**PB-11**

**Isolation of N₂O-producing fungi from upland field soil applied with organic pelleted fertilizer**

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Key word: Nitrous oxide emission, upland field soil, organic pelleted fertilizer, Fungi

High N₂O emission was observed in Andisol upland soils. Surface-applied with organic pelleted fertilizer, when the pellets were apparently surrounded by fungal mycelia after the rainfall. This strongly suggested that N₂O production through fungal denitrification. Objectives of this study were to examine the effect of organic pelleted fertilizer application on fungal community of the field soil, and to isolate the fungi responsible for N₂O production. When above-mentioned N₂O emission was observed, surface soil containing organic pelleted fertilizer was collected, and separated into the organic fertilizer pellets and residual soil. Fungal population (CFU) in separated fertilizer pellets was ca. 10 times larger than that in residual and control soil. DGGE analysis revealed that the fungal population structure in soil was obviously influenced by the pelleted fertilizer application; genus *Fusarium, Mucor* and *Nectria* dominate the fungal community in separated fertilizer pellets and residual soil. Strains belonging to genus *Fusarium* were most frequently isolated, followed by *Mucor* and *Nectria*. *F. oxysporum* and *F. solani* strains from separated fertilizer pellets showed strong capability to produce N₂O through denitrification. These fungal strains might be responsible, at least in part, for the N₂O emission observed in Andisol field soil applied with organic pelleted fertilizer.

**PB-12**

**A novel method for RNA extraction from soil revealed amo4 genes expressed in Andosols in response to ammonium sulfate treatment**

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Key word: Soil, RNA extraction, Andosol, amo4

The lack of a universal method to extract RNA from soil hinders the progress of studies related to nitrification in soil, which is an important step in the nitrogen cycle. It is particularly difficult to extract RNA from certain types of soils such as Andosols (volcanic ash soils), which is the dominant agricultural in Japan, because of RNA adsorption by soil. To obtain RNA from these challenging soils to study the bacteria involved in nitrification, we developed a soil RNA extraction method for gene expression analysis. Auto clave casein was added to an RNA extraction buffer to recover RNA from soil and high quality RNA was successfully extracted from eight types of agricultural soils that were significantly different in their physicochemical characteristics. To detect bacterial ammonia monooxygenase subunit A gene (amo4) transcripts, bacterial genomic DNA and mRNA were co-extracted from two different types of Andosols during incubation with ammonium sulfate. PCR-DGGE and RT-PCR-DGGE analyses of amo4 in soil microcosms revealed that only few amo4, which had the highest similarities to those in *Nitrosospira multiformis*, were expressed in these soils after treatment with ammonium sulfate although multiple amo4 genes were present in the soil microcosms examined.

**PB-13**

**Sequence analysis of a fosmid clone possessing AOA amo4 and 16S rRNA genes from agricultural soil**

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Key word: AOA amo4, 16S rDNA, fosmid clone, soil

There is little information about soil organisms that contribute to the global N-cycle in natural ecosystems, because most of them cannot be cultivated in the laboratory. To study members or functions of such organisms containing ammonia-oxidizing archaea, PCR products of 16S rRNA genes (rDNA) or some functional genes such as ammonia monooxygenases (amo) amplified from DNA extracted from soils have been used. In such studies, it is very difficult to relate some detected functional genes to rDNA sequences.

In this study, fosmid library was constructed using DNA extracted from a spinach field soil. From the library, one clone, KO6osH4E8 was found to have both genes encoding ammonia monoxygenases of ammonia-oxidizing archaea (AOA amo4) and 16S rDNA. Nucleotide sequence of 35 kb insert in clone KO6osH4E8 was analyzed and compared with that of reported fosmid clone $546_\text{sp}$ derived from a calcareous grassland soil which also had AOA amo4 and 16S rDNA. Similarity of nucleotide sequences between AOA amo4 and 16S rDNA from each clone were 94.8% and 89.3%, respectively. Diversities of AOA amo4 and 16S rDNA in the same spinach field soil were analyzed by sequencing the PCR products. This analysis indicated that AOA amo4 and 16S rDNA of clone KO6osH4E8 was one of dominant group in the spinach field soil.


**PB-14**

**Analysis of community structure of ammonia oxidizing bacteria and archaea in agricultural soils under long-term application of chemical fertilizer and cow manure**


Key word: ammonium-oxidizing bacteria, ammonia-oxidizing archaea, community analysis, cow manure, pyrosequence

Ammonium-oxidizing bacteria (AOB) and archaea (AOA) play an important role in agriculture ecosystems. The aim of this study was to clarify the influence of long-term application of chemical fertilizer and cow manure on community structures of AOB and AOA in agricultural fields. The fertilizer experiment included 6 treatments: chemical fertilizer (CF), combination of chemical fertilizer and cow manure (OC), cow manure (CM), threefold cow manure (3CM), poultry manure (PM) and the control (without fertilization, CT). AOB and AOA amo4 genes in total DNA extracted from soils were amplified by PCR with the specific primer sets. The PCR products were analyzed using DGGE and pyrosequencing methods. DGGE profiles and deep sequencing analysis showed that the community structures of AOB and AOA in CM and 3CM treatments were similar to those in CT and PM treatments but differed from those in CF and OC treatments. The community structures of AOB and AOA in the used manures were not reflected in those in the manure-treated soils. Abundance of AOA and AOB was determined by quantitative PCR targeting amo4. The AOB abundance in 3CM treatment was ten times higher than in CT. The abundance was approximately the same as those in CF and OC treatments. These data suggest that cow manure do not shift the AOB community structure but increase AOB abundance in soils after long-term application.