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SUMMARY OF DOCTORAL THESIS

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Title:

STUDY ON CHARACTERIZATION OF ENDOPHYTIC BACTERIAL COMMUNITIES IN SWEET POTATO (*Ipomoea batatas*) CULTIVATED IN DIFFERENT CLIMATIC CONDITIONS

（異なる気候条件下で栽培されたサツマイモ(*Ipomoea batatas*) の内生細菌群集に関する研究）

Sweet potato (*Ipomoea batatas* L.) is a resilient, easily propagated crop which grows well in marginal lands and can be cultivated in low-fertile soils, and takes up more nitrogen than other root crops. There are reports that sweet potato harbors different types of endophytic bacteria and the effects of climatic conditions on the endophytic bacterial community compositions are limited. In the context of Nepal, there is no information on the sweet potato endophytes till date. On the other hand, Nepal, a small Himalayan country, lies along the southern slopes of the Himalayan Mountains between China and India and varies in topography, climate and vegetation; the elevation ranges from 68 to 8,848 masl in a just 150 to 250-km south–north transect.

Therefore, we aimed to examine bacterial community of sweet potato endophytes in Nepal in relation to the environmental parameters and characterize their plant growth promoting traits. As synergistic effect of mixed cultures of plant growth promoting bacteria was reported, we also examined their potential by inoculating combined isolates from each location. Sweet potato tubers were collected from three months old plants during the autumn of 2015 representing 12 sweet potato growing sites in Nepal, six from subtropical and six from temperate regions. Then the sweet potato samples were washed with tap water, shade dried and kept at room temperature until the isolation of the endophytic bacteria. The sweet potato samples were washed again with running tap water for 10 min. Each sample was cut transversely when its diameter was more than 10 mm otherwise cut longitudinally. Then, the cut surface was stamped on the modified MR agar medium, and incubated for 2 days at 26^{0}C. The appeared colonies were grouped based on their morphologies and the representative colonies reflecting their relative abundance were purified for further analysis as endophytes. The partial 16S rRNA genes of the isolated endophytic bacteria were amplified using the universal primers (fD1 and rP2) to the domain bacteria. In addition the isolates were examined for their indole-3-acetic acid (IAA) producing ability, nitrogenase gene, antagonistic effect against bacterial and fungal pathogens, and cellulase and pectinase activities in in vitro condition. As a result, we isolated 243 endophytic bacteria belonging to 34 genera in six classes from 12 locations of Nepal. Among them, the predominant classes were Bacilli and γ-Proteobacteria. The principal component analysis revealed that the composition of bacterial classes was unrelated to the environmental parameters of the sampling sites. Regarding their plant growth promoting potentials, 57% of the strains demonstrated IAA producing ability while 5% strains had nitrogen fixing gene (*nifH*) and acetylene reduction assay (ARA) activity. The representative strains in all six classes showed antagonistic effect against bacterial pathogens while only *Bacillus* strain showed the effect against fungal pathogen. For endophytic traits, cellulase activity was observed in 5 classes, while pectinase activity was only in Proteobacteria. Fresh weight and vine length of sweet potato increased by inoculating mixed cultures of the isolates from each location. In

this study, we observed that the cultivating locations did not affect the sweet potato endophytic community and distinction of the effects between the climate and the soil was not clear.

So we designed next experiment with an aim to examine the effects of soil and climatic conditions on the endophytic bacterial communities of sweet potato by using the same soil at different locations and applying culture-dependent and independent approaches. For culture-dependent analysis, one tuber from each cultivation conditions was used for analysis. The tubers were washed in a running tap water for 10 min and then rinsed with sterilized distilled water. Then, cork-borer was perpendicularly inserted into the six different parts across the longitudinal axis of the tuber, each ca. 0.5 g making a total of ca. 3 g tuber samples. The samples were then placed in a sterilized mortar and macerated with 6 mL sterilized distilled water under aseptic conditions. Further, serial 10-fold dilutions were prepared up to 10^{-7}, and each 0.1 mL aliquot was taken and spread on modified MR media supplemented with 0.1 g NH_4NO_3/L and incubated at 26^0C for further analysis. As a result, sixty two colonies were isolated and identified. γ-Proteobacteria (96%), β-Proteobacteria (87%) and Actinobacteria (88%) dominated in the samples cultivated in Fukagawa, Matsue and Miyazaki soils at the corresponding locations, respectively. When the soil samples were used in the different locations, the above mentioned location-specific phyla increased at the new sites. The endophytic bacterial population was also affected by the cultivating locations. It was suggested that the location rather than the soil influenced on the endophytic community and population.

In culture-independent approach, locked-nucleic acid-PCR clamping technique and next-generation technology were used to examine the effect of the soils and the locations on the whole community. Briefly, each ca. 5 g of frozen sweet potato tuber was taken from each pot, and macerated to a fine powder with a sterile mortar and pestle in liquid nitrogen, and then total DNA was extracted using ISOPLANT II plant DNA extraction kit according to the manufacturer's instruction. The extracted DNA was applied to selective amplification of bacterial DNA using LNA-PCR clamping technique. The LNA PCR products were applied for metagenomic analysis and the 16S rRNA gene amplicon libraries were prepared following the "16S Metagenomic Sequencing Library Preparation Protocol" (Illumina) using the Nextera XT index kit. The study revealed that Proteobacteria (85.0%), Bacteroidetes (6.6%) and Actinobacteria (6.3%) were the three most dominant phyla, accounting for 97.9 % of the reads, and γ-Proteobacteria (66.3%) being the most abundant. The overall endophytic communities were similar among the samples and top 10 genera represented 81.2% of the overall reads in which *Pseudomonas* (31.9-45.0%) being the most predominant operational taxonomic units. Principal component analysis and Shannon diversity indices showed a tendency that the location was more important than the soil to determine the sweet potato endophytic bacterial community.

In conclusion, sweet potato tubers were dominated by specific but different endophytic bacteria with plant growth promoting potentials, and the endophytic bacterial compositions were unrelated with the cultivating locations. Effect of climate and soil on endophytic bacterial compositions was analyzed by culture-dependent and independent methods showed different results. The former method showed that the climate of the specific locations influenced on the endophytic community compositions, while the later indicated that the endophytic community compositions were similar among the samples cultivated in different climate and soil conditions.

Further study on why the specific groups of endophytes dominate the sweet potato tubers, and how the climate and the soil effect on the endophytic community compositions and population is to be explored.