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SHORT COMMUNICATION

# Eukaryotic communities associated with the decomposition of rice straw compost in a Japanese rice paddy field estimated by DGGE analysis

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**Abstract** To estimate the succession and phylogenetic composition of the eukaryotic communities responsible for the decomposition of rice straw compost under flooded conditions during the cultivation period of paddy rice, denaturing gradient gel electrophoresis (DGGE) analysis targeting 18S rDNA followed by sequencing was conducted in a Japanese paddy field. The eukaryotic communities in rice straw compost incorporated into the flooded paddy field were influenced by the mid-season drainage and mainly composed of fungi (Ascomycota, Zygomycota, and Chytridiomycota) and protozoa (Ciliophora, Euglyphida, and Dactylopodida), most of which existed continuously during the cultivation period of paddy rice. The results indicated that these eukaryotic members were associated with the decomposition of rice straw compost in paddy field soil directly or indirectly.

**Keywords** Decomposition · Denaturing gradient gel electrophoresis analysis · Eukaryotic community · Paddy field · Rice straw compost

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## Introduction

Rice straw compost is most commonly applied to paddy fields to improve soil fertility and increase rice yield. Application of rice straw compost to paddy field soil generally increases microbial number, microbial biomass, and enzyme activity (Hasebe et al. 1985; Hayano et al. 1995; Shiota et al. 1987). The changes in composition of microbial communities during the decomposition of rice straw compost into a Japanese paddy field were monitored by phospholipid fatty acid (PLFA; Tanahashi et al. 2004) and 16S rDNA polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE; Tanahashi et al. 2005) analyses. Not only bacteria but also eukaryotes, e.g., fungi and protozoa, could contribute to the decomposition of rice straw compost in paddy field soil. Cahyani et al. (2004) studied the eukaryotic community structure in the composting process of rice straw by 18S rDNA PCR-DGGE analysis and reported that this process affected composition of fungi, protozoa, algae, and nematodes communities. The PLFA analysis by Tanahashi et al. (2004) detected considerable amounts of the biomarker fatty acids for eukaryotes, straight polyunsaturated PLFAs, indicating the presence of eukaryotic communities in the rice straw compost incorporated into paddy field soil. However, the PLFA analysis cannot allow determining species in the decomposition process of rice straw compost in paddy field soil (Nannipieri et al. 2003).

In this communication, we report DGGE analyses targeting 18S rDNA followed by sequencing for the rice straw compost samples buried into the Japanese paddy field and already used in the previous studies (Tanahashi et al.

2004, 2005). The aim was to describe the succession and phylogenetic composition of the eukaryotic communities associated with the decomposition of rice straw compost after incorporation into soil under flooded conditions during the cultivation period of paddy rice. The data obtained in this study on the community structure were compared with those on PLFA analysis (Tanahashi et al. 2004) and those on the composting process of rice straw by DGGE analysis (Cahyani et al. 2004).

## Materials and methods

The rice straw compost samples used in the present study were the same as those used for the PLFA (Tanahashi et al. 2004) and DGGE (Tanahashi et al. 2005) analyses of microbiota in rice straw compost placed into a Japanese flooded paddy field. These samples were collected from the same composting yard as that used in the study by Cahyani et al. (2002, 2003, 2004). Thus, the experimental field and the field experiments as well as composting process have been already reported (Tanahashi et al. 2004; Cahyani et al. 2002). The soil showed the following characteristics: total C, 12 g $kg^{-1}$; total N, 1.3 g $kg^{-1}$; pH ($H_2O$), 5.8.

*Rice straw compost samples and date of sampling* Rice straw compost of the curing stage was obtained from the composting pile located on May 24, 2002. About 80 g (wet weight; corresponding to about 15 g of dry matter) of the sample was cut into 1-cm segments and interleaved between glass fiber sheets (Whatman GF/D, diameter 15 cm). The margin of the filter sheets was sewed with a nylon string, and the sample in the glass fiber sheets was packed into a nylon 1-mm mesh bag. In total, 21 samples were buried at 5- to 10-cm depths in the rice field on June 3, 2002. The schedule of field management was as follows: transplanting, May 27; mid-season drainage, from July 4 to July 25; drainage, September 20; and harvest, September 24, 2002. Three nylon mesh bags containing rice straw compost in the glass fiber sheets were taken out seven times for the DGGE analysis on June 11, June 17, July 1, July 15, July 29, August 26, and September 24, 2002. The rice straw compost samples were removed from the glass fiber sheets and mixed well. A portion of the sample (about 6 g wet weight; corresponding to about 0.9–1.9 g of dry matter) was taken into a 15-ml polypropylene tube and then kept at −80°C until analysis.

*DNA extraction, PCR amplification, and DGGE analysis* Three replicates of the rice straw compost sample taken from each set of glass fiber sheets were homogenized separately using a mortar and a pestle under liquid nitrogen. Then, DNA was extracted from about 0.1 g (wet weight; corresponding to about 15–32 mg of dry matter) of the sample. The methods for DNA extraction from the rice straw compost samples and the subsequent purification have been previously described (Tanahashi et al. 2005). The extracted DNA was kept at −20°C until use. Although DNA from triplicate samples of the compost were used for the DGGE in the preliminary experiments to check the reproducibility of the patterns, two of the triplicate samples were subjected to the DGGE analysis due to shortage of the amount of DNA in the rest of the sample. PCR amplification and DGGE analysis targeting 18S rDNA was carried out as reported by Cahyani et al. (2004). Approximate amounts of DNA used for PCR and DGGE were 12–18 and 200–250 ng, respectively.

*Statistical analysis* Cluster analysis and principal component analysis were performed for the data obtained from the DGGE patterns, based on the band intensity (0, no band; 1, weak [<5 ng]; 2, moderate [5–8 ng]; 3, strong [>8 ng]; values in parentheses are approximate amounts of DNA) and position. Principal component analysis was performed using Excel Statistics 97 for Windows (SRI, Tokyo, Japan). A correlation matrix was used in the analysis. Cluster analysis was performed using a Black box program (Aoki 1996; http://aoki2.si.gunma-u.ac.jp/Blackbox/Blackbox.html) with the Ward method.

*Sequencing of the characteristic DGGE bands* The characteristic DGGE bands were excised from two different lanes, and the re-amplified fragments were subjected to direct sequencing after the mobility was confirmed to be same as the original one by DGGE as described above. Two bands (G and M; see below) failed to be sequenced by direct sequencing and were subjected to cloning. These steps were performed as described by Cahyani et al. (2003, 2004). The sequencing was conducted using the DYEnamic™ ET Terminator Cycle Sequencing Kit, according to the manufacturer's instructions (Amersham, Tokyo, Japan) with a 373S DNA Sequencer (ABI, Chiba, Japan).

*Phylogenetic analysis* Sequences of the DGGE bands were identified based on the 18S rDNA sequences obtained with the basic local alignment search tool (BLAST) from the database of the DNA Data Bank of Japan (DDBJ; http//:www.ddbj.nig.ac.jp/E-mail/homology.html).

*Nucleotide sequence accession number* The sequences of 18S rDNAs obtained in this study are available in the DDBJ database under the accession numbers from AB295006 to AB295022.

## Results and discussion

### DGGE patterns of eukaryotic communities in rice straw compost during decomposition in paddy field soil

The DGGE patterns of the eukaryotic communities in the rice straw compost collected from paddy field soil for two of the triplicate samples are shown in Fig. 1. Slight differences in number of bands (one to four) and intensity of the band were observed among the DGGE patterns from the triplicates at each sampling time, especially before the mid-season drainage (data not shown). The number of total different bands was 71, and 30 bands were commonly present throughout the period; the number of the bands ranged from 41 to 54 at each sampling occasion. These findings indicate that most of the eukaryotic species in the rice straw compost persisted during the degradation periods. The number of DGGE bands decreased gradually after the mid-season drainage, as observed for the number of DGGE bands of bacterial 16S rRNA extracted from the same rice straw compost (Tanahashi et al. 2005). The DGGE patterns changed markedly after the mid-season drainage while showing a small difference between before and after placement in the field (Fig. 1). The differences were mainly due to the increase or decrease of the band intensity rather than the number of bands. The number of bands remained almost similar among the sampling periods, and more than one half to two thirds of the bands in the respective samples were commonly present throughout the period as mentioned above. Tanahashi et al. (2005) reported that the DGGE patterns of bacterial communities in the rice straw compost placed into paddy field soil also changed after the mid-season drainage.

Cluster analysis of the DGGE patterns separated the samples into two groups before and after the mid-season drainage (Fig. 2a) and confirmed the changes in the eukaryotic communities after the mid-season drainage. This suggested that the mid-season drainage affected the eukaryotic communities in rice straw compost during its decomposition in paddy fields. Tanahashi et al. (2004, 2005) reported that the succession of the microbial communities in the rice straw compost placed in flooded paddy field was gradual, except for the period of mid-season drainage, as evidenced by the PLFA composition of microbiota and DGGE band patterns of bacterial DNA/RNA, probably due to the slow decomposition (about 20% for 3 months) of the compost under flooded conditions. These results indicate that mid-season drainage is the common important factor that primarily influences the microbial communities in rice straw compost incorporated into paddy fields.

Principal component analysis of the DGGE patterns also separated the rice straw compost samples into two groups,

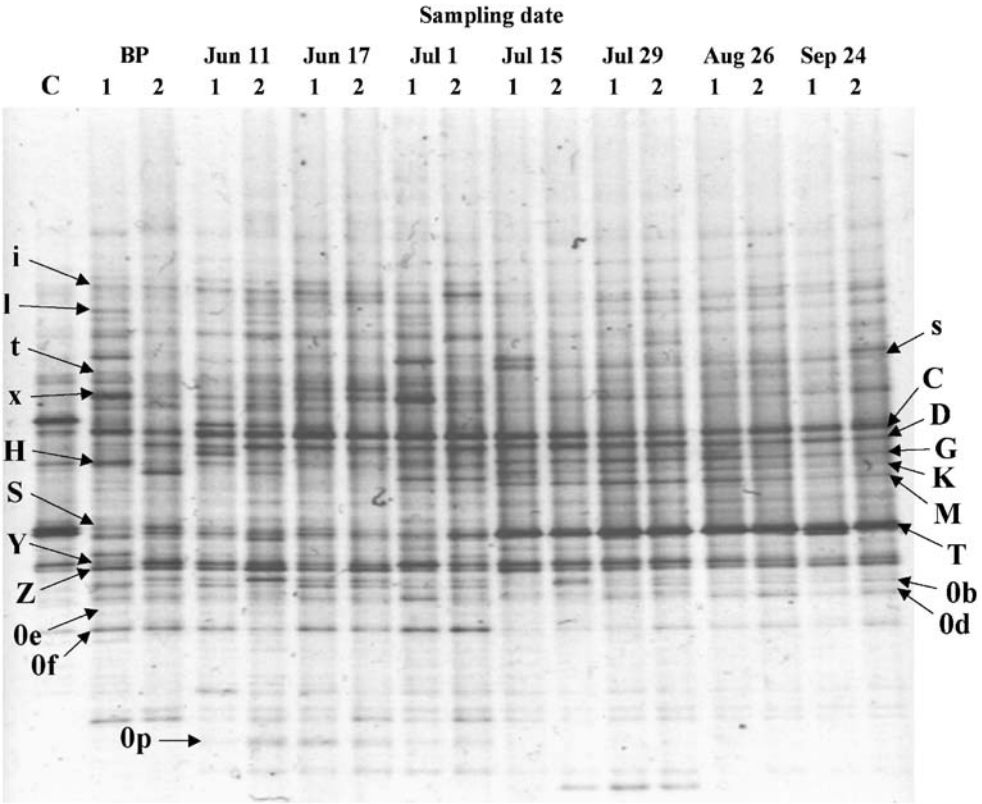


**Fig. 1** DGGE pattern of eukaryotic communities in rice straw compost in paddy field soil. *C*, DGGE pattern of rice straw compost at the curing stage (Cahyani et al. 2004); *BP*, before incorporation into the paddy field; *1* and *2*, first and second replicates

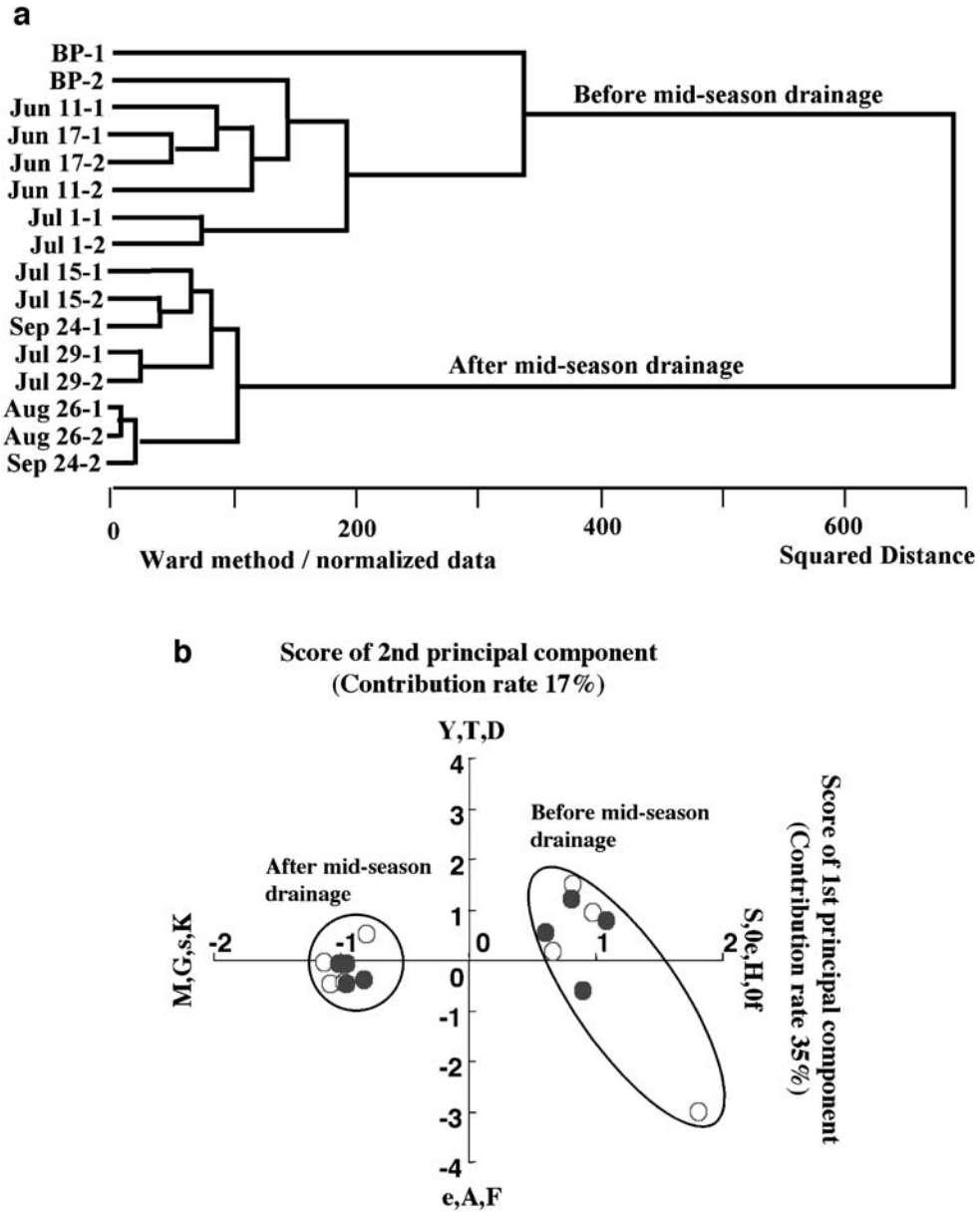


**Fig. 2** **a** Cluster analysis of the DGGE pattern of the eukaryotic communities in rice straw compost in paddy field soil. *BP*, before placement; *1* and *2*, first and second replicates, respectively. **b** Principal component analysis of the DGGE pattern of the eukaryotic communities in rice straw compost in paddy field soil. *Open* and *solid circles* denote first and second replicates, respectively. Bands contributing to the principal components are indicated at the end of the axes

namely before and after the mid-season drainage, in score plots of the first principal component (Fig. 2b), as was revealed by cluster analysis. Bands S, 0e, H, and 0f characterized the samples before mid-season drainage, while bands M, G, s, and K did the samples after mid-season drainage.

## Phylogenetic position of characteristic DGGE bands

Closest relatives of DGGE bands characterizing the eukaryotic communities described above (except for bands 0e, G, and M) together with some of the commonly existing bands (bands D, T, Y, Z, 0b, i, l, t, x, C, and 0d) and another band (0p) are listed in Table 1. All of the sequenced DGGE bands from the two positions with the same mobility (except for bands G and M) showed the identical sequences. Close relatives of the commonly present bands belonged to fungi (Ascomycota [D, T, Y and Z] and Chyridiomycota [0b]), protozoa (Ciliophora [i, l, t and C] and Euglyphida [x]), and bear animalcule (Tardigrada [0d]). These eukaryotic members inhabited the rice straw compost throughout the period and thus seemed to be characteristic in the eukaryotic communities of rice straw compost in paddy field soil. Six sequenced bands (H, S, T, Y, Z, and 0f) were also observed in the rice straw compost at the curing stage (lane C in Fig. 1; Cahyani et al. 2004); bands T and 0f showed identical sequences with those of DGGE bands Z and j, respectively, determined by Cahyani et al. (2004). Half of the bands (T, Y, and Z) were present throughout the period and the remaining half (S, H, and 0f) were detected until the mid-season

**Table 1** Closest relatives of the bands in the DGGE analysis of rice straw compost in paddy field soil

| DGGE band | PCA eigenvalue | Sequence base pair | Closest relatives | | | Similarity (%) | Alignment |
|---|---|---|---|---|---|---|---|
| | | | Microorganisms | Phylogenetic affiliations | Accession number | | |
| D | | 165 | *Tetracladium marchalianum* strain CBL27 | Ascomycota | AY204619 | 92 | 153/165 |
| T | | 162 | Uncultured eukaryote 18S rRNA gene | Eukaryote | AB120159 | 100 | 162/162 |
| | | 162 | *Coniochaeta ligniaria* | Ascomycota | AY198389 | 99 | 161/162 |
| Y | | 162 | Uncultured eukaryote 18S rRNA gene | Eukaryote | AB120159 | 96 | 154/160 |
| | | 162 | *Coniochaeta ligniaria* | Ascomycota | AY198389 | 95 | 153/160 |
| Z | | 158 | *Coniochaeta ligniaria* | Ascomycota | AY198389 | 96 | 153/159 |
| 0b | | 163 | *Diplochytridium lagenarium* | Chytridiomycota | AF164286 | 97 | 159/163 |
| i | | 169 | *Bresslaua vorax* | Ciliophora | AF060453 | 96 | 163/169 |
| l | | 157 | *Dileptus* sp. | Ciliophora | AF029764 | 98 | 154/157 |
| t | | 152 | *Metopus palaeformis* clone 3 | Ciliophora | AY007452 | 92 | 139/150 |
| x | | 168 | *Tracheleuglypha dentata* | Euglyphida | AJ418790 | 99 | 167/168 |
| C | | 162 | *Orthoamphisiella breviseries* | Ciliophora | AY498654 | 100 | 162/162 |
| 0d | | 163 | *Thulinia stephniae* | Tardigrada | AF056023 | 94 | 155/164 |
| S | | 164 | *Monoblepharella* sp. M15 | Chytridiomycota | AY546682 | 98 | 162/164 |
| H | | 161 | Uncultured eukaryote 18S rRNA gene | Eukaryote | AB120152 | 96 | 153/159 |
| | 1st PC(+) | 161 | *Basidiobolus haptosporus* | Zygomycota | AF113413 | 90 | 145/161 |
| 0f | | 160 | Uncultured eukaryote 18S rRNA gene | Eukaryote | AB120169 | 100 | 160/160 |
| | | 160 | *Beauveria felina* strain CBS 250.34 | Ascomycota | AY261369 | 98 | 157/160 |
| s | | 163 | *Mayorella* sp. JJP-2003 | Dactylopodida | AY294143 | 86 | 142/164 |
| K | 1st PC(-) | 161 | *Oxytricha* sp. Steamboat Hot Springs | Ciliophora | AF508769 | 94 | 147/155 |
| 0p | | 157 | *Batrachideidae* gen. sp. | Arthropoda | Z97631 | 70 | 113/160 |

PCA, Principal component analysis; 1st PC(+) and (−), positive and negative large eigenvalue in the first principal component

drainage. All of them belonged to fungi (Ascomycota, Zygomycota, and Chyridiomycota). Those fungal members may have inhabited the rice straw compost before and after the placement and been associated with the decomposition both in the composting process and in the flooded soil. Tanahashi et al. (2005) also reported some similarities for several members in the bacterial communities of the rice straw compost after incorporation into flooded paddy field and the middle and curing stages. These results may indicate that some microbial inhabitants of rice straw compost are carried over and retained in flooded soil after its incorporation.

The DGGE bands (S, H, and 0f) that characterized the communities before the mid-season drainage belonged to fungi (Chyridiomycota, Zygomycota, and Ascomycota) as described above. Bands S and H disappeared, and band 0f decreased its intensity after the mid-season drainage (Fig. 1). Members of Chyridiomycota, related to band S, were exposed to unfavorable condition because water content in the rice straw compost decreased markedly during the mid-season drainage (Tanahashi et al. 2004) and the fungi are aquatic (James et al. 2000). Tanahashi et al. (2004) reported that total amount of PLFAs as an indicator of microbial biomass, proportion of straight poly-unsaturated PLFAs as a biomarker for eukaryotes, and proportion of a biomarker PLFA (18:2ω6c) for fungi declined for the rice straw compost under drained condition and suggested the decrease of some fungal populations in the period. These findings indicate that the above fungal members related to bands S, H, and 0f may not have adapted themselves to the drained conditions. The band 0p, which appeared after incorporation, was related to Arthropoda, but the similarity of sequence was low. Detailed taxonomic affiliation of the eukaryote corresponding to band 0p was impossible, and the characteristics of the eukaryote could not be expected.

The DGGE bands that were characteristic to the communities after the mid-season drainage were closely related to Amoebozoa (band s) and Ciliophora (band K). Bands M and G contained several fragments that exhibited the same mobility but were affiliated to distant phylogenetic taxa: Acantharea, Alveolata, and Ciliophora for band M and Euglyphida, Dactylopodida, Athalamea, and Cercomonadida for band G, where six and four clones were analyzed for bands G and M, respectively (data not shown). Thus, the phylogenetic positions of these bands could not be defined. Members of Ciliophora, which were observed as commonly present eukaryotes, together with Amoebozoa may have fed on bacteria proliferated in the rice straw compost and might have indirectly contributed to the decomposition of rice straw compost as a result, as most protozoa feed on bacteria (Darbyshire 1994; Ekelund and Ronn 1994; Foissner 1987).

The eukaryotic communities in the rice straw compost incorporated into a flooded paddy field were influenced by the mid-season drainage and mainly composed of fungi and protozoa, most of which occurred commonly during the cultivation period of paddy rice. Those eukaryotic members may have been associated with the decomposition of rice straw compost in paddy field soil directly or indirectly. Active members in the eukaryotic communities and their roles remain to be investigated in the future by RNA-based analysis and microscopic observation.

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