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SUMMARY OF DOCTORAL THESIS

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Title: Effects of Inoculation of Plant Growth-Promoting Bacteria on Plant Growth, Bacterial Endophytic Community Structure and Colonization
(植物成長促進細菌の接種が植物成長、内生細菌の群集構造および植物内定着に及ぼす影響)

Sweet potato (*Ipomoea batatas* L.) grows well even in infertile and nitrogen-limited fields, and endophytic bacterial communities have been proposed to be responsible for this ability. Plant-growth-promoting bacteria are considered eco-friendly and are used in agriculture, but their application can interact with endophytic communities in many ways. In this study, a commercial biofertilizer, OYK, consisting of a *Bacillus* sp., was applied as PGPR (about 8E+9 CFU/mL) to two cultivars of sweet potato, and the effects on indigenous endophytic bacterial communities in field conditions were examined. One milliliter of OYK solution was diluted to 4L with sterilized distilled water, and twelve seedlings of each cultivar were dipped in the solution for 60 h. The same numbers of the seedlings were soaked in distilled water as a control. At harvest, the fresh weights of the shoots and tubers of each sweet potato plant were measured. The surface of each tuber sample was washed with running tap water for 10 min and cut longitudinally with a sterilized knife at its middle part after wiping off the water with a sterilized paper towel. Then, the cut surface was stamped on modified MR agar medium, with and without the supplementation of ammonium nitrate as a nitrogen source in a petri dish. Originally, 269 bacterial colonies appeared on the agar plates in total, of which 232 strains were successfully isolated. Based on their morphological relative abundance, 1–3 representative isolates were selected from each group, comprising 30–81% of the original isolates; as a result, 109 isolates were selected, in total, for further analysis. Among the 109 selected endophytic bacterial isolates, 101 strains were successfully sequenced for the partial 16S rRNA gene. The isolates belonged to 25 bacterial genera in 9 classes, which showed 97–100% homology.

Although the inoculated OYK was not detected and significant plant-growth-promoting effects were not observed, the inoculation changed the endophytic bacterial composition, and the changes differed between the cultivars, as follows: *Novosphingobium* in α-Proteobacteria was dominant; it remained dominant in Beniharuka after the inoculation of OYK, while it disappeared in Beniazuma, with an increase in *Sphingomonas* and *Sphingobium* in α-Proteobacteria as well as *Chryseobacterium* and *Acinetobacter* in Flavobacteria. The behavior of Bacilli and Actinobacteria also differed between the cultivars. The Shannon diversity index (H) increased after inoculation in all conditions, and the values were similar between the cultivars. Competition of the inoculant with indigenous rhizobacteria and endophytes may determine the fates of the inoculant and the endophytic community. When the commercial biofertilizer, OYK, consisting of a *Bacillus* sp., was applied to two cultivars of sweet potato, the inoculation changed the culturable indigenous endophytic bacterial communities, differently between the cultivars, and increased the diversity of the bacterial communities, although the inoculated OYK was not detected and significant plant-growth-promoting effects were not observed. Competition of the inoculant with indigenous rhizobacteria and endophytes may determine the fates of the inoculant and the endophytic community. Origin of the inoculant, which was isolated from soil, was expected as the possible reasons for the lack of the endophytic potential.

As many endophytic *Bacillus* strains have already been reported in several plants, it is assumed that endophytic bacteria have some colonization strategies in interaction

with plants. For efficient and practical use of PGPR, it is essential to understand its colonizing behavior and abilities to compete with co-existing bacteria. Though several studies have been reported on the effects of co-inoculation with multiple bacteria on plant growth, their effects on colonization have not been extensively studied yet. Then we designed a new experiment intending to evaluate the colonization properties of *Bacillus* sp. OYK, which was isolated from a soil, in relation to its origin by comparing it with those of the other *Bacillus* sp. strains isolated from plant endosphere and rhizosphere, and then to elucidate the effects of co-inoculation of the endophytic *Bacillus* sp. strain with the other endophytes on their colonization and plant growth-promoting activities.

In addition to *Bacillus* sp. OYK, three strains of *Bacillus* sp.: two strains (*Bacillus* sp. RF-12 and RF-37) isolated from the rhizosphere of sweet potato and another one (*Bacillus* sp. F-33) as an endophyte of the same plant cultivated in Japan, and three strains of endophytes: *Herbaspirillum* sp. Sal 6, *Klebsiella* sp. Sal 1, and *Enterobacter* sp. Sal 3, isolated from Nepalese sweet potato were used in this study. Surface-sterilized seeds of tomato (*Solanum lycopersicum* L. cv. Chika F1 hybrid, Takii & Co., Ltd., Kyoto, Japan) were sown in the sterilized vermiculite in a Leonard jar supplied with the sterilized Hoagland solution, and 1ml of the four each *Bacillus* sp. strains were inoculated (at ca. 10^8 CFU/ml) onto the seed zone. After cultivation in a phytotron at 28/25°C (16h/8h, day/night), plant growth parameters and population of the inoculants in the root, shoot, and rhizosphere were determined. Statistical analysis of the data on the plant growth and population of the inoculant obtained in each twice-repeated experiment was performed using the MSTAT-C 6.1.4 software package. Data were subjected to Tukey's test after one-way ANOVA. In addition, effects of co-inoculation and time interval inoculation of *Bacillus* sp. F-33 with the other endophytes were examined. All *Bacillus* sp. strains promoted plant growth except for *Bacillus* sp. RF-37, and populations of the rhizospheric and endophytic *Bacillus* sp. strains were 1.4–2.8 orders higher in the tomato plant than that of *Bacillus* sp. OYK. The plant growth promotion by *Bacillus* sp. F-33 was reduced by co-inoculation with the other endophytic strains: *Klebsiella* sp. Sal 1, *Enterobacter* sp. Sal 3, and *Herbaspirillum* sp. Sal 6., though the population of *Bacillus* sp. F-33 maintained or slightly decreased. When *Klebsiella* sp. Sal 1 was inoculated after *Bacillus* sp. F-33, the plant growth-promoting effects by *Bacillus* sp. F-33 were reduced without a reduction of its population, while when *Bacillus* sp. F-33 was inoculated after *Klebsiella* sp. Sal 1, the effects were increased in spite of the reduction of its population. *Klebsiella* sp. Sal 1 colonized dominantly under both conditions.

In expansion to plant growth-promoting properties, the colonization potential should be considered as important criteria when assessing their suitability for commercial development. The lower population of *Bacillus* sp. OYK, which was isolated from soil, than the other *Bacillus* sp. strains, which were isolated from either the rhizosphere or endosphere of plant samples, suggests the importance of the origin of the strains for their colonization. The plant growth promotion and colonization potentials were independently affected by the co-existing microorganisms. Further studies are necessary to evaluate the colonization potential of PGPR under field conditions where diverse microorganisms exist.