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SUMMARY OF DOCTORAL THESIS

Name: Sabitri Adhikari Dhungana

Title: Study on plant growth promoting properties of endophytic bacteria affected by their abiotic and biotic environments
(非生物的および生物的環境条件に影響される細菌エンドファイトの植物成長促進特性に関する研究)

Endophytic bacteria inhabit the internal tissue of the plants and generally cause no harmful effects to the host plants. Many of them promote the growth of the host plant through biological nitrogen fixation, synthesis of plant hormones, and so on. Such functions seem to be affected positively or negatively by the host environment and interaction within microbial community. Many researchers have studied the beneficial endophytic properties and their effect on plant growth, but it has not been extensively examined how the endophytic environments and their interaction affect the plant growth.

In our previous study, diverse endophytic bacterial strains were isolated from sweet potatoes cultivated in Nepal, and inoculation of a mixed culture of the isolates improved fresh weight and vine length of sweet potato in a growth chamber, however, responsible strains have remained unclear. In this study, we selected the isolated eight bacterial endophytes from Salyan location, and examined their plant growth promoting properties in relation to effects of nitrogen level in the culture and interaction in the endophytic community in the plants.

The first purpose of this study was to specify the responsible strains among the selected strains, and examine the effects of nitrogen level on their production of indole-3-acetic acid (IAA) and nitrogen fixation activity and on the plant growth promotion by the endophytes. Among the selected eight strains, *Klebsiella* sp. Sal 1, *Enterobacter* sp. Sal 3, *Rhizobium* sp. Sal 4, *Agrobacterium* Sal 7 and *Microbacterium* sp. Sal 4 produced IAA at 65, 40, 20, 13 and 4 μg/mL, respectively, in 0.1g/L NH_4NO_3 amended Modified Rannie (MR) medium. In the two *nif*H gene containing strains, *Klebsiella* sp. Sal 1 showed higher acetylene reduction activity than *Herbaspirillum* sp. Sal 6in MR medium. Inoculation of the strains showed positive effects on the growth of sweet potato in three different experiments in nitrogen non-limiting (1/2MS) conditions cultivated in vermiculite pot and agar tube. Based on the plant growth promoting properties, *Klebsiella* sp. Sal 1 and *Enterobacter* sp. Sal 3 were selected for further study. The high IAA producers, *Klebsiella* sp. Sal 1 and *Enterobacter* sp. Sal 3, showed optimum concentrations at 15–60 mg NH_4NO_3/L for IAA production in plant growth basal medium. For the acetylene reduction activity, *Klebsiella* sp. Sal 1 showed the higher activity at 0–6.25mg NH_4NO_3/Lin MS medium, while no activity was observed in *Herbaspirillum* sp. Sal 6. Under the nitrogen-limiting conditions (1/2MS with NH_4NO_3 at 120 mg/L), inoculation of the two selected strains showed positive effects on the root number of the sweet potato cuttings. In the tomato seedlings grown on Kimwipes in the liquid medium test tube cultivation conditions, the inoculation of the endophytes showed the tendency to increase total fresh weight and root fresh weight but not shoot and root lengths under both nitrogen-limiting and non-limiting conditions. While in the gelritepetri dish cultivation conditions, the effects of the inoculation were apparent in all of the growth parameters under nitrogen

non-limiting conditions. As both the inoculants colonized rhizosphere, root and shoot part of inoculated tomato seedlings, it was supposed that the endophytes produced IAA in plant where lower nitrogen levels were expected. *Klebsiella* sp. Sal 1 also improved the growth of strawberry seedlings under the nitrogen non-limiting gelrite petridish conditions with high colonization of the inoculant in rhizosphere, root and leaf parts.

Bacterial production of IAA and its effects on plant growth have been much studied but less is about the ecology of IAA degrading bacteria. Some studies have shown that plants harbor not only IAA producing bacteria, but also IAA degraders as member of epiphytic and rhizospheric bacterial community.

The second purpose of this study was to examine the IAA degrading ability of the selected eight endophytic isolates and to elucidate the interaction between the IAA producer and degrader by their co-inoculation. All of the strains including five IAA producers showed the IAA degrading ability, among which *Herbaspirillum* sp. Sal 6 had the highest activity. Large difference in growth of Sal 6 in the media with and without IAA suggested that the IAA degrader utilized IAA as a source of energy. When IAA-producing *Klebsiella* sp. Sal 1 and *Enterobacter* sp. Sal 3 were co-cultivated with IAA degrading *Herbaspirillum* sp. Sal 6 in tryptophan amended medium, IAA concentrations detected were decreased compared with the single inoculation of the IAA producers. As *Herbaspirillum* sp. Sal 6 showed high activity for degrading tryptophan, it was suggested that the co-inoculation of the strain Sal 6 decreased the concentration of IAA by degrading IAA produced by the IAA producers, and/or by degrading tryptophan, a precursor of IAA for the IAA producers. When the IAA producer and degrader were individually or co-inoculated to tomato plant to examine the interactive effects on the plant growth, inoculation of the IAA producing strain *Klebsiella* sp. Sal 1 increased the root fresh weight, but the effect was reduced by co-inoculation of the IAA degrading strain *Herbaspirillum* sp. Sal 6. Root length and fresh plant weight were not affected by the any inoculations. In radish plant, inoculation of the IAA producer increased the root fresh weight and total fresh weight, and the effect was reduced by co-inoculation of the IAA degrader as the tomato experiment. In the individual inoculation, both *Klebsiella* sp. Sal 1 and *Herbaspirillum* sp. Sal 6 colonized tomato and radish plants in high populations. Colonization in rhizosphere was 2-3 order higher than root and leaf in both plants. Population of Sal 1 in tomato was 13 times higher in root than leaf, whereas Sal 6 was 7 times higher in leaf than root. In case of radish, the populations in root and leaf were almost same in both strains. In the co-inoculation, the rhizosphere, root and leaf were colonized by the bacteria in the similar way as in the individual inoculation. Relative percentage of the population of Sal 6 was higher in all plant parts (75-95%) than Sal 1 except for the root of tomato (33%). High colonization of the inoculated strains suggested that the IAA degrader decreased IAA levels in the plants by degrading IAA and/or its precursor tryptophan. Similar response of tomato to exogenous IAA with that caused by the IAA producing *Klebsiella* sp. Sal 1 suggesting that the root growth promotion observed in the inoculation of *Klebsiella* sp. Sal 1 was due to IAA produced by the strain.

In this study, colonization of the plant tissue by the endophytes at high population suggested their potential to establish symbiotic relationship with the host plants. Considering their usage as biofertilizer for actual agriculture, their probable positive and negative interactions with existing indigenous endophytic microbial community should be considered. Therefore, the observed effects of plant growth promotion by the inoculants in laboratory experiments would not act in the same way under the field conditions. Further study on understanding the mechanisms of the interactions with several biotic and abiotic environmental factors could help to achieve the positive potential of the efficient endophytes in agriculture.