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Succession of Microbiota Estimated by Phospholipid Fatty Acid Analysis and Changes in Organic Constituents during the Composting Process of Rice Straw

Vita Ratri Cahyani, Akira Watanabe, Kazuo Matsuya*, Susumu Asakawa, and Makoto Kimura

*Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464–8601 Japan; and *Laboratory of Crop Science, Aichi-ken Anjo Research and Extension Center, Anjo, 446–0073 Japan*

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The succession of responsible microbiota during the composting process of rice straw (RS) was studied for 145 d in relation to the changes in the organic constituents of RS. During the composting process, the C / N ratio of RS decreased from 56 to 22. On a C basis, the relative contents of lipid, water-soluble organic matter (WSOM), hemicellulose, cellulose, and lignin fractions in RS changed from 5.6, 8.9, 32.9, 17.9, and 34.0%, to 7.3, 5.8, 30.7, 3.8, and 51.1%, respectively, indicating that the cellulose fraction was mainly decomposed in the composting process. Biomass C accounted for 18.3% (on day 75 when the total amount of phospholipid fatty acids (PLFAs) reached a peak) and 11.5% (at the end of composting) of the total C of RS under the composting process. As for PLFAs, the biomarkers of fungi and Gram-negative bacteria predominated in the RS material used. At the thermophilic stage (the first 2 weeks), biomarkers of Gram-positive bacteria and actinomycetes predominated. After the thermophilic stage, biomarkers of other Gram-positive bacteria became dominant. Finally, at the curing stage, the proportion of the biomarkers of Gram-negative bacteria and eukaryotes increased, indicating the co-contribution of Gram-positive and Gram-negative bacteria and fungi in the decomposition process at this stage. The *trans / cis* ratio of 16 : 1ω7 PLFA of RS under the composting process ranged from 0.18 to 0.30, indicating that the composting process of RS prepared a significantly lower environmental stress ($p < 0.01$) compared to the decomposition of RS in a submerged paddy soil.

***Key Words*: compost, microbial community, phospholipid fatty acid, proximate analysis, rice straw.**

Application of organic fertilizers to paddy fields for yield increase has a long history in Asian countries. One of the most common applied is rice straw (RS) compost. Although the importance of compost application for sustainable agriculture has been well documented by long-term fertilizer trials (Inoko 1984; Leita et al. 1999), little attention had been paid to the composting process from the viewpoint of soil microbiology.

There have been several studies on the microbiota responsible for the decomposition of RS under submerged soil conditions. Kimura and Tun (1999) and Tun and Kimura (2000) examined the decomposition of leaf sheaths and leaf blades of rice plants that were placed in a flooded paddy field with a scanning electron microscope, and found that the decomposition of the leaf blades was faster than that of the leaf sheaths. Phospholipid fatty acid (PLFA) composition has been used as a bio-marker for identifying specific groups of microorganisms: straight mono-unsaturated PLFAs for Gram-negative bacteria (Ratledge and Wilkinson 1988), branched-chain PLFAs for Gram-positive bacteria (Haack et al. 1994), 10 Me-PLFAs for actinomycetes (Kroppenstedt 1992), and straight poly-unsaturated PLFAs for eukaryotes including fungi (Zelles 1999). The PLFA composition in decomposing RS under submerged incubation conditions showed that Gram-positive bacteria were mainly responsible for the RS decomposition in the submerged paddy soil irrespective of the incubation temperature and nitrogen status (Kimura et al. 2001).

The process of enzymatic decomposition of organic residues by microbiota in composting is similar to that of plant residues left in and on soils in which organic constituents with different chemical properties are de-

composed independently (Poincelot 1972; Miller and Donahue 1990; Devevre and Horwath 2000). It is necessary to gain a better understanding of the succession of the microbiota responsible for the decomposition of RS in a composting system compared to that observed in the decomposition in soil.

In the composting system, the examination of the succession of microbial community structure using PLFA analysis with materials consisting of a mixture of *Miscanthus* straw and pig slurry (Klamer and Bååth 1998) and various feed materials from municipal solid waste (Herrmann and Shann 1997) indicated a similar pattern. At the initial, mesophilic stage of composting, fungi and Gram-negative bacteria occurred, and during the subsequent, thermophilic stage, the number of Gram-positive bacteria increased and they remained as the dominant community. The final or curing stage was defined by the persistence of Gram-positive bacteria and by the conspicuous reappearance of fungi and Gram-negative bacteria.

Although many investigations on the responsible microorganisms in the composting process have focused on the temperature pattern, especially at the thermophilic stage (Nakasaki et al. 1985; Strom 1985a, b; Beffa et al. 1996; Herrmann and Shann 1997; Klamer and Bååth 1998), no studies have been conducted to elucidate the relationship between the changes in the composition of organic constituents of the composting materials and the succession of responsible microbiota during the composting process.

The purpose of this study was to investigate the changes in the organic constituents of RS and the succession of responsible microbiota throughout the composting period. This is the first study on microbial succession during the composting process of RS by the application of a technique of modern biochemistry (PLFA analysis) which enables to reveal the community structure of microbiota including not only bacteria but also eukaryotes (Zelles 1999). In the present experiment, the composting process was studied based on the conventional method used in Japan, and the compost had been applied to the field of a long-term fertilizer trial for at least more than 50 years.

MATERIALS AND METHODS

Experimental site. A long-term fertilizer trial had been conducted in a paddy field at Aichi-ken Anjo Research and Extension Center, Central Japan (latitude 34°8′ N, longitude 137°5′ E) since 1925, and the compost used in the present study was prepared based on a conventional method from the beginning of the trial, and was applied to the compost plot. Composting of RS was conducted in a storehouse from January 11 to June 5, 2001.

Composting procedure. As compost material, 300 kg of air-dried RS was cut into 2–3 cm pieces using a cutter machine and moistened by supplementing water to reach a moisture content of about 70%. It was piled on the floor in the storehouse and covered with plastic sheets to avoid moisture loss. The size of the compost pile was approximately as follows: 210 cm long, 180 cm wide, and 80 cm high. At the time of the first turning, 2 weeks after the onset of composting, ammonium sulfate ($(NH_4)_2SO_4$) was applied as N source at the rate of 10 kg Mg^{-1} of air-dried RS. To ensure oxic decomposition and homogeneous decomposition of RS, the compost pile was turned once every month. At the time of turning, water was added to maintain adequate moisture conditions. The composting process was terminated after 145 d on June 5, 2001.

Sampling. Dried RS (compost material) stored in a storehouse was sampled twice before composting on November 27, 2000 and January 11, 2001, 6 weeks after harvest (or 45 d before the onset of composting) and 12 weeks after harvest (on the day of composting), respectively.

During the composting process, samples were taken 12 times, at 7, 14 d (just before turning and adding water), 21, 28, 39, 47 d (just before turning and adding water), 61, 75 d (just before turning and adding water), 89, 103 d (just before turning and adding water), 124 and 145 d, respectively, after the onset of compost piling. At each sampling time, five subsamples were collected from randomized positions at a depth of about 30 cm, where the measured temperature was not different from that of the center. The samples were then stored at a temperature of −20°C.

Determination of pH and organic matter content of compost samples. The compost pH was measured at a compost : water ratio of 1 : 20 (g wet compost : mL distilled water) using a pH meter (F-7ssII, HITACHI-HORIBA, Kyoto, Japan). The moisture content was determined after drying of the sample at 105°C for 24 h. The organic matter content was calculated as the loss of ignition at 550°C for 24 h from the dried samples. In addition, the compost samples were dried and ground for the determination of the contents of total C and total N, and the C / N ratio using an elemental analyzer (NC 2500, ThermoQuest, Milan, Italy).

Estimation of organic constituents of RS under the composting process. The compost samples were dried at 70°C for 48 h and cut in 0.5 cm pieces. To fractionate organic constituents in the samples, a modification of proximate analysis (Waksman and Stevens 1930; Stevenson 1965; Watanabe et al. 1993) was performed.

The lipid fraction was extracted with benzene–ethanol (1 : 1) in a Soxhlet apparatus and estimated based on the decrease of the C content after extraction. To obtain the water-soluble organic matter (WSOM) fraction, the lipid-free residue sample was extracted by boiling with distilled water for 2 h at 105°C. The filtrate was subjected to the determination of the total organic C content using a total organic carbon analyzer (TOC-500, Shimadzu, Kyoto, Japan). The total organic C content in the filtrate was designated as WSOM fraction.

The hemicellulose fraction was removed by hydrolysis of the lipid-free residue sample with 0.65 M HCl for 5 h at 105°C. As WSOM was also extracted in this hydrolysis procedure, the hemicellulose fraction was estimated by subtracting the total organic C content in the WSOM fraction described above from that in the HCl-hydrolyzable fraction.

The cellulose fraction was removed by hydrolysis of the lipid-free residue sample with 15 M H_2SO_4 at room temperature for 2.5 h and then with 0.42 M H_2SO_4 at 105°C for 5 h. The cellulose fraction was determined by subtracting the total organic C content in the HCl-hydrolyzable fraction from that in the H_2SO_4-hydrolyzable fraction. The lignin fraction was estimated from the C content in the residue after H_2SO_4 hydrolysis.

PLFA analysis. The representative portions of the subsamples were pooled, cut in 0.5 cm pieces, and mixed. The PLFAs were analyzed from 0.5 g wet weight of compost samples and determined in triplicate.

Analysis of PLFAs was performed by the method of Okabe et al. (2000), after a modification of the methods of Bligh and Dyer (1959) and White et al. (1979). Briefly, the lipids were extracted from the compost samples in a one-phase mixture of methanol, chloroform, and phosphate buffer solution. The total lipids contained in the chloroform phase were fractionated into neutral lipids, glycolipids, and phospholipids on a solid phase extraction column (Sep-Pack® Cartridges Silica, Waters, Milford, USA) by eluting them with chloroform, acetone, and methanol, sequentially. The phospholipid fraction was subjected to methanolysis using Hydrogen Chloride-Methanol Reagent 10 (Tokyo Chemical Industry, Tokyo, Japan), to synthesize fatty acid methyl esters (FAMEs) (Arao et al. 1998). The analysis of FAMEs was performed using a gas chromatograph equipped with FID (5890 series II, Hewlett Packard, CA, USA). The GC operation was conducted according to the method of Okabe et al. (2000).

Nomenclature of fatty acids. Fatty acids were designated based on the total number of carbon atoms : number of double bonds, followed by the position of the double bond from the methyl end (aliphatic end (ω)) of the molecule. The *cis* and *trans* configurations were indicated by "c" and "t," respectively. The prefixes "ai" and "i" referred to anteiso and iso branchings, respectively, 10Me referred to a methyl group on the 10th carbon atom from the carboxyl end of the molecule, and "cy" referred to a cyclopropane fatty acid.

Statistical analysis. Characterization of the microbial communities present in the samples was based on the identification of the PLFAs. To estimate the succession of responsible microorganisms during the composting process of RS, changes in the composition and content of the PLFAs extracted from the samples were analyzed by principal component analysis (PCA) and cluster analysis. Principal component analysis was performed using EXCEL STATISTICS 97 for Windows (SRI, Tokyo). Correlation matrix was used in the analysis. Cluster analysis was performed according to the Blackbox program (Aoki 1996). Ward method was used in the analysis.

RESULTS AND DISCUSSION

Temperature, pH (H_2O), and moisture content during the composting process

As shown in Fig. 1, the room temperature was low (3–10°C) during the first 2 months because the composting process started from mid-winter. However, the

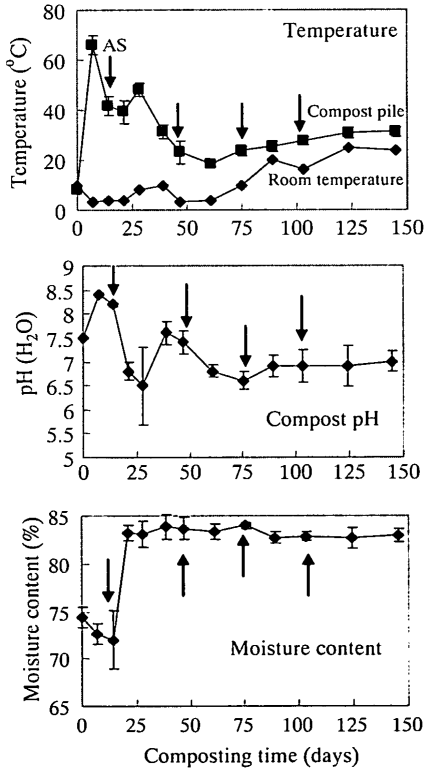

Fig. 1. Temperature, pH, and moisture content of rice straw under the composting process. ↓, turning time of compost pile; AS, ammonium sulfate application.

highest temperature of the compost pile, 66°C, was observed at the first sampling, 1 week after the onset of compost piling. As it was generally observed that the peak of temperature appeared within the first week (frequently in 2 or 3 d) after the onset of composting (Poincelot 1972; Hellmann et al. 1997; Lei and VanderGheynst 2000; Eiland et al. 2001), the highest temperature of the compost pile seemed to be higher than the detected level of 66°C (Fig. 1). As heating of the compost pile had decreased until day 61, the gradual increase of the temperature of the compost pile after 61 d was attributed to the effect of the increase of the room temperature during this period. As $(NH_4)_2SO_4$ was applied 2 weeks after the onset of piling, heating of the compost pile at the thermophilic stage occurred spontaneously under adequate moisture and aeration conditions without the addition of a N source. By mixing *Miscanthus ogiformis* Honda "*Giganteus*" with liquid pig manure from the beginning of the composting process, Eiland et al. (2001) reported that the duration of heat development (temperature above 40°C) ranged from day 1 to 10 in the box and from day 3 to 11 in the reactor systems. In the present study, application of $(NH_4)_2SO_4$ in the second week appeared to have extended the duration of heat development until the 28th day of composting, probably because the application resulted in the stimulation of microbial activities in the process of RS decomposition.

The compost pH (H_2O) was higher than 8 at the initial stage (Fig. 1). High initial pH values (above 8) were also reported in the composting process of *Miscanthus* straw by Klamer and Bååth (1998) and Eiland et al. (2001). Ammonia that was liberated from the decomposition of RS during the composting process increased the pH, shifting the NH_4^+-NH_3 equilibrium toward ammonia. The high temperature and high pH at this stage seemed to have resulted in extensive ammonia volatilization and loss of total N as was observed in the study of *Miscanthus* compost (Eiland et al. 2001). After the application of $(NH_4)_2SO_4$ upon the first turning, the pH decreased to 6.5, but it shortly increased again by about 1 pH unit and remained at around 7 afterwards. The decrease in the compost pH after the application of $(NH_4)_2SO_4$ was due to the acidification by SO_4^{-2}.

On the day of composting, the moisture content of the compost material was 74% after watering and piling (Fig. 1). After the first turning, the moisture content increased to 82.5% by watering, and then remained generally constant until the end of the composting process. This finding indicated that there were no specific fluctuations in the moisture content during the composting process and that the moisture content was sufficient for the microbiota throughout the period of composting. Hence, it was considered that the moisture content was not a factor that regulated the succession of microbiota during the composting process.

Decomposition rate and changes in organic matter constituents of RS during the composting process

Total organic matter content in RS decreased from 87 to 74% and the C / N ratio decreased from 56 to 22 during the 145-d period of composting (Figs. 2 and 4), indicating that the present study covered the whole period of composting. The content of total organic C during the composting of RS decreased in parallel with the decomposition of RS, whereas the total N content increased during the composting process (Fig. 2). The increase in the content of total N was ascribed to: 1) active decomposition of labile organic-C compounds, 2) immobilization of inorganic N, and 3) N fixation (Bernal et al. 1996; Paredes et al. 2000). Composting of RS resulted in a 9.2% loss of C and 117% gain of N from the initial values on the day of composting.

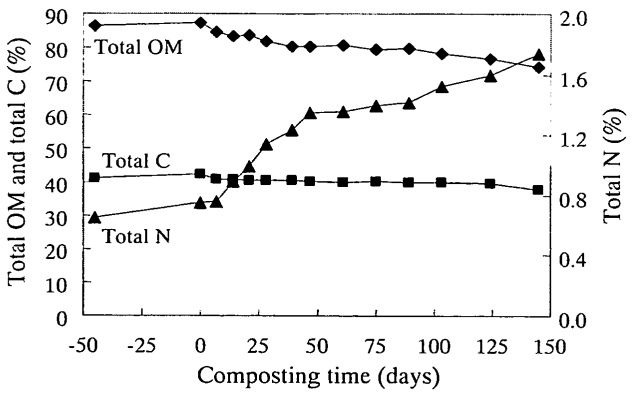

Fig. 2. Total organic matter (OM), total C, and total N contents of rice straw under the composting process.

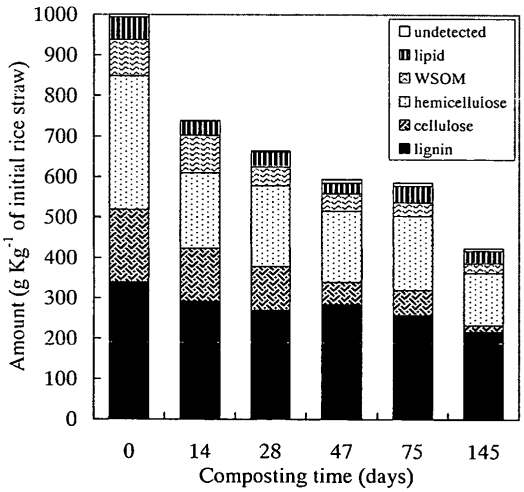

Fig. 3. Decomposition of rice straw constituents under the composting process (taking the ash content as constant).

On a C basis, the relative contents of lipid, WSOM, hemicellulose, cellulose, and lignin fractions in RS changed from 5.6, 8.9, 32.9, 17.9, and 34.0% on day 0 to 7.3, 5.8, 30.7, 3.8, and 51.1% on day 145, respectively. To estimate the rate of RS decomposition, the ash content was assumed to be constant, as shown in Fig. 3. All of the organic fractions including the most recalcitrant fraction (lignin) were gradually decomposed, and the cellulose fraction was mainly decomposed in the composting process of RS at a constant decomposition rate. As shown in Fig. 3, a fast decomposition of the hemicellulose fraction was observed at the thermophilic stage (the first 2 weeks), followed by a fast decomposition of the WSOM fraction in the middle of the composting process (hereafter referred to as "the middle stage") (from day 14 to 47), and then the lignin fraction content considerably decreased at the curing stage. Similar findings of drastic cellulose decomposition in the process of composting of RS were reported by Inoko (1984) and Tsutsuki and Ponnamperuma (1987). However, a different pattern of decomposition during the composting process was reported for the residues of another Gramineae plant, *Miscanthus* straw (Eiland et al. 2001), in which the hemicellulose fraction was decomposed nearly completely while the cellulose fraction decreased only by 64–70%. The reason for the difference in the decomposition patterns between RS and *Miscanthus* straw during the composting process could not be elucidated.

Total amount and composition of PLFAs in RS materials

Two kinds of RS materials were subjected to analysis: one sampled 6 weeks after harvest (45 d before composting) and the other sample taken 12 weeks after harvest or on the day of compost piling (day 0 of composting). The total amount of PLFAs in the first RS sample was 1.32 μmol g^{-1} DW and it increased to 3.99 μmol g^{-1} DW in the second sample (Fig. 4). As shown in Fig. 5, more than half (58%) of the total PLFAs in RS stored for 6 weeks after harvest consisted of straight saturated PLFAs, especially 16 : 0 PLFA (43%). Straight mono-unsaturated and straight poly-unsaturated PLFAs in RS showed similar proportions, 20 and 16%, respectively. The proportion of straight saturated PLFAs decreased appreciably in RS after 12-week storage from that in RS after 6-week storage, especially 16 : 0 PLFA, to the level of 20%. In contrast, the proportions of the other groups of PLFAs increased, especially 18 : 2ω6c PLFA (a biomarker of fungi) which increased considerably from 15.2% in RS after 6-week storage to 36.4% in RS after 12-week storage (Fig. 6).

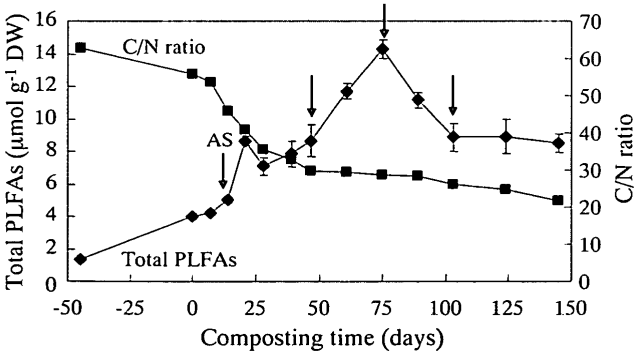

Fig. 4. Total amount of PLFAs and C / N ratio of rice straw under the composting process. ↓, turning time of compost pile; AS, ammonium sulfate application; DW, dry weight.

Amount of PLFAs in RS under the composting process

The total amounts of PLFAs in the RS materials were 1.32 and 3.99 μmol g^{-1} DW, at 45 and 0 d before the onset of piling, respectively. The total amount increased steadily during the composting period until day 75 with a small peak on day 21 (Fig. 4). Then the amount gradually decreased until the end of the experiment, when the total amount of PLFAs was still more than double that in RS at the time of compost piling (day 0 of composting). This finding was different from that in the composting of *Miscanthus* straw (Klamer and Bååth 1998), in which the peak appeared 1 d after the onset of composting, probably because in the present study the first peak during the thermophilic stage was omitted. The proportions of hemicellulose, cellulose, and lignin fractions were 31.2, 10.7, and 44.0%, respectively, at the time when the total amount of PLFAs showed a peak (day 75). In Fig. 4, the growth of the microbiota responsible for the composting process of RS is represented by the total amount of PLFAs and the decomposition of RS during compost-

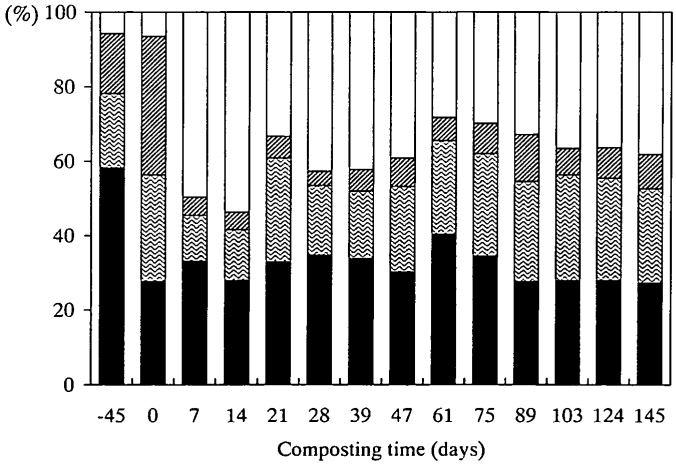

Fig. 5. Percentage distribution of PLFAs in rice straw under the composting process. ■, straight, saturated; ▨, straight, mono-unsaturated; ▨, straight, poly-unsaturated; □, branched-chain.

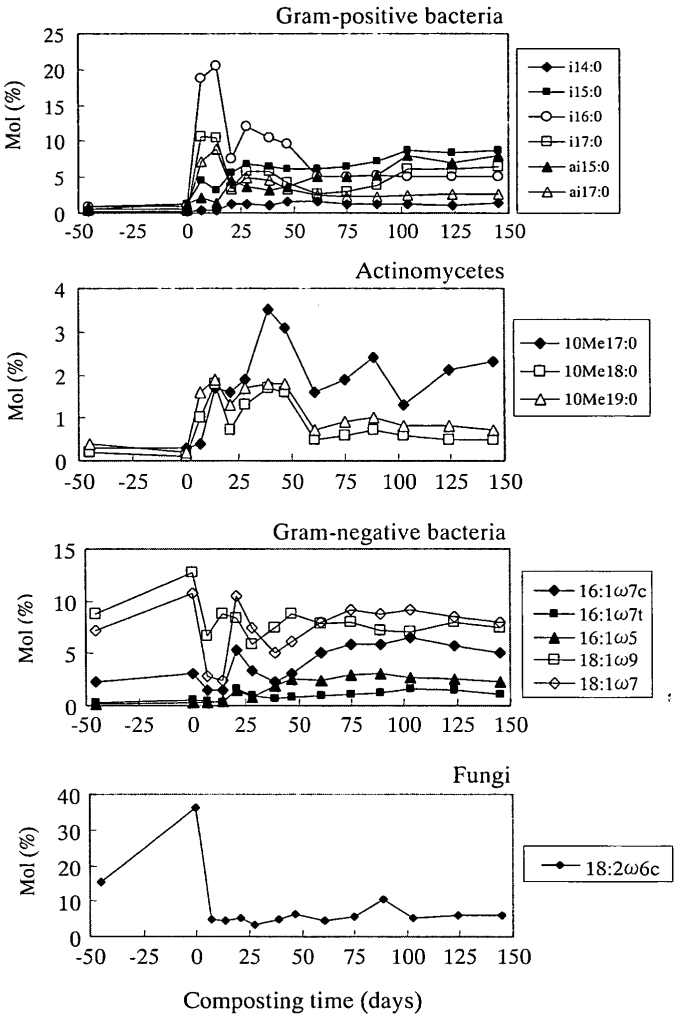

Fig. 6. Succession of microbiota responsible for the composting of rice straw (based on PLFA profile).

ing is represented by the decrease of the C / N ratio.

The total amount of PLFAs is considered to be an indicator of microbial biomass (Zelles 1999). Using the conversion factor of Balkwill et al. (1988) and Arao et al. (1998) in which 100 μmol of PLFAs was equivalent to 1 g dry weight of bacterial cells, and assuming that the microbiota in decomposing RS contained 50% of C, as was observed in bacterial cells (Miller and Donahue 1990), the total biomass C was estimated to account for 18.3% (day 75) and 11.5% (day 145 at the end of composting) of the total C of RS under the composting process. This estimation suggested that the total biomass C in the RS compost was considerably higher than that in agricultural soils which ranged from 0.27 to 4.8% of the total soil C (Anderson and Domsch 1980).

Changes in the composition of PLFAs in RS under the composting process

The RS material that was taken 45 d before the onset of piling was characterized by a PLFA composition consisting of Gram-negative bacterial biomarkers, 16 : 1ω7c (2%), 18 : 1ω9 (9%), and 18 : 1ω7 (7%), and of a fungal biomarker, 18 : 2ω6c (15%) (Fig. 6). The second sample of the RS material that was taken on the day of piling consisted of PLFAs mainly from Gram-negative bacterial biomarkers, namely 16 : 1ω7c (3%), 18 : 1ω9 (13%), and 18 : 1ω7 (11%), and of a fungal biomarker, 18 : 2ω6c (36%) (Fig. 6). Thus, the marked increase in the total amount of PLFAs in the second sample was ascribed to the growth of fungi.

Over the period of composting, the branched-chain PLFAs predominated, especially at the thermophilic stage (7 and 14 d after the onset of piling) (Fig. 5), followed by the straight mono-unsaturated PLFAs. At the curing stage (from 61 d after the onset of piling), this group showed a similar proportion to that of the branched-chain PLFAs (Fig. 5).

In the first week after the onset of composting, when the temperature reached a peak (thermophilic stage), the PLFA composition showed considerable changes from that in the RS material (Figs. 5 and 6). The proportion of branched-chain PLFAs increased markedly from 6.5 to 50% (Fig. 5). The biomarkers of actinomycetes (10Me PLFAs) that were included in this group also increased from 0.53 to 2.95% (Fig. 6). The proportion of straight mono-unsaturated PLFAs decreased from 29 to 12% (Fig. 5). The proportion of straight poly-unsaturated PLFAs, especially 18 : 2ω6c PLFA (a biomarker of fungi) decreased quickly from 36 to 4.8% (Fig. 6), while the proportion of straight saturated PLFAs showed a slight increase from 28 to 33% (Fig. 5). Abundant PLFAs at the peak temperature were i16 : 0 (18.8%), i17 : 0 (10.6%), and ai17 : 0 (7.1%) (Fig. 6).

A similar increase in the proportion of branched-chain PLFAs representing Gram-positive bacteria (iso- and anteiso-PLFAs) and actinomycetes (10Me PLFAs) during the thermophilic stage was reported for municipal waste compost (Herrmann and Shann 1997). In contrast, Klamer and Bååth (1998) reported the contribution of only Gram-positive bacteria with the same biomarkers (i16 : 0, i17 : 0, and ai17 : 0 PLFAs) at the thermophilic stage of the composting of *Miscanthus*. Contribution of actinomycetes was not observed in their study. In the second week, although the temperature of the compost had decreased, the proportion of PLFAs still showed a similar pattern to that in the first week sample in which branched-chain PLFAs (biomarkers of Gram-positive bacteria) remained the predominant PLFA group. After the thermophilic phase until the end of composting, the proportion of the respective branched-chain PLFAs showed specific fluctuations: the proportions of i15 : 0, ai15 : 0, and i14 : 0 PLFAs increased, while those of i16 : 0, i17 : 0, and ai17 : 0 (Fig. 6) decreased. It is important to determine the contribution of these two

groups of Gram-positive bacteria to the composting process of RS.

The increase in the proportion of 10Me17 : 0, 10Me18 : 0, and 10Me19 : 0 PLFAs reflected the growth of actinomycetes from the first week when the temperature peaked. Their largest proportion was observed at the end of the thermophilic stage (at 14 d after the onset of piling). The 10Me19 : 0 PLFA which predominated during the thermophilic stage, was replaced by 10Me17 : 0 PLFA at the following stage, reflecting the actinomycete succession during the composting process (Fig. 6).

The proportion of straight mono-unsaturated PLFAs increased after the thermophilic stage from day 21 after the onset of piling, especially for 16 : 1ω7c, 18 : 1ω7, and 18 : 1ω9 PLFAs (Fig. 6).

During the experimental period, within the straight poly-unsaturated PLFAs, 18 : 2ω6c PLFA, a biomarker PLFA of fungi always predominated, accounting for around 3.4 to 10.3% of the total PLFAs (Fig. 6). Although at a low level (accounting for around 0.4 to 1.6%), 20 : 4ω6c PLFA, a biomarker of protozoa (Vestal and White 1989; Herrmann and Shann 1997) was observed continuously from day 39 after the onset of piling (data not shown). The importance of fungi in composting, especially during the late curing stage, was also suggested by Strom (1985b), Herrmann and Shann (1997), and Klamer and Bååth (1998).

Interesting similarities and differences were found when the succession of microbiota responsible for the decomposition of RS in the composting system was compared with that observed under submerged soil conditions (Kimura et al. 2001). The dominant biomarkers of Gram-positive bacteria and actinomycetes throughout the period after the thermophilic stage of composting (i15 : 0, ai15 : 0, and 10Me17 : 0 PLFAs) also predominated in decomposing RS throughout the incubation period under submerged soil conditions, irrespective of the incubation temperature and nitrogen status. However, Gram-negative bacteria showed a different development pattern under submerged soil conditions, in which the proportion of 16 : 1ω7c, 18 : 1ω9, and 18 : 1ω7 PLFAs decreased during the incubation period, whereas that of 17 : 1ω8 and 17 : 1ω6 PLFAs increased. The growth of the fungi was suppressed under submerged soil conditions, as shown in the decrease of the proportion during the incubation period from 5.3 to 2.7% and from 5.6 to 1.3% under 22 and 30°C incubation conditions, respectively (Kimura et al. 2001).

Statistical analysis of the PLFA composition of RS under the composting process

Principal component analysis of all the data of the PLFA composition during the composting process showed that the total percentages contributed by the primary and secondary principal components were 34.6 and 25.5%, respectively (Fig. 7). PLFAs with highly positive and negative loads in the 1st principal component were i16 : 0, 17 : 0, ai17 : 0, 18 : 0, 10Me19 : 0, 10Me18 : 0, i16 : 1, and ai17 : 1 PLFAs (biomarkers of Gram-positive bacteria and actinomycetes), and 16 : 1ω7c, 20 : 4ω6c, 18 : 1ω7, and 18 : 3ω6c PLFAs (biomarkers of Gram-negative bacteria and eukaryotes), respectively. On the other hand, PLFAs with highly positive and negative loads in the 2nd principal component were i15 : 0, i14 : 0, 10Me17 : 0, and 10Me19 : 0 PLFAs (biomarkers of Gram-positive bacteria and actinomycetes), and 18 : 2ω6c, 17 : 1ω8, 18 : 1ω9, 12 : 0, and 16 : 0 PLFAs (18 : 2ω6c PLFA is a biomarker of fungi), respectively. Score plots of PLFA composition shifted anti-clockwise along with the duration of the composting process from the lower-middle side to the upper-right side, and then to the upper-left side in the figure, which reflected the succession of the four stages observed in this composting process.

The RS materials before composting that were located on the lower-middle side were characterized by a high proportion of 18 : 2ω6c, 17 : 1ω8, and 18 : 1ω9 PLFAs (biomarkers of fungi and Gram-negative bacteria). The first shift of the score plots from the lower-middle side to the middle-right side resulted from the increase in the proportion of biomarkers of Gram-positive bacteria (i16 : 0, ai17 : 0, i16 : 1, and ai17 : 1 PLFAs) and actinomycetes (10Me19 : 0 PLFA) as well as the decrease in the proportion of biomarkers of fungi and Gram-negative bacteria. Then, the score plots shifted to the upper-middle side due mainly to the increase in the proportion of other branched-chain PLFAs (i15 : 0, i14 : 0, 10Me17 : 0

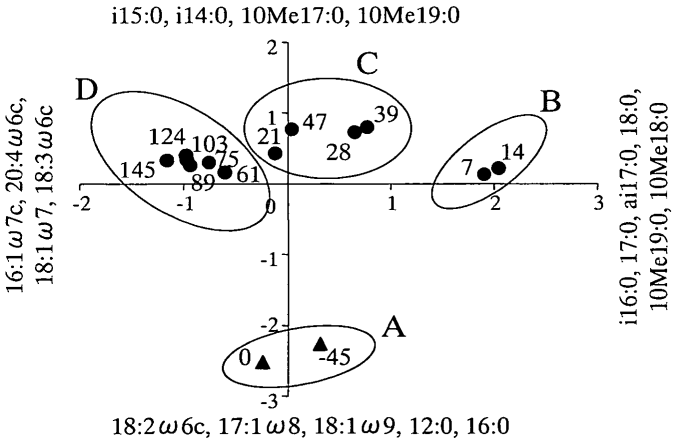

Fig. 7. Principal component analysis of PLFA composition in the rice straw under the composting process. A: Rice straw materials. B: Rice straw compost at the thermophilic stage. C: Rice straw compost at the middle stage. D: Rice straw compost at the curing stage. Numbers next to the symbol denote the composting day.

PLFAs) as well as the decrease in the proportion of the previous biomarkers of PLFAs. Finally, the score plots of the PLFA composition reached the middle-left side due to the increase in the proportion of biomarkers of Gram-negative bacteria (16 : 1ω7c, 18 : 1ω7 PLFAs) and of eukaryotes (20 : 4ω6c, 18 : 3ω6c PLFAs) as well as the decrease in the proportion of branched-chain PLFAs (biomarkers of Gram-positive bacteria).

Cluster analysis enabled to identify four clusters for the PLFA composition of the RS materials under the composting process (data not shown). The PLFA composition was divided first into two clusters, and both of them were further divided into two sub-clusters. The PLFA composition of stored RS and RS at the thermophilic stage corresponded to the first cluster. And RS formed different sub-clusters in the second cluster at the middle and the final / curing stages during the composting process. Domains A to D shown in Fig. 7 corresponded to the respective sub-clusters obtained in the cluster analysis. The shifting of the score plots of PLFA composition reflected the succession of the microbiota during the composting process. Different subsets of the microbial communities seemed to have contributed sequentially to the gradual decomposition of RS, being moreover responsible for the specific variations in the decomposition rates among the organic constituents of RS as described above.

Stress factor

The *trans / cis* ratio of 16 : 1ω7 PLFA was used as an indicator of environmental stresses such as substrate shortage in peat soils and in other soil types (Borga et al. 1994; Frostegård et al. 1997; Reichardt et al. 1997; Bossio and Scow 1998), heavy metal contamination in soils (Frostegård et al. 1993, 1996), and anaerobic stress in estuarine sediments (Guckert et al. 1985). The *trans / cis* ratio of 16 : 1ω7 PLFA of RS under the composting process ranged from 0.18 to 0.30 (Fig. 8). Thus, the degree of environmental stress indicated by the *trans / cis* ratio of 16 : 1ω7 PLFA in RS under the composting process was significantly ($p < 0.01$) lower than that in decomposing RS under submerged soil conditions which ranged from 0.21 to 0.58 (Kimura et al. 2001). Although the difference in the level of the *trans / cis* ratio of 16 : 1ω7 PLFA between the composting process of RS and RS decomposition in submerged soil might reflect differences in the physiological state of microbiota as a response to differences in physiochemical conditions (environmental factors) between them, a definite reason for this significant difference could not be determined.

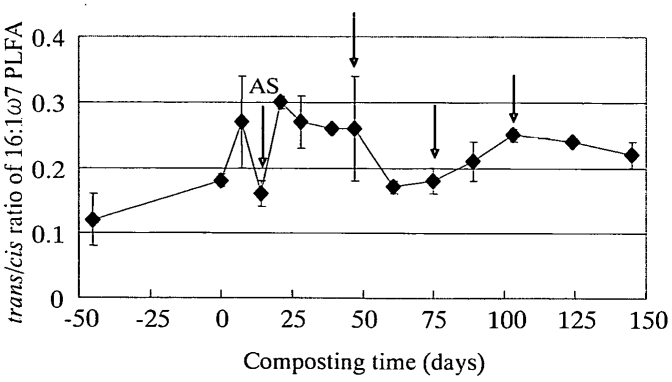

Fig. 8. *Trans / cis* ratio of 16 : 1ω7 PLFA in the rice straw under the composting process. ↓, turning time of compost pile; AS, ammonium sulfate application.

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