Analysis of determining factors on community structure of soil bacteria in volcano ash soil (Kanto Loam) farming field using PCR-DGGE method

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ABSTRACT

Soil bacteria were analyzed to discuss factors affecting structure and diversity of their community in two farming fields with different soil types (red loam and Andosol) originated from volcano ash soil, Kanto Loam, in Japan. Soybean, barley and maize were grown in rotation with different fertilization from 2007 to 2008. The bacterial 16S rDNA sequences extracted from the individual soil sample was amplified by PCR and detected by denaturing gradient gel electrophoresis (DGGE). Similarity of bacterial community structure among the samples was analyzed by principal component (PC) analysis on the detected DNA band patterns. The crop species affected much on the detected band patterns. The largest number of bands was detected from maize soil, suggesting high diversity of bacterial species in the community, while the smallest number of bands was from soybean soil. Mapping of the soil samples by the PC1, PC2 and PC3 also demonstrated the clear differences in structure of bacterial communities among three crop species. In maize and barley, growth stage of crops was the primary major factor determining the structure of bacterial communities, and the soil type was the second one. In soybean soil, the band pattern was rather stable comparing with maize and barley. The effects of fertilization were not obvious in any crops. The results indicate that the large effects of plant species in crop rotation and their growth stages have large effects on the communities of soil bacteria, while there also were rhizosphere effects.

KEYWORDS: Barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), PCR-DGGE method, soil bacteria, soybean (*Glycine max* (L.) Merr.)

1. INTRODUCTION

Global-scale increase of population has still been continuing in this century and there is necessity for stable food-supply to feed them. At that time environment-friendly and sustainable agriculture is required. One of possible tools to establish such agricultural system of crop production is to utilize soil microorganisms effectively. Studies on microorganisms in soil, however, have difficulties, mainly because most of them are almost impossible to be cultured. Therefore, we used the molecular biological method (Muyzer et al., 1993) instead of culture method to examine DNA of soil bacteria extracted from soil (Hasebe et al., 2003). The objective of this study is to investigate structure and diversity of soil bacterial community in both rhizosphere and non-rhizosphere with reference to crop species, their growth stages, soil types and management practices including fertilization in the field of rotation for about 30 years using principle component analysis.

2. MATERIALS AND METHODS

2.1. Plant cultivation and soil sampling

Soil samples for extraction of bacterial DNA were taken from two fields with different types of

top soil, red loam soil and Andosol, where soybean-barley-maize-barley have been rotated under same three fertilization, namely chemical fertilizer, compost and no fertilization, from 1980 to 2007 (Yamagishi and Matsuzaki, 1998). Soil samples were taken from each experimental plot at flowering and harvesting for soybean (summer in 2007, cultivar Enrei), booting stage and ripening period for barley (winter in 2007-2008, cultivar Doriru-mugi) and tassel booting stage and ripening stage for maize (summer in 2008, cultivar Gold Dent Hybrid KD720). At that time, rhizosphere soil was defined as adhered to root and non-rhizosphere soil was taken from inter-row space. The same cultivar of maize was also grown in 1/5000 a Wagner pots with topdressing nitrogen or phosphorous fertilizer 30 days after planting. After topdressing, soil samples were taken to analyze community structure of soil bacteria in the rhizosphere for ten days. Also non-rhizosphere soil samples were taken from pots with no plants.

2.2. Analysis of soil bacteria using PCR-DGGE method

DNA of soil bacteria was extracted from soil for PCR-DGGE analysis. Skimmed milk was added into soil samples to prevent adsorption of extracted bacterial DNA by soil particles as recommended by Takada-Hoshino and Matsumoto (2004). At that time soil sample was treated by using bead beating kit (ISOIL for Beads Beating, Nippon gene Inc.) to penetrate skimmed milk through destroying soil structure. A detailed method followed the protocol of the kit. The V_3 region of bacterial 16S rDNA extracted from soil samples was amplified by PCR. PCR products were analyzed by 30-60% denaturing gradient gel electrophoresis (DGGE) (Doi *et al.* 2007). Community structure of soil bacteria was compared among the soil samples by the principal component (PC) analysis based on the detected DNA bands patterns.

3. RESULTS AND DISCUSSION

3.1. Effects of crops, growth stage, soil types and fertilization on bacterial community structure

Application of skimmed milk with bead beating treatment for the extraction of soil bacterial DNA enabled clear observation of PCR-amplified 16S rDNA bands separated by DGGE. The crop species affected much on the detected 16S rDNA band patterns. The largest and smallest numbers of DNA bands were detected in soil samples from maize (Figure 1) and soybean, respectively. Mapping of the bacterial community structure characterized by the combinations of PC1-PC4 showed clear differences among the three crop species (Figure 2). In maize and barley, growth stages and soil types were the first and second major factors affecting structure and diversity of bacterial community structure. The effects of fertilization were not obvious in any crops. The influence of soil types was more significant comparing with fertilization, probably because nitrogen and the carbon contents of the Andosol soil were higher than that of the red loam soil.

3.2. Rhizosphere effect in the pot experiment of maize

Although there was no significant difference between community structures of soil bacteria in rhizosphere and non-rhizosphere soils of maize field in the field experiment, the pot experiment showed more numbers of DNA bands in rhizosphere suggesting rhizosphere effect by maize roots that could enhance the diversity of bacteria. Effect of topdressing on the soil bacteria was obscure in the pot experiment as in the field experiment.
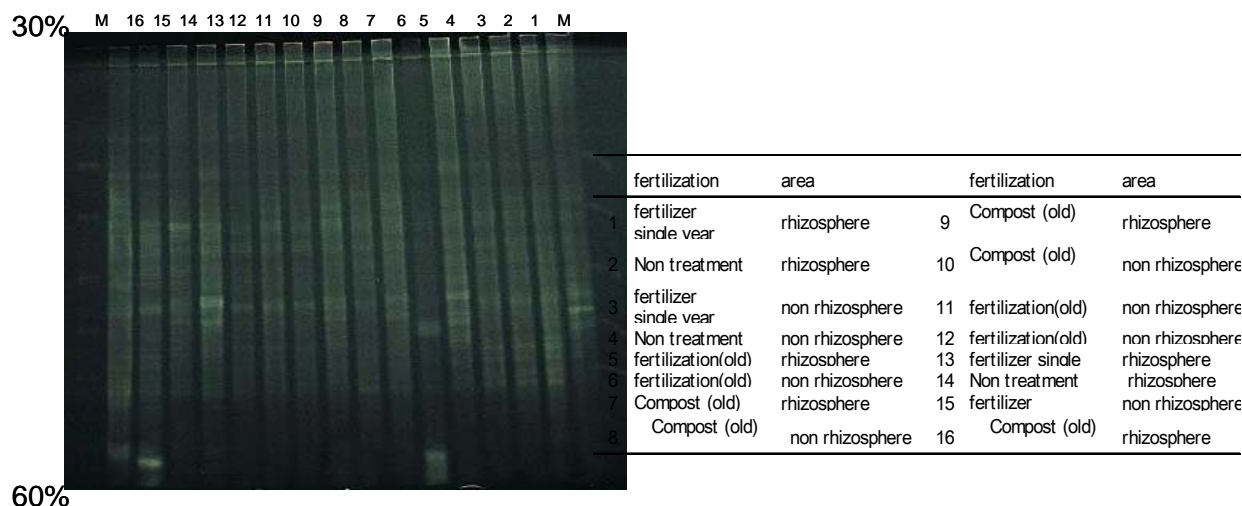
	fertilization	area		fertilization	area
1	fertilizer single year	rhizosphere	9	Compost (old)	rhizosphere
2	Non treatment	rhizosphere	10	Compost (old)	non rhizosphere
3	fertilizer single year	non rhizosphere	11	fertilization(old)	non rhizosphere
4	Non treatment	non rhizosphere	12	fertilization(old)	non rhizosphere
5	fertilization(old)	rhizosphere	13	fertilizer single	rhizosphere
6	fertilization(old)	non rhizosphere	14	Non treatment	rhizosphere
7	Compost (old)	rhizosphere	15	fertilizer	non rhizosphere
8	Compost (old)	non rhizosphere	16	Compost (old)	rhizosphere

Figure 1. 16S rDNA band patterns of soil bacteria in maize field obtained by PCR-DGGE method. Tassel booting stage, Summer 2008.

[Crops] S: soybean, B: barley, M: maize, [Soil] L: Red loam soil, A: Andosol, [Fertilizer] C/c: no fertilizer, F/f/i: chemical fertilizer, O/o: compost, [Site] R/N: rhizosphere/non-rhizospere soils, [Year] 7: 2007, 8:2008, [Growth stage] 1/2: first and second sampling times
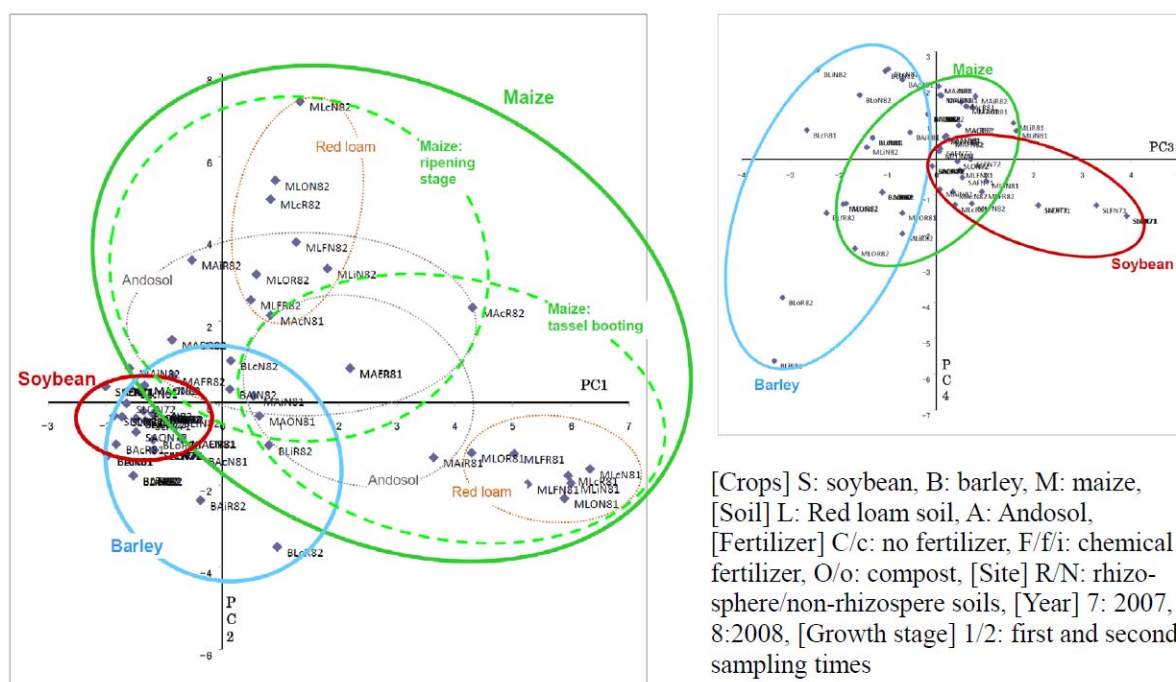
Figure 2. Analysis of principal component (PC) on the basis of bacterial DNA band patterns obtained by PCR-DGGE. Similarity among the soil samples was analyzed by PC1 and PC2 (a) and PC3 and PC4 (b) .

CONCLUSION

Results from this study show that crop species, soil types and rhizosphere affect great influences on structure and diversity of bacterial community, though both short- and long-term effects of

fertilization were not significant. It is partly because the amount and components of root exudate, which depends on crop species and growth stages, can affect soil bacteria (Vancura, 1964), and partly because differences in amount of organic matter and available nutrient, both of which depend on soil types, have strong influences on soil bacteria. In addition, soil physical and chemical properties that are affected by seasonal climate change and soil fertility should be taken account to discuss the structure and diversity of bacterial community in arable lands.

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