# Identification and Selection of Myanmar Indigenous Soybean-nodulating Bradyrhizobia for Biofertilizer Production with Streptomyces griseoflavus P4

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Title : Identification and Selection of Myanmar Indigenous Soybean-nodulating Bradyrhizobia for Biofertilizer Production with *Streptomyces griseoflavus* P4
（*Streptomyces griseoflavus* P4 を用いたバイオ肥料製造のためのミャンマーの土着のダイズ根粒菌 Bradyrhizobia の同定と選択）

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## Thesis Summary

Rhizobia play an important role in plant nutrition by fixing nitrogen that is subsequently available for uptake by plants. In this study, 120 strains were isolated from five major soybean-growing regions of Myanmar. The strains were characterized based on sequence analysis of the 16S-23S rRNA ITS region. Determination of nodulation types of native isolates was based upon their compatibility between strain and soybean cultivars by inoculation test. Sequence analysis indicated that all isolates belonged in the *Bradyrhizobium* genus and were conspecific with *B. liaoningense*, *B. elkanii*, *Bradyrhizobium* spp., *B. japonicum* and *B. yuanmingense*. A phylogenetic tree showed that 40, 33.3, 19.2, 5 and 2.5% of the isolates were related to *B. liaoningense*, *B. elkanii*, *Bradyrhizobium* spp., *B. japonicum* and *B. yuanmingense*, respectively. Among tested isolates, type A strains were accounted for 74% of the isolates, while type B and C strains accounted for 22 and 4%, respectively.

The selection of effective rhizobia for higher efficiency nitrogen fixation is one of the most important steps for inoculum production. Screenings of type A and type B strains using the specific soybean varieties were done with a completely randomized design and three replications in this study. In the first and second screening experiments, type A and type B strains with higher nitrogen fixation and proper nodulation on their respective soybean cultivars were selected. In the third screening experiment, *B. japonicum* SAY3-7 and *B. elkanii* BLY3-8, which had higher nitrogen fixing potential and proper nodulation, were selected as effective isolates. Based on the results of the screening experiment, these two strains were tested for their symbiotic efficacy in Futsukaichi soil. This study shows that inoculation treatment of SAY3-7 and BLY3-8 significantly increased plant growth, nodulation, and $N_2$ fixation at the V6, R3.5 and R8 stages in Yezin-3 ($Rj_4$) and/or Yezin-6 (non-*Rj*), and the seed yield at R8 stage, in Yezin-3 ($Rj_4$) and Yezin-6 (non-*Rj*) soybean varieties compared with the control treatment.

Co-inoculation of nitrogen-fixing bacteria with plant growth promoting-bacteria has become more popular than single inoculation of rhizobia or plant growth-promoting bacteria because of the synergy of these bacteria in increasing soybean yield and nitrogen fixation. However, proper inoculation density is important for successful nodulation and competition with indigenous rhizobia existing in the soil. In this present study, low density co-inoculation of soybean with *S. griseoflavus* P4 and SAY3-7 ($10^5$ cells $mL^{-1}$) were very effective in terms of nodulation, $N_2$ fixation, and plant

growth in a pot condition, suggesting that low inoculation density was optimal for soybean. In this study, low density co-inoculation of SAY3-7 (type A) and P4 significantly improved nodule number, nodule dry weight, shoot and root biomass, $N_2$ fixation, nutrient (N, P, K, Ca and Mg) uptake at various growth stages and seed yield in Yezin-6 (non-*Rj*) soybean cultivar compared with the control, but not the single inoculation treatments. Significant differences in plant growth, nodulation, $N_2$ fixation, nutrient uptake and yield between co-inoculation and control, not between single inoculation and control, suggests that there is a synergetic effect due to co-inoculation of SAY3-7 (type A) and P4.

The effects of co-inoculation of BLY3-8 (type B) with P4 on symbiotic effectiveness of $Rj_4$ soybean varieties was studied using Futsukaichi soil under natural environmental conditions. This study shows that co-inoculation of BLY3-8 and P4 significantly increased $N_2$ fixation at V6 stage; plant growth, nodulation, $N_2$ fixation and N uptake at R3.5 stage, and shoot growth, N uptake and seed yield at R8 stage, in $Rj_4$ soybean varieties compared with the control. Significant difference in plant growth, nodulation, $N_2$ fixation, N uptake and yield between co-inoculation and control, not between single inoculation and control, suggests that there is a synergetic effect due to co-inoculation of BLY3-8 (type B) and P4.

Biofertilizer production using rhizobia together with plant growth promoting bacteria has become popular because promotion of plant growth and nitrogen fixation are induced by the synergetic functions of different bacterial species. Before producing biofertilizer, synergetic effects of SAY3-7 (type A) plus BLY3-8 (type B) and P4 was studied. In this study, synergetic effects for nitrogen fixation was induced by combined inoculation of SAY3-7 plus BLY3-8 with P4 in all tested soybean varieties. Therefore, biofertilizer was produced using these effective bacteria: SAY3-7, BLY3-8 and P4. This biofertilizer was found to be significantly promoted plant growth, nodulation, nitrogen fixation, N and P uptakes, and seed yield in Yezin-3 and/or Yezin-6 soybean varieties at some growth stages cultivated in Futsukaichi soil.