy). The cohabitants mostly comprised *B. japonicum* and *S. fredii* genotypes. The proportion of each genotype appeared almost in accordance with their relative abundances in the mixtures. The cells of various genotypes would be incorporated randomly into nodules; resulting relative abundance-dependent infection.

Regarding to select the more efficient strains in the field environment, diversity of nodulating rhizobia isolated from nodules on 15 different soybean cultivars in the same field were analyzed by phenotypic and genotypic characteristics (chapter 5). Overall, slow-growing and alkali-producing *B. japonicum* accounted for 59.7% of the isolates followed by fast growing and acid-producing *S. fredii* at 33.8%. No cultivar was infected solely by either *B. japonicum* or *S. fredii*. The frequency of multiple occupancy was host-dependent and highest in Sachiyutaka. No specific combination was found for multiple occupancy formation. The diversity of infecting rhizobia was at lowest for Fukuyutaka cultivar followed by Kyushu 151 cultivar and at highest in Sachiyutaka cultivar following diversity indices of Simpson's *D* and Shannon- Weaver's *H'*. In addition, similar trend was observed for the frequency of multiple occupancy nodules; none in Kyushu 151 cultivar followed by Fukuyutaka cultivar and at highest in Sachiyutaka cultivar. Putative factors for the diversity might be explained as the combination of the occupation rate by *S. fredii* and the proportion of nodules with multiple occupancy.

In conclusions, the results obtained in this study suggested that the frequency of isolation of *S. fredii* strains correlated to the formation of multiple occupancy in field growth soybean. The tendency to form single or multiple occupancy nodules depends on the competing potential of each bacterial member in the inoculated mixtures. The competition ability of a certain rhizobial strain would affect the overall diversity of infecting rhizobial flora and multiple occupancy formation in synergy of plant preference. An increase in the isolate diversity occurred with the increase in relative abundance of isolates belonging to *S. fredii* (*Ensifer fredii*) and in the proportion of multiple occupancy among nodules.