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SUMMARY OF DOCTORAL THESIS

Name: Dinesh Adhikari

Title: Genetic diversity and phenotypic characterization of soybean- and common bean-nodulating rhizobia in Nepalese soils in relation to biogeographical distribution

(ネパール土壌におけるダイズおよびインゲン根粒菌の生物地理学的分布と遺伝的多様性および表現型特性との関係)

Nepal is one of the richest centers of genetic diversity, where many crops including soybean (*Glycine max* L.) and common beans (*Phaseolus vulgaris* L) are cultivated over a wide range of climatic and geographical regions. Soybean and common beans are among the important food legumes in the world. Most of the legumes, including soybean and common beans make a symbiotic association with soil inhabiting bacteria capable of forming nodules in the host plants, called rhizobia. Due to the symbiotic nitrogen fixation abilities, productivity of legume crops can be increased by inoculation of rhizobia, and understanding the diversity of these bacteria in relation to biogeographical conditions could be of great use to improve the inoculation efficiency. This thesis reported the genetic diversity of nodule forming rhizobia in soybean and common beans in various agroecological regions in Nepal, and also reported the physiological and symbiotic properties of various genotypes relation to environmental factors.

To characterize the soybean nodulating rhizobia, a total of 102 rhizobial strains were isolated from the root nodules of soybean collected from 12 different sites in Nepal. Identification, genetic diversity and phylogenetic relationships of the isolated strains were elucidated based on the partial sequences of 16S rDNA, 16S-23S rDNA inter-transcribed spacer (ITS) regions, *nodC* and *nifH* genes. Of the isolated strains, 43, 38, 16 and 5 were grouped under *Bradyrhizobium japonicum*, *Bradyrhizobium elkanii*, *Bradyrhizobium yuanmingense* and *Bradyrhizobium liaoningense*, respectively, based on the homology of the 16S rDNA and ITS sequences with the reference strains in the database. Population of each of four species differed along with climate and soil pH across the country. In temperate region, strains of *B. japonicum* dominated the total population, and in subtropical locations, relative occupancy depended on the soil pH, where *B. elkanii* was dominant in acid soils (pH 4.8 to 5.1), *B. yuanmingense* in moderately acid soils (pH 5.7 to 6.4) and *B. liaoningense* in a slightly alkaline soil (pH 7.4). Phylogenetic analysis of 16S rRNA genes of 102 strains divided the strains in eight clusters, of which clusters I to III were belonged to *B. elkanii* and rest to *B. japonicum*, *B. yuanmingense* and *B. liaoningense*. ITS phylogeny separated the strains into species level which were undifferentiated in 16S rDNA phylogeny. Differences in symbiotic genes among the strains were lesser compared to the ribosomal genes, but the closer similarity between phylogenies of *nodC* and *nifH* genes suggested co-evolution of the symbiotic genes probably through lateral transfer.

The biogeographical distribution of soybean rhizobial strains in Nepal indicated that each of the four species has a specific climatic and pH preference to make one species more

competent than the other for nodulation. To reveal the relationship between relative nodule occupancies of various species and their physiological properties, the growth of some representative dominant and minor strains in some locations were examined at various levels of temperature and pH in the liquid media. Results showed a characteristic difference in the growth among the strains of *B. japonicum*, *B. elkanii* and *B. yuanmingense* at various pH and temperature, although the differences could not explain their relative occupancies at different climate and soil pH. Strains of *B. elkanii* were relatively tolerant to wide range of pH from acidity to alkalinity. However, this property was opposite to the higher nodule occupancy of *B. yuanmingense* at pH 5.8 to 6.4 than those of *B. elkanii*. In a case, superior growth of a representative *B. yuanmingense* strain Mug-2 matched to its dominance over *B. elkanii* at a subtropical location (Muglin). When growth was compared under different pH, temperature and shaking conditions, shaking resulted into higher growth. Furthermore, different strains varied in nodulation ability and plant growth when inoculated in the soybean, but the difference was unrelated to species and their relative nodule occupancies in the fields.

Common bean nodulating rhizobial diversity was examined by isolating a total of 63 strains from the nodules of common bean grown in the soils collected from seven bean fields in Nepal and analyzing their partial sequences of 16S–23S ITS regions, 16S rDNA, and *nodC* and *nifH* genes. Of the isolated strains, 33 belonged to *Rhizobium leguminosarum*, 14 to *Rhizobium etli*, 9 to *Rhizobium phaseoli* and remaining 7 to an unknown *Rhizobium* lineage. The most abundant species was *R. leguminosarum*, which showed its presence in four temperate locations from east to far-west, followed by *R. etli* in two western temperate locations, and each of *R. phaseoli* and unknown *Rhizobium* lineage in one subtropical location.

Nine ITS genotypes of common bean rhizobia were detected mainly corresponding to a single site, including a dominant group at three sites harboring highly diverse multiple ITS sequences. Based on the *nodC* and *nifH* genotype, all isolates belonged to a common sv. *phaseoli*. Three symbiotic genotypes were detected and each corresponded to a geographical region. The variation in symbiotic genotype among the strains irrespective to the ribosomal DNA group suggested the lateral transfer of symbiotic genes separately in each geographical region.

Common beans are reported to be poor in nitrogen fixation compared to soybean and many other legumes, and among the cultivars determinate bushy type are more inferior compared to indeterminate climbing type cultivars in Nepal and other countries. Effect of inoculation of some Nepalese common bean rhizobial strains was examined under different pH conditions in different cultivars of the common beans. All of the tested strains formed nodules and effectively fixed the nitrogen both in climbing and bushy type cultivars. The activity was superior at neutral pH, but reduced at acidic and alkaline conditions even when the pH was similar to the soil pH where they were isolated.

There are a few studies of diversity of rhizobia that nodulate soybean in Nepalese soils. This research detected highly diverse genotypes of soybean rhizobia closely related with environmental factors. Likewise, this is the first study to report the diversity of common bean rhizobial populations in Nepal. This study provided the evidence of correlation between nodule occupancy of different rhizobial genotypes and environmental conditions. Difference in physiological and symbiotic abilities among various genotypes did not correlate to their relative presence at different environments in Nepal. In future, it is important to clarify the genetic and physiological background of the rhizobia involved in the traits related to the nodulation preference under various environmental conditions. If it is revealed, the results could be used to specify the suitable inoculants for each environmental condition in Nepal.