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SUMMARY OF DOCTORAL THESIS

Name: Md Hafizur Rahman Hafiz

Title: LATITUDINAL CHARACTERISTIC NODULE COMPOSITION OF *Bradyrhizobium* spp. AFFECTED BY THEIR TEMPERATURE-DEPENDENT PROLIFERATION IN SOIL AND INFECTION

（ダイズ根粒菌の温度依存的な土壌中の生息と感染に影響を受ける緯度特異的な根粒内組成）

Soybean (*Glycine max* [L] Merr.) belongs to the Leguminosae family, and is an important grain legume. Several soybean-nodulating bacteria, which can fix atmospheric nitrogen symbiotically in the nodules of the host plant, have been reported worldwide. Effective nitrogen fixation mainly depends on the potential of the rhizobial strain and their competition for proliferation and infection among indigenous rhizobia in a soil. Because effective nitrogen fixation depends on the potential of an individual rhizobial strain, competition for proliferation and infection among the rhizobial strains in a soil is crucial subject. In addition, their behaviours need to be considered based on environmental conditions.

Bradyrhizobium japonicum and *B. elkanii* are the major soybean nodulating rhizobia and it has been reported that *B. japonicum* and *B. elkanii* dominate in nodules of soybean cultivated in latitudinally northern and southern fields, respectively. Previous reports suggest that temperature-dependent infection and proliferation in a soil determine their nodule compositions. But it has not been elucidated which factor is more responsible for the latitudinally characteristic nodule composition of the bradyrhizobia.

To examine the contribution of two factors in fields under the local climate conditions, we selected three study locations, Fukagawa with temperate continental climate, and Matsue and Miyazaki with humid sub-tropical climate in Japan. Each soil sample was transported to the other study locations, and soybean cv. Orihime (non-Rj) was pot-cultivated using three soils at three study locations for successive two years. Three healthy soybean plants per pot were cultivated in triplicate for ca. 2–3 months without fertilization, and fresh plant weight and number of nodules were measured at harvest. Ten nodules were randomly selected from one plant for each replication, and rhizobial strains were isolated and phylogenetically characterized based on their partial 16S rRNA and 16S–23S rRNA ITS gene sequences, and the nodule composition of *Bradyrhizobium* spp. was determined.

The fresh plant weight and the nodule numbers were not significantly different among soils in both years in all locations with a few exceptions. Overall, the fresh plant weight increased from northern to southern study locations, whereas the nodule number showed the opposite tendency. Reduction in nodules at higher temperature might be due to strain-specific properties.

In this study, two *Bradyrhizobium japonicum* (Bj11 and BjS10J) and one *B. elkanii* (BeL7) were isolated, and Bj11 and BjS10J were phylogenetically sub-grouped into two (Bj11-1-2) and four clusters (BjS10J-1-4) based on their ITS sequence, respectively. Bj11-1 was characterized as slow grower and isolated primarily in the Fukagawa soil, while Bj11-2 was fast grower and isolated in the Fukagawa and Matsue soils. Regarding BjS10J, BjS10J-1 and BjS10J-3 were originated from the Matsue soil, while BjS10J-2 and BjS10J-4 were primarily isolated from the Miyazaki soil. *B. elkanii* L7 was ubiquitous in all soils.

In the Fukagawa soil, Bj11-1 dominated (87%) in the Fukagawa location, and the dominancy was not changed in the Matsue (80%) and Miyazaki (83%) locations. In the Matsue soil, the composition was similar in the Matsue and Miyazaki locations, in which BeL7 was dominated (70–73%) and Bj11-2 was minor (17-20%). While in the Fukagawa location, BeL7 decreased to 53% along with the increase of Bj11-1 (17%) and BjS10J-3 (13%). In the Miyazaki soil, BeL7 dominated at 77%, and BeL7 decreased to 13% and 33% in the Fukagawa and Matsue locations, respectively, while BjS10J-2 (53-73%) and BjS10J-4 (13%) increased.

These results suggested that the *B. japonicum* strain preferably proliferated in the Fukagawa location, leading to its nodule dominancy because the nodule composition was not changed in the Matsue and Miyazaki locations. While in the Miyazaki location, *B. elkanii* dominated in the nodules, but the dominant strain changed to *B. japonicum* in the Matsue and Fukagawa locations, suggesting that the temperature-dependent infection would lead to the nodule dominancy of *B. elkanii* in the Miyazaki location. In the Matsue location, because the nodule composition partially changed in the Fukagawa location, both factors would be involved in the determination of the nodule composition. In the second year, BeL7 found to be increased in all soils and locations, which might be due to the little higher temperature during one month after sowing in the second year.

Growth and competitive infection behaviors of two sets of *Bradyrhizobium* spp. strains were examined at different temperatures to explain the strain-specific soybean nodulation in Fukagawa and Miyazaki by using the *Bradyrhizobium* spp. strains isolated from the corresponding soils and locations. Each set consisting of three strains was as follows: *B. japonicum* Hh 16-9 (Bj11-1), *B. japonicum* Hh 16-25 (Bj11-2) and *B. elkanii* Hk 16-7 (BeL7); *B. japonicum* Kh 16-43 (Bj10J-2), *B. japonicum* Kh 16-64 (Bj10J-4) and *B. elkanii* Kh 16-7 (BeL7), which were isolated from the soybean nodules cultivated in the Fukagawa and Miyazaki soils, respectively. The growth of each strain was evaluated in yeast mannitol (YM) liquid medium at 15, 20, 25, 30 and 35 °C with shaking at 125 rpm for one week while measuring their OD_{660} daily. In the competitive infection experiment, each set of the strains was inoculated in sterilized vermiculite followed by sowing surface sterilized soybean seeds, and they were cultivated at 20/18 °C and 30/28 °C in 16/8 h (day/night) cycle in a phytotron for three weeks. After three weeks plant length and weight of shoot and root were measured, and number of nodules were counted. Then nodule composition of the inoculated strains was examined using randomly selected ten nodules per plant. The nodule compositions were determined based on the partial 16S-23R rRNA ITS gene sequence of the DNA extracted from the nodules.

The optimum growth temperatures were at 15–20°C for all *B. japonicum* strains, while they were at 25–35 °C for all *B. elkanii* strains. The shoot and root lengths, and the shoot and root weights were significantly higher at 30/28 °C than 20/18 °C in all treatments except for the root lengths of the soybeans inoculated with the Miyazaki strains. While the nodule numbers were not significantly different between the different temperature conditions. In the Fukagawa strains, Bj11-1 and BeL7 dominated in the nodules at the low and high temperatures, respectively. In the Miyazaki strains, BjS10J-2 and BeL7 dominated at the low and high temperatures, respectively.

In the Fukagawa soil, because *B. elkanii* BeL7, which has higher ability to grow and nodulate than the *B. japonicum* strains (Bj11-1 and 2) at the high temperature, did not appear in the Matsue and Miyazaki locations, it was suggested that the *B. japonicum* strains preferably proliferated in the Fukagawa soil, and Bj11-1 nodulated more preferably than Bj11-2. In the Miyazaki soil, *B. elkanii* BeL7 dominated in the nodules, but the *B. japonicum* strains BjS10J-2 and BjS10J-4 increased in the Fukagawa and Matsue locations, suggesting that both *B. japonicum* and *B. elkanii* proliferate in the Miyazaki soil, and *B. elkanii* (BeL7) dominated in nodules due to their temperature-dependent infection.