Succession of Bacterial Populations during Plant Residue Decomposition in Rice Field Soil^{▽†}

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Received 25 March 2009/Accepted 13 May 2009

The incorporation of rice residues into paddy fields strongly enhances methane production and emissions. Although the decomposition processes of plant residues in rice field soil has been documented, the structure and dynamics of the microbial communities involved are poorly understood. The purpose of the present study was to determine the dynamics of short-chain fatty acids and the structure of bacterial communities during residue decomposition in a rice field soil. The soil was anaerobically incubated with the incorporation of rice root or straw residues for 90 days at three temperatures (15, 30, and 45°C). The dynamics of fatty acid intermediates showed an initial cumulative phase followed by a rapid consumption phase and a low-concentration quasi-steady state. Correspondingly, the bacterial populations displayed distinct successions during residue decomposition. Temperature showed a strong effect on the dynamics of bacterial populations. Members of *Clostridium* (clusters I and III) were most dominant in the incubations, particularly in the early successions. *Bacteroidetes* and *Chlorobi* were abundant in the later successions at 15 and 30°C, while *Acidobacteria* were selected at 45°C. We suggest that the early successional groups are responsible for the decomposition of the easily degradable fraction of residues, while the late successional groups become more important in decomposing the less-degradable or resistant fraction of plant residues. The bacterial succession probably is related to resource availability during residue decomposition. The fast-growing organisms are favored at the beginning, while the slow-growing bacteria are better adapted in the later stages, when substrate availability is limiting.

Rice residues, including root and straw residues, serve as the major carbon source in paddy fields. It has been estimated that the amounts of organic matter supplied annually to paddy fields range from 1,700 to 3,470 kg ha⁻¹, and more than 65% of them were derived from plant residues (19, 23). The incorporation of rice residues into paddy fields helps sustain soil organic matter, improve physical and chemical properties, and increase nutrient availability (15, 39). However, it also strongly enhances methane production and emissions (6, 45, 46).

Numerous studies have been carried out on residue decomposition and CH₄ production in rice field soils (11–13, 22). The rate of decomposition usually is separated into a fast phase and a slowdown phase (24). According to the dynamics of the intermediates H₂ and fatty acids and the activities of polysaccharolytic enzymes, Glissmann and Conrad (13) proposed five stages for residue decomposition, including the production and consumption of reducing sugars, the production and consumption of H₂ and fatty acids, and the production of CH₄. The rate of decomposition was affected by the composition of residues (11, 18). Root residues generally are decomposed slower than straw residues (24). The fermentation pathway also could differ depending on the residue material, resulting in different fatty acid intermediates (13).

A complex microbial assemblage consisting of hydrolytic,

fermenting, homoacetogenic, syntrophic, and methanogenic microorganisms are involved in the anaerobic decomposition of organic residues (5, 41, 48). Plant residues are composed of complex components. With the decomposition process, the proportion of labile component decreases while the resistant components relatively accumulate. Changes in the activity and structure of the microbial community thus are anticipated during the processes of residue decomposition. However, little has been known about the dynamics of microbial populations during residue decomposition in anoxic rice soil. Using culture-independent methods, Weber et al. (47) showed that the structure of the bacterial community shifted between early and late stages. Microscopic observation revealed the differential colonizations of bacterial populations on different parts of straw residue, indicating the effects of residue quality and niche condition (18). The microbial community appears to form a spatially well organized architecture, with the fermenting bacteria colonizing the residue particles, while the syntrophic bacteria and methanogens mainly inhabit the adjacent soil during the decomposition process (12).

Air temperature exhibits a large seasonal variation in south-eastern Asia, where rice is widely cultivated. The lowest and highest records in our research site, for instance, were –5 and 40°C, respectively, in 2006, when we collected the soil samples. It has been demonstrated that temperature has a strong effect on residue decomposition and CH₄ production (8, 22, 33). However, it is uncertain whether the effect also is reflected in the structure and function of degrading microbial communities in the soil. Therefore, the purpose of our project was to determine the effect of temperature on microbial communities during the processes of plant residue decomposition in a Chi-

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† Supplemental material for this article may be found at <http://aem.asm.org/>.

▽ Published ahead of print on 22 May 2009.

nese rice field soil. The dynamics of methanogenesis and methanogenic archaea have been reported previously (33). Here, we show the results on fatty acid intermediates and the responsible bacterial communities.

MATERIALS AND METHODS

Preparation of soil and residue samples. The preparation of soil and residue samples has been reported previously (33). In brief, rice soil was collected from an experimental farm at China National Rice Research Institute in Hangzhou, China (30°04'37"N, 119°54'37"E). The soil had the following properties, as measured by the standard methods (32): pH 6.7, cation-exchange capacity of 14.4 cmol kg⁻¹, organic C of 24.2 g kg⁻¹, and total N of 2.3 g kg⁻¹. Anaerobic incubations were prepared in 100-ml glass bottles filled with 10 g dry soil and 20 ml autoclaved and degassed water. After the addition of 100 mg (dry weight) of straw or root residues, the bottles were closed with butyl stoppers. Soil slurries then were anaerobically incubated for 90 days at 15, 30, and 45°C. Each treatment was carried out in triplicate.

Analyses for short-chain fatty acids. Liquid samples (0.5 ml) were taken twice a week from soil slurries using sterile syringes. Prior to sampling, bottles were vigorously shaken for 1 min. The liquid samples were centrifuged at 17,949 × g for 15 min, and the supernatants were collected and filtered through 0.45-μm membrane filters. The solution samples then were stored at -20°C until analysis. The concentrations of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate were determined with an HP 6890N gas chromatograph (Agilent Technologies) using a free fatty acid phase column (15 m by 0.32 mm by 0.25 μm; Agilent Technologies). Other intermediates such as ethanol were not determined, because they were hardly detected according to previous studies (11).

Soil sampling and DNA extraction. The procedures for soil sampling and genomic DNA extraction have been described previously (33).

T-RFLP analyses. PCR amplification and terminal restriction fragment polymorphism (T-RFLP) analyses of bacterial 16S rRNA gene fragments followed the protocols described previously (25, 29). In brief, the amplification was performed using primers 27f and 907r. The 5' end of the forward primer was labeled with 6-carboxyfluorescein (FAM). The 100-μl reaction mixture contained 1 μl of DNA template (in a 1:10 dilution of original extracts), 10 μl of 10× buffers (Tiangen Biotech, Beijing), 7.6 μl of 25 mM MgCl₂, 2 μl of 10 mM deoxynucleoside triphosphates (Tiangen), 1 μl of each 50 μM primer (Tiangen), and 2.5 U *Taq* DNA polymerase (TakaRa). The thermal profile for amplification was 2 min at 94°C; 30 cycles of 30 s at 94°C, 45 s at 52.5°C, and 80 s at 72°C; and finally 10 min at 72°C. The FAM-labeled PCR product was purified using an agarose gel DNA extraction kit (TakaRa) and digested at 37°C for 5 h by *Msp*I (Fermentas, Canada). The digestion products were further purified with SigmaSpin Post-Reaction Clean-Up columns (Sigma) and then were size separated using the 3130xl Genetic Analyzer (Applied Biosystems) as described previously (33).

Cloning, sequencing, and phylogenetic analyses. Clone libraries of the bacterial 16S rRNA genes were constructed. The PCR amplification used the same primers as those indicated above. PCR products were purified and ligated into the pMD19-T Vector (TakaRa) according to the manufacturer's instructions. Plasmids were transformed into *Escherichia coli* cells, and clones were randomly selected and sequenced with an ABI 3730xl sequencer using BigDye Terminator cycle sequencing chemistry (Applied Biosystems).

The sequence data were analyzed using the ARB software package (<http://www.arb-home.de>) (27). The 16S rRNA gene sequences were integrated into the ARB database, which contains 39,790 complete or partial 16S rRNA sequences. Sequence data from GenBank were imported to include the closest matches that were not available in the ARB database. Phylogenetic trees were constructed using the neighbor-joining algorithm by following the procedure of Lueders and Friedrich (28). Highly variable nucleotide positions and those with possible alignment errors were excluded from the phylogenetic analysis by using only positions present in at least 50% of all of the sequences analyzed in this study.

Ordination analyses of T-RFLP fingerprints. The relative abundance (*A_p*) of terminal restriction fragments (T-RFs) was calculated as the percentage of an individual T-RF in the sum of all concerned peak heights in a given T-RFLP profile. The ordination analyses of T-RFLP profiles were performed using CANOCO 4.5 software (Microcomputer Power, Ithaca, NY) (42). We first ran the detrended correspondence analysis to estimate the gradient length of variables. It was found that the longest gradient was shorter than 3.0 in most cases. Thus, the linear model approaches, i.e., the principle component analysis (PCA) and redundancy analysis (RDA), were chosen for analysis, because they performed better than the unimodal approaches under such conditions according to

the CANOCO manual (42). The untransformed data of *A_p* means (in triplicate) were used in the analysis, with the plot scaling focused on sample distances. The Euclidean distance between samples roughly corresponds to their dissimilarity (21).

The temporal effect on bacterial dynamics at different temperatures was analyzed using partial RDA with permutation tests (1,499 replicate runs) of significance on the trace values. The untransformed data of *A_p* means were used in analyses, and the plot scaling was focused on species distance (each T-RF was defined as a species in RDA). The species scores were divided by species standard deviations. The correlations between one species and another species or the explanatory variable (time) could be estimated by perpendicularly projecting the arrow tip of a species of interest onto the arrow of another species or explanatory variable (21).

Nucleotide sequence accession numbers. All sequence data generated from this study have been deposited in the EMBL nucleotide sequence database under accession numbers FM956721 to FM957001.

RESULTS

Dynamics of fatty acids. Fatty acids were produced within 2 days after the anaerobic incubation. Three phases of fatty acid dynamics could be distinguished (Fig. 1). In the first phase, fatty acids accumulated rapidly. Two peaks of transitional accumulations were detected for most of the fatty acids, with the first peak on days 1 to 3 and the second peak on days 10 to 14. The fatty acid concentrations sharply decreased in the third week (second phase), which was in correspondence with the rapid increase of CH₄ production (33). In the last phase (day 22 onwards), the concentrations of all fatty acids remained at low levels without further fluctuation until the end of the experiment. Acetate showed the highest concentrations (Fig. 1A, B), followed by propionate (Fig. 1C, D) and butyrate (Fig. 1E, F), the concentrations of which were approximately one order lower than that of acetate. Isobutyrate, valerate, and isovalerate showed the lowest concentrations (Fig. 1G to L), being about one order lower further than those of propionate and butyrate. The accumulation of fatty acids was higher in straw than root residue. Incubation at 45°C strongly enhanced the accumulation.

Composition of bacterial populations. Four clone libraries of bacterial 16S rRNA genes were constructed to determine the composition of the bacterial community during plant residue decomposition (Table 1). Phylogenetic analyses of 281 clones showed that the bacterial community in rice field soil during the residue decomposition consisted of *Firmicutes*, *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, *Bacteroidetes*, *Chlorobi*, *Actinobacteria*, *Verrucomicrobia*, *Cyanobacteria*, *Nitrospira*, *Planctomycetes*, *Gemmatimonadetes*, *Fibrobacteria*, and *Deinococcus-Thermus* (see Fig. S1 to S3 in the supplemental material). *Firmicutes* were the most abundant organisms (111 of 281 clones), followed by *Proteobacteria* (47 clones), *Chloroflexi* (28 clones), *Actinobacteria* (22 clones), *Acidobacteria* (20 clones), and *Bacteroidetes* (15 clones).

Dynamics of bacterial populations. T-RFLP fingerprinting was used to determine the dynamics of the bacterial community during the 90-day incubations. In total, 108 T-RFLP profiles (2 residues × 3 temperatures × 3 replicates × 6 sampling times) were analyzed, and 56 T-RFs in total were detected across the profiles. Only those with an *A_p* higher than 5% in at least 1 of 108 profiles were selected as the signature T-RFs for analyses of bacterial dynamics. This cutoff was adopted from a previous study (31), which determined the bacterial successions in a rice field soil with a similar set of T-RFLP profiles.

FIG. 1. Dynamics of short-chain fatty acids during residue decomposition in rice field soil. Rice root (left) and straw (right) residues were incorporated (1%, wt/vol) into soil and incubated anaerobically for 90 days at 15, 30, and 45°C. Acetate (A, B), propionate (C, D), butyrate (E, F), isobutyrate (G, H), valerate (I, J), and isovalerate (K, L) were determined during the incubation period. The acetate data were published previously (33). We included these data for the complete picture of the fatty acids. Data are means from three replications. Bars indicate the positive standard errors.

TABLE 1. Phylogenetic affiliation and percentage of clone numbers of bacterial 16S rRNA genes retrieved from anoxic rice soil during the degradation of rice root or straw residue at different times and temperatures

Clone library	Residue type	Temp (°C)	Time point (day)	No. of clones							
				Total	<i>Proteobacteria</i>	<i>Acidobacteria</i>	<i>Verrucomicrobia</i>	<i>Chloroflexi</i>	<i>Cyanobacteria</i>	<i>Firmicutes</i>	<i>Actinobacteria</i>
R15	Root	15	14	83	21.7	4.8	1.2	10.8	1.2	39.8	6
R30	Root	30	14	34	26.5	11.8	0	0	0	23.5	17.6
R45	Root	45	21	94	9.6	6.4	0	4.3	0	60.6	9.6
S30	Straw	30	41	70	15.7	8.6	2.9	21.4	0	18.6	2.9

Twenty-six T-RFs thus were selected. This screening neglected the contribution of low-abundance T-RFs, which consisted of no more than 15% of total signals in the present study. A combination of in silico analyses of 281 sequences and the T-RFLP fingerprinting of the representative clones was used to assign 26 T-RFs to individual bacterial lineages (Table 2). Most T-RFs were affiliated with a single group of bacteria. However, three T-RFs (133, 150, and 160 bp) showed affiliations with two different groups.

T-RFLP profiles revealed clear successions of bacterial populations during the 90-day incubations. According to temporal

patterns of the relative abundances, 26 T-RFs could be divided into three successional groups: the early group, the late group, and the nonsuccessional group. The structure and dynamics of the bacterial community also were influenced strongly by temperature and moderately by residue type.

(i) Early successional group. The early successional group was characterized by a rapid increase in abundance at the beginning, followed by a sharp decrease in the later stages (Fig. 2). At 15°C, eight T-RFs (165, 274, 436, 512, 518, 522, 531, and 540 bp) in root treatment and six T-RFs (81, 274, 512, 518, 522, and 540 bp) in straw treatment were indicative of the early successions in the incubations (Fig. 2A, B). The early successional group at 30°C was less diverse than that at 15°C and included mainly the T-RFs of 120, 165, 522, and 540 bp in the incubations (Fig. 2C, D). The early successional group at 45°C differed greatly from those at 15 and 30°C. The 206-bp T-RF was exclusively dominant, accounting for approximately 36% (Ap) in straw incubations and 20% (Ap) in root incubations at its maxima (Fig. 2E, F). In the root incubations, the T-RFs of 81, 120, and 140 bp also were abundant in the early successions (Fig. 2E).

(ii) Late successional group. The relative abundances of the late successional group were low at the beginning, gradually increased until around day 41, and then remained at high levels until the end of incubation (Fig. 3). In incubations at 15°C, the T-RFs of 89, 93, 140, and 201 bp were abundant in the late successions (Fig. 3A, B). The 170-bp T-RF also increased in abundance in the late stages in straw incubations. At 30°C, only the T-RFs of 89 and 93 bp (and 140 bp in straw) were abundant in the late successions (Fig. 3C, D). Similarly to the early successional group, the late successional group at 45°C showed a significant difference from the abundances at 15 and 30°C (Fig. 3E, F). The 152-bp T-RF was exclusively dominant, accounting for at its maxima of 29% in root and 20% in straw incubations. In addition, the 100- and 150-bp T-RFs increased in the late successions in root and straw incubations, respectively.

(iii) Nonsuccessional group. Most of the nonsuccessional group showed low Ap values that did not change during the 90-day incubations. Depending on the temperature, many T-RFs in this group can display different characteristics of either early or late successional phases. For instance, T-RFs of 206 and 152 bp were dominant at 45°C in the early and late successions, respectively. However, both T-RFs were characterized as the nonsuccessional group at 15°C (data not shown). Similarly, though T-RFs of 140, 512, and 522 bp showed substantial changes between early and late stages at 15 and 30°C,

TABLE 2. Assignment of selected T-RFs to defined bacterial taxa and successional groups at different temperatures

T-RF ^a	Affiliation ^b	Succession at ^c :		
		15°C	30°C	45°C
81	<i>Clostridium</i> (cluster III)	E	L	E
89	<i>Chlorobi</i>	L	L	
93	<i>Bacteroidetes</i> (<i>Flavobacteria</i> / <i>Cytophaga</i>)	L	L	E
100	<i>Clostridiaceae</i>			L
120	<i>Lactobacillales</i>		E	E
125	<i>Acidobacteria</i>			E
133	<i>Actinobacteria</i> / <i>Deltaproteobacteria</i>			
140	<i>Clostridiaceae</i>	L	L	E
143	<i>Bacteroidetes</i> (<i>Sphingobacteriales</i>)		E	E
150	<i>Clostridium</i> (cluster XIVa)/ <i>Acidobacteria</i>			L
152	<i>Acidobacteria</i>			L
160	<i>Alphaproteobacteria</i> / <i>Geobacter</i>			E
163	<i>Geobacter</i>			
165	<i>Actinobacteria</i>	E	E	L
170	<i>Actinobacteria</i>	L	L	E
201	<i>Bacteroidetes</i> (<i>Sphingobacteriales</i>)	L		E
206	<i>Clostridium</i> (cluster III)		L	E
274	<i>Clostridium</i> (cluster III)	E	E	E
305	<i>Clostridium</i> (cluster XIVa)		E	
436	<i>Gammaproteobacteria</i>	E	E	
488	<i>Betaproteobacteria</i>			L
512	<i>Clostridium</i> (cluster I)	E		
518	<i>Clostridium</i> (cluster I)	E	E	
522	<i>Clostridium</i> (cluster I)	E	E	
531	<i>Clostridiaceae</i>	E		E
540	<i>Bacteroidetes</i> (<i>Sphingobacteriales</i>)	E	E	

^a T-RF sizes are in base pairs.

^b The assignment of the selected T-RFs to bacterial groups is based on the phylogenetic analyses of environmental 16S rRNA gene sequences retrieved from the rice field soil. The phylogenetic trees are shown in Fig. S1 to S3 the supplemental material. Three T-RFs (133, 150, and 160 bp) have affiliations for two bacterial groups.

^c The assignment of T-RFs to the early successional group (E) or the late successional group (L) is based on the partial redundancy analyses. Data of root and straw incubations were integrated at each temperature. The assignments are left blank if the T-RFs are related to the nonsuccessional groups or show only low relative abundances in the respective incubations.

TABLE 1—Continued

No. of clones							
<i>Bacteroidetes</i>	<i>Chlorobi</i>	<i>Planctomycetes</i>	<i>Gemmatimonadetes</i>	<i>Nitrospira</i>	<i>Fibrobacteria</i>	<i>Deinococcus-Thermus</i>	Unclassified
7.2	1.2	1.2	1.2	1.2	1.2	0	1.2
8.8	2.9	0	0	0	0	0	8.8
1.1	0	2.1	1.1	1.1	0	0	4.3
7.1	8	1.4	1.4	2.9	0	1.4	4.3

they remained at low abundances without significant fluctuations at 45°C.

Ordination analyses of T-RFLP profiles. To further statistically assess the succession of the bacterial community and the effects of temperature and residue type during the residue decomposition, we performed two ordination analyses (PCA and RDA) for the T-RFLP profiles.

PCA analysis showed significant effects of temperature and incubation time on T-RFLP profiles. In the two-dimensional plot (Fig. 4), the samples from incubations at 15 and 30°C were clearly separated from that at 45°C, although the difference between 15 and 30°C was indiscernible. The samples at 15 and

30°C in the early stage (days 8 and 14) were clustered with day-0 samples, but the difference between the late and early samples increased with time. For incubations at 45°C, samples on days 8 and 14 were separated from those at day 0, indicating a shift of the bacterial community right at the beginning of the incubation.

The partial RDA analyses were performed for T-RFLP profiles at each temperature, with time as the sole explanatory variable. Since the effect of the residue type was less obvious relative to that of temperature, the datasets of root and straw residues were combined for analyses. The results showed that in incubations at 15°C, T-RFs of 274, 512, 518, and 522 bp were

FIG. 2. T-RFs that showed rapid increases followed by decreases during the early stages of incubation. These T-RFs are indicative of the early succession phase during rice residue decomposition. Root (left) and straw (right) were incorporated into soil and incubated anaerobically at 15 (A, B), 30 (C, D), and 45°C (E, F). Data are the means from three replications. Bars indicate the positive standard errors.

FIG. 3. T-RFs that showed low abundances at the beginning but gradually increased in the later periods of incubation. These T-RFs were indicative of the late succession phase during rice residue decomposition. Root (left) and straw (right) were incorporated into soil and incubated anaerobically at 15 (A, B), 30 (C, D), and 45°C (E, F). Data are means from three replications. Bars indicate the positive standard errors.

grouped as the early successional group, while T-RFs of 89, 93, 140, and 170 bp were identified as the late successional group (Table 2). Similarly, in incubations at 30°C, T-RFs of 120, 143, 165, 305, and 522 bp belonged to the early group, while T-RFs of 81, 89, 93, 170, and 206 bp were the late group. In incubations at 45°C, the early group was composed of T-RFs of 93, 120, 143, 160, 201, 206, 274, and 531 bp, while the late group consisted of T-RFs of 100, 150, 152, 165, and 488 bp. These results of partial RDA analyses were corroborated with the classification of T-RFs based on the temporal patterns of the relative abundances (Table 2).

DISCUSSION

Knowledge of organic residue decomposition is crucial for understanding CH_4 production and emission from paddy fields. We previously reported the effects of temperature on the dynamics of methanogenesis and the methanogenic archaeal community during residue decomposition in anoxic rice soil (33). From the cumulative patterns of CO_2 and CH_4 in the headspaces of incubations, it was clear that the decomposition processes were separated into a rapid phase and a slowdown

phase (see Fig. 1 in reference 33). Consistently with this, the present study showed that the dynamics of fatty acid intermediates could be separated into a fast cumulative phase, a rapid consumption phase, and a low-concentration quasi-steady-state phase. The organic components of plant residues conceptually can be divided into an easily degradable fraction and a less-degradable or resistant fraction, such as cellulose and lignin (16, 34). Apparently, the decomposition of the easily degradable fraction of residues caused a rapid accumulation of fatty acids during the first 2 weeks. Later on, the production rate decreased due to the gradual consumption of easily degradable organic components. However, the activity of methanogens, in particular those of acetoclastic *Methanosarcina* species, was stimulated substantially by the accumulated acetate (33). Therefore, a sharp decrease of fatty acids occurred in the second phase, with an accompanying burst of CH_4 production (33). In the last phase, acetate was gradually depleted, resulting in a slowdown of CH_4 production (33). The fast-growing *Methanosarcina* species gradually were replaced by the slow-growing *Methanosaeta* species. The production and consumption of fatty acids was in equilibrium at low levels, reaching the quasi-steady states. Rice straw residues contain more

FIG. 4. Two-dimensional plots of PCA for the entire T-RFLP profiles. Different symbols indicate different treatments (root [R] or straw decomposition [S]) at 15, 30, and 45°C, and different colors indicate time points (days 0 to 90 [D0 to D90]). The eigenvalues of the first two axes are 0.340 and 0.145, respectively.

degradable components than root residues (1, 43, 44), hence producing a higher accumulation of fatty acids (Fig. 1) as well as CH_4 production (33). The enhanced accumulation of fatty acids at 45°C probably was due to the faster decomposition and/or the time required for the thermophilic consumers to be activated (33).

In correspondence with the dynamics of fatty acids, the bacterial community showed a distinct succession during the course of residue decomposition. Recently, it has been shown that bacterial succession occurred due to the spatial-temporal gradients of nutrients in rice soil (31). The succession was considered to be driven by the life strategies of different organisms. The fast-growing organisms (r-strategists) usually dominate in the early succession, while the maintenance-preferred K-strategists gradually replaced them in the late succession (31, 37). The spatial-temporal gradient of resource availability typically is formed when a substrate is spiked into soil. Thus, it is very possible that similar mechanisms of bacterial succession operated during residue decomposition in the present study. The decomposition of easily degradable components and the accumulation of fatty acids stimulated the growth of the early successional group, which probably used the r-strategy, while the decreases of resource availability (degradable residue components and fatty acids) resulted in the gradual replacement by the late successional group, which used the K-strategy. The successions of bacterial communities have been reported in various environments (10, 14, 30, 38). The

decomposition of plant residues in rice field soil probably provides a typical example.

Thirteen T-RFs in total were identified to be indicative of early succession during residue decomposition. Eight of them were assigned to *Clostridia*. The other five were assigned to *Lactobacillales* (120 bp), *Acidobacteria* (125 bp), *Actinobacteria* (165 bp), *Gammaproteobacteria* (436 bp), and *Sphingobacteriales* (540 bp). Among T-RFs assigned to *Clostridia*, three (512, 518, and 522 bp) were related to clostridial cluster I (according to reference 4), three (81, 206, and 274 bp) were affiliated with clostridial cluster III (4), and two (140 and 531 bp) belonged to other groups. Obviously, *Clostridium* spp. dominated the early successions in the residue decomposition.

Clostridia form a physiologically very diverse group of anaerobic, gram-positive, and endospore-forming bacteria (4, 40). Previous studies showed that *Clostridia* were the dominant members of the bacterial community in rice field soil (3, 17, 26, 47). We detected at least five clostridial clusters (based on reference 4) in residue decomposition incubations. The members of clostridial cluster I typically are fast growing and ferment various carbohydrates to fatty acids. Apparently, these organisms were responsible for the decomposition of easily degradable residue components and the fast accumulation of fatty acids in the early stages at 15 and 30°C. The 206-bp T-RF that represented the members of clostridial cluster III dominated in the early succession at 45°C. This group probably was adapted to a higher temperature in the early stages.

Eight T-RFs were characteristic for the late successional group. Three of them were affiliated with clostridial cluster XIVa (150 bp) and other groups (100 and 140 bp) of *Clostridia*. The rest could be assigned to *Bacteroidetes* (93 and 201 bp), *Chlorobi* (89 bp), *Acidobacteria* (150 and 152 bp), and *Actinobacteria* (170 bp), respectively.

Bacteroidetes and *Chlorobi* predominated at 15 and 30°C, while *Acidobacteria* species were selected at 45°C in the late successions. Both *Bacteroidetes* and *Acidobacteria* frequently were detected using a 16S rRNA gene survey in diverse environments (2, 7, 14). *Bacteroidetes* species have the ability to digest and grow on a variety of complex substrates, such as cellulose, chitin, and agar (20, 35). In a study of the fermentation of resistant carbohydrates in the human intestine, Sharp and Macfarlane (36) revealed that *Bacteroidetes* were better adapted at low substrate availability than members of clostridial cluster I. Apparently, the similar adaptation probably occurred during the plant residue decomposition in the rice field soil. The ecological functions of *Acidobacteria* are largely unknown due to the paucity of cultured representatives. A few isolation studies reveal that these organisms are slow growing, contain only one or two rRNA operons, and generally adapt to oligotrophic environments (7, 9). Our study indicates that both *Bacteroidetes* and *Acidobacteria* were dominant in the late successions, when the substrate availability possibly was limiting and hence presumably were active in the decomposition of less-degradable or resistant residue components. *Bacteroidetes* appeared to be better adapted at 30°C, while *Acidobacteria* are selected at a higher temperature (45°C).

ACKNOWLEDGMENTS

We thank the anonymous reviewers for valuable comments and suggestions that improved the manuscript.

This study was financially supported by the Natural Science Foundation of China (grant no. 40571080 and 40625003).

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