（第３号様式）(Form No. 3)

学 位 論 文 要 旨
Dissertation Abstract

氏名： Md. Rokunuzzaman
Name

学位論文題目：
Title of Dissertation

Study on soil bacterial communities in response to different agricultural activities
（農業活動による土壌細菌叢の変動に関する研究）

学位論文要旨：
Dissertation Abstract

Soils are considered to be complex and dynamic living substances with diverse microbial habitats in Earth. Soil bacterial communities are very important especially for agriculture owing to play an essential role to maintain soil ecosystem functions and biogeochemical cycle by decomposing organic matters, nutrient recycling and mineralization processes. In this study, I studied soil bacterial communities in response to three different aspects of agricultural management. I used PCR-DGGE and amplicon pyrosequencing using 16S rRNA gene partial sequences for analyzing bacterial communities.

Little is known about the effect of soil disinfection on bacterial communities. I used four chemical and biological materials against natural farm soil. Soil was treated with wheat bran and ethanol for soil reduction, chloropicrin for chemical fumigation, and mustard greens (*Brassica juncea)* for soil reduction plus biofumigation. Tomato seedlings were planted after disinfection treatment. Total organic carbon, which reflects microbial biomass, was significantly low in chloropicrin-applied soils. The overall bacterial community structures were similar based on DGGE analyses among soils except chloropicrin treatment. Several chloropicrin-treated specific bands were observed. Bacterial species richness in chloropicrin-applied soils was about one-half of those in other soils and recovered to some extent after planting tomato. The two most abundant phyla were *Acidobacteria* and *Proteobacteria* in farm soil. Soil reduction with ethanol, wheat bran, and mustard greens did not change phylum structure very much. However, chemical fumigation with chloropicrin made the phylum *Firmicutes* dominant by occupying more than 80%. After planting tomato, the proportion of *Firmicutes* was reduced to basal level and the phyla *Bacteroidetes* and *Proteobacteria* became dominant. A taxonomic classification on OTU level supported this observation. Beta diversity analyzed by Principal Coordinate Analysis (PCoA) showed bacterial communities in soils except chloropicrin treatment formed a cluster. A community of chloropicrin-applied soil was quite different from those of other soils and also distinct after planting tomato, assuming that bacterial community structure was dramatically altered by chemical chloropicrin fumigation and shifted over time, while it did not go

back to original state.

Most of the terraced rice-fields were abandoned or have been changed to citron fields and planted forests in the hilly and mountainous area at Otoyo, Kochi, Japan. DGGE band patterns of each soil were quite similar, although the several paddy-field specific DNA bands were observed. Based on pyrosequencing data, bacterial species richness and diversity was slightly higher in paddy fields than in other soils. While overall bacterial community structures of soils at different land uses were similar, beta-diversity analysis clearly indicated soils were divided into three clusters. Detail bacterial community structure of paddy fields was different from those of non-paddy field lands. Interestingly, community structures of crop fields, which had not been used as paddy field, were different from those of at least one-time paddy fields. This might reflect the history of land use.

Aseptically grown seedlings of tobacco and eggplant were planted in pots filled with soil prepared from normal experimental greenhouse of Kochi University and grown under 16h light and 8h dark at 25 °C. Although no plants were planted on the same soil as a control, weeds happened to grow on several pots. This might be because of preexisting seeds in greenhouse soil. I used these pots as another control. As expected, overall bacterial community structures of five soils, original dried soil, water-fed soil without plants, soil with tobacco, eggplant, and weed, did not show much differences. Several species of cyanobacteria belonging to genus *Bacillariophyta* became dominant only in wet soil. Fed fertilizer might cause the emergence of these bacteria under light. Pyrosequencing analysis indicated that the original dried soil was mainly dominated by bacterial species belonging to phyla *Firmicutes*, *Actinobacteria* and *Proteobacteria*, which are quite normal bacterial communities. In other four wet soils, cyanobacteria OTU1280 belonging to genus *Bacillariophyta* was dominant. DGGE and pyrosequencing analyzed the different hypervariable regions of 16S rRNA gene. Although it is difficult to identify the two dominant cyanobacteria found in both DGGE and pyrosequencing, it seemed to be the same clone. OTU2821 belonging to genus *Sediminibacter* was found only in eggplant rhizosphere. The closest clone to OTU2821 is identified in tomato soil, indicating that this clone is indeed specific in eggplant rhizosphere. OTU3300 belonging to Gammaproteobacteria was dominant only in tobacco rhizosphere. OTU263 belonging to order *Rhodospirillales* was not detected in control, indicating that this clone could be a common bacterium residing in rhizosphere. Interestingly dominant clones only in weed rhizosphere were not observed. This might indicate that weed grows routinely in greenhouse soil and adapts to the environment. As a result, weed-root associated bacteria are already fixed in the soil.