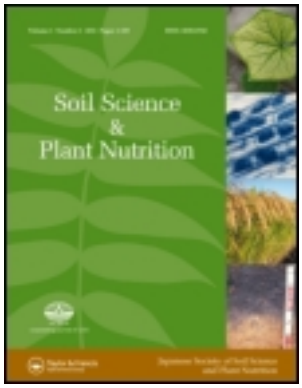

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Effect of application of rice straw and compost on the community structure of bacteria associated with microcrustaceans in the floodwater of a paddy field microcosm

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Effect of Application of Rice Straw and Compost on the Community Structure of Bacteria Associated with Microcrustaceans in the Floodwater of a Paddy Field Microcosm

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A microcosm experiment was conducted to study the effect of the application of rice straw and compost to a soil on the bacterial communities associated with five species of microcrustaceans in the floodwater of paddy fields based on RFLP analysis. PCR products for bacterial 16S rDNA were digested with four restriction endonucleases (*Hin*f I, *Sau*3A I, *Hae* III, *Eco*R I). The results showed that the RFLP patterns of the bacterial communities were distinctly different among the microcrustaceans, although DNA fragments common to *Tanycypris* sp., *Cypretta* sp., and *Heterocypris* sp. were recognized. Bacterial communities associated with *Tanycypris* sp. showed the smallest number of fragments among the microcrustaceans studied. Rice straw and compost application exerted a negligible effect on the band patterns from *Cypretta* sp. and *Moina* sp., respectively. Cluster analysis and principal component analysis enabled to separate the band patterns into 2 groups with a total of 4 subgroups: I-1. *Moina* sp., I-2. *Mesocyclops* sp., II-1. *Tanycypris* sp. and *Cypretta* sp., and II-2. *Heterocypris* sp. The results indicated that the bacterial association with the microcrustaceans was primarily determined by the genus and species of hosts. Effects of rice straw and compost application on the bacterial communities associated with *Moina* sp. and *Cypretta* sp. and the duration of flooding on the bacterial communities associated with all the microcrustaceans were also observed, although the effects were not appreciable compared to those of the kind of host microcrustaceans.

Key Words: **application of organic materials, bacterial communities, floodwater paddy field, microcrustaceans, RFLP.**

Microcrustaceans (Cladocerans, Copepods, and Ostracods) are common inhabitants of the floodwater of paddy fields (Ali 1990; Kuwabara 1999; Yamazaki et al. 2001). Microcrustaceans and their associated bacteria that inhabit the floodwater of paddy fields may play a role in nutrient cycling and decomposition of organic materials. Wilson et al. (1980) and Grant et al. (1983) reported that microcrustaceans exerted a significant effect on N_2 fixation in the floodwater of a paddy field by grazing nitrogen-fixing blue-green algae. Microcrustaceans are important as hosts of some functional groups of microbiota in aquatic environments. For example, microcrustacean copepods were reported to be associated with nitrogen-fixing bacteria (Proctor 1997; Zehr et al. 1998; Braun et al. 1999), methanogens (Traganza et al. 1979; Cynar and Yayanos 1991; de Angelis and Lee 1994), and *Vibrio* sp. (Huq et al. 1984; Carli et al. 1993; Dumontet et al. 1996; Pruzzo et al. 1996; Montanari et al. 1999) in seawater and freshwater environments. There have been, however, few studies dealing with bacterial communities associated with microcrustaceans in the floodwater of paddy fields (Taniguchi et al. 1997a, b, 1999; Niswati et al. 2002).

A specific bacterial community is considered to develop in association with microcrustaceans. By scanning electron microscopy, Taniguchi et al. (1997a, b, 1999), observed specific colonization patterns of epibiotic microorganisms on microcrustaceans obtained from the floodwater of Japanese and Philippine paddy fields. Niswati et al. (2002) recently compared the bacterial communities associated with aquatic organisms in a paddy field including microcrustaceans (Cyclopoida and Cladocera) by using a molecular ecological technique (PCR-RFLP), and suggested that unique bacterial communities developed for the respective microcrustaceans. Epibiotic bacteria may use debris and excreta from host microcrustaceans which could contribute to the development of specific epibionts (Holland and Her-

genrader 1981; Carman 1994).

Application of organic materials such as rice straw and rice straw compost, which is a common practice in rice cultivation, affected the community structure of the free-living bacteria in the floodwater of paddy fields (Okabe et al. 2000; Kimura et al. 2002). Yamazaki et al. (2001) reported that the frequency of appearance of microcrustaceans in floodwater also differed depending on the kind of fertilizer applied. There is limited information, however, about the effect of the application of organic materials on the community structure of bacteria associated with microcrustaceans in the floodwater of paddy fields.

The purpose of this study was to determine the effect of rice straw and compost application on the community structure of bacteria associated with five kinds of microcrustaceans (three species of Podocopida, one species of Cladocera, and one species of Cyclopoida) in the floodwater of a paddy field microcosm based on the estimation of the PCR-RFLP patterns of 16S rDNA. Those microcrustaceans were common inhabitants of floodwater of Japanese paddy fields (Yamazaki et al. 2001).

MATERIALS AND METHODS

Set-up of a paddy field microcosm. Soil used in the experiment was taken from a paddy field located at Aichi-ken Anjo Research and Extension Center, central Japan (latitude 34°48′N, longitude 137°30′E). Properties of the soil sample (Anthraquic Yellow Soil; an Oxiaquic Dystrochrepts) were as follows: total C content, 13.3 g kg^{-1}; total N content, 0.9 g kg^{-1}; pH(H_2O), 6.0; pH(KCl), 4.9. One kilogram of soil that passed through a 4-mm mesh screen was mixed thoroughly with chemical fertilizers consisting of $(NH_4)_2SO_4$, $Ca(H_2PO_4)_2 \cdot H_2O$, and KCl at the rates of 0.5, 0.5, and 0.2 g kg^{-1}, respectively. Rice straw segments about 2 cm long with a C / N ratio of 40.0 or rice straw compost with a C / N ratio of 14.0 were mixed thoroughly into the fertilized soil (10 g kg^{-1} on a dry weight basis). Fertilized soil without amendment of organic materials was also prepared as a control. To avoid the development of indigenous microcrustaceans, the soil was heated by autoclaving at 80°C for 2 h. All the treatments were prepared in triplicate. One kilogram of the soil prepared as indicated above was put into a container (length × width × height of 20 cm × 13 cm × 14 cm, respectively) and was submerged with 3,000 mL of distilled water. The containers were placed in the Koitotron (Koito Industries, Ltd., Yokohama, Japan) at temperatures ranging from 30°C in the daytime (4 A.M. to 8 P.M.) to 20°C at night (8 P.M. to 4 P.M.). During the incubation period, the depth of water was maintained at 10 cm.

Preparation of microcrustaceans. Three species of Podocopida (*Tanycypris* sp., *Cypretta* sp., and *Heterocypris* sp.), one species of Cladocera (*Moina* sp.), and one species of Cyclopoida (*Mesocyclops* sp.) were collected from the floodwater of a paddy field located at Aichi-ken Anjo Research and Extension Center. Each microcrustacean was cultured separately in an aqueous medium containing 5 g L^{-1} chicken manure (N, 2.58%; P_2O_5, 8.11%; K_2O, 3.37%; CaO, 19.21%) and 0.1 g L^{-1} baker yeast with periodical supply of *Chlorella*.

Inoculation and sampling of microcrustaceans. About 150 individuals of adult microcrustaceans that were harvested from the culture medium described above (easily distinguishable from juveniles by size) were inoculated to the paddy field microcosms one day after the set-up. One hundred adult individuals of microcrustaceans were weekly collected with a plankton net. They were cleaned at least three times in sterile distilled water to minimize the bacterial contamination from bulk water, and stored in a 1.5 mL tube with 1 mL of sterile ultra-pure water at −20°C until DNA extraction.

DNA extraction for 16S ribosomal DNA analysis. One hundred individuals of the respective microcrustaceans were macerated with a sterile pipette tip in the 1.5 mL tube. DNA was immediately extracted from the microcrustaceans using a high temperature, salt, and sodium dodecyl sulfate (SDS)-based lysis method (Zhou et al. 1996). This protocol had been a slightly modified for the amount of reagent suitable for the extraction of small volume of microcrustaceans (Niswati et al. 2002). DNA was extracted in duplicate. The suspension was stored at 4°C for immediate use or at −20°C for long storage.

PCR amplification. The 16S rDNA target in the microcrustaceans was amplified by using the eubacterial pair of primers 27f and 1401r under the PCR conditions described in the previous study (Niswati et al. 2002). PCR products from some of the samples were purified prior to RFLP analysis by the Ultrafree-DA procedure (Amicon, Bedford, MA, USA).

Aliquot of the PCR product was precipitated with twice the volume of pure ice-cold ethanol and one-tenth volume of 3 M NaOAc at pH 5.2. It was centrifuged, washed with 0.7 L L^{-1} ice-cold ethanol, dried up, and suspended again in sterile ultra-pure water.

RFLP analysis. The PCR products were digested with four kinds of restriction endonucleases (*Eco*R I., *Hae* III, *Hinf* I, and *Sau*3A I: Takara Shuzo Co., Ltd., Shiga, Japan), according to the manufacturer's specifications. The digests were resolved by electrophoresis on 20 g L^{-1} MetaPhor® agarose (BMA, Rockland, ME, USA) gels containing 20 mL L^{-1} of 50 × TAE buffer (40 mM Tris-acetate, 2 mM EDTA) and 0.5 μg mL^{-1}

ethidium bromide. A 100 bp ladder (BioLabs Inc., New England) was used as a DNA marker. The gel was electrophoresed at 50 V for 1 h, scanned by UV illumination, and photographed. The RFLP analysis was conducted in triplicate. Since the PCR-RFLP patterns were the same among the replications, data from one representative pattern were subjected to analysis for each treatment. The size and amount of the fragments that were visualized by UV illumination were estimated based on the mobility of the DNA marker and the band intensity using 4 grades (0, no band; 1, weak (0–40 ng); 2, medium (40–95 ng); and 3, strong (>95 ng)), respectively. The fragments that were shorter than 100 bp were not considered in the following analysis because their diffusion was too wide to determine their fragment sizes.

Statistical analysis. To estimate the effect of the soil treatments on the bacterial communities associated with microcrustaceans, cluster analysis and principal component analysis were performed based on the data obtained from RFLP analysis. All the fragments obtained by digestion with four kinds of restriction enzymes were used for the calculation. Cluster analysis was performed by the Blackbox program using the Ward method (Aoki 1996). The values were normalized for principal component analysis and cluster analysis. Principal component analysis was performed using Sristat program in EXCEL STATISTICS 97 for windows (SRI, Tokyo). Correlation matrix was used for this analysis.

RESULTS

Community structure of bacteria associated with microcrustaceans

The RFLP patterns of sample DNA were sufficiently reproducible to enable to distinguish differences in the patterns among microcrustaceans and treatments. As exemplified in Fig. 1, the RFLP patterns of the bacterial communities associated with the respective microcrustaceans were different from each other, although fragments common to *Tanycypris* sp., *Cypretta* sp., and *Heterocypris* sp. were observed. The sizes of the fragments were 880 and 180 bp for the *Hae* III digestion samples, 700, 330, and 310 bp for the *Hinf* I digestion samples, and 550 and 310 bp for the *Sau*3A I digestion samples, respectively, regardless of the sampling time and treatments.

Digestion of PCR products from *Moina* sp. with *Hae* III produced three major DNA fragments of 900, 230,

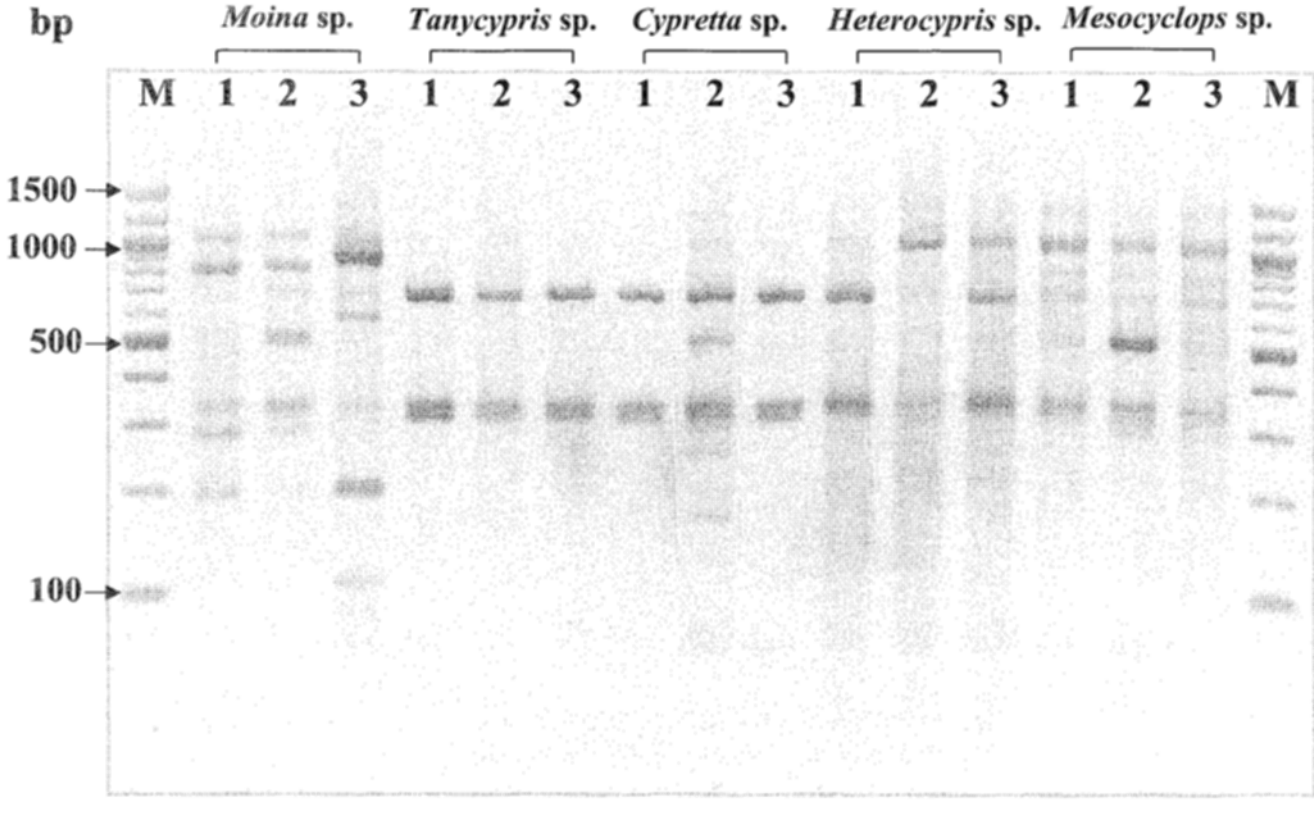

Fig. 1. Representative PCR-RFLP patterns of 16S rDNA of the bacterial communities associated with microcrustaceans in the floodwater of a paddy field microcosm at 6 weeks after flooding based on digestion with *Hinf* I restriction endonuclease. 1, soil treated with NPK only; 2, NPK + rice straw; 3, NPK + compost, respectively. M indicated the DNA marker in base pairs.

and 180 bp, digestion with *Hinf* I produced four major DNA fragments of 1,000, 800, 500, and 330 bp, and digestion with *Sau*3A I produced three major DNA fragments of 1,150, 500, and 310 bp.

Digestion of PCR products from *Mesocyclops* sp. with *Hae* III produced four major DNA fragments of 900, 500, 230, and 180 bp, digestion with *Hinf* I produced three major DNA fragments of 1,350, 1,000, and 330 bp, and digestion with *Sau*3A I produced three major DNA fragments of 1,350, 1,150 and 1,000 bp. Digestion of all the samples with *Eco*R I produced three major DNA fragments of 1,400, 720, and 680 bp (data not shown).

The smallest number of fragments was observed for the bacterial communities associated with *Tanycypris* sp. with the average number of 4, 4.6, 5, and 4.6 when digested with *Eco*R I, *Hae* III, *Hinf* I, and *Sau*3A I, respectively, followed by *Cypretta* sp. (3.5, 8, 6, and 7), *Heterocypris* sp. (4, 8.5, 7, and 9), *Moina* sp. (4, 12, 7.5, and 10), and *Mesocyclops* sp. (4, 9.5, 8, and 9).

Both principal component analysis and cluster analysis of the RFLP patterns of the DNA products enabled to separated the bacterial communities into four groups (Fig. 2); *Moina* sp. group, *Mesocyclops* sp. group, *Heterocypris* sp. group, and *Tanycypris* sp. and *Cypretta* sp. group. As the effect of the treatments on the RFLP patterns was negligible, the treatments were not specified in Fig. 2. The contribution percentages of the primary and secondary components were 16.7 and 12.1%, respectively. Principal component analysis indicated that the community structure of the bacteria associated with *Mesocyclops* sp. and *Moina* sp. was characterized by microorganisms with *Hae* III 1150 (enzyme used and fragment size), *Hae* III 900, *Hinf* I 800, *Hae* III 500, *Hae* III 600, and *Hae* III 760, that associated with *Heterocypris* sp. was characterized by bacteria with *Hae* III 160, *Sau*3A I 700, *Hae* III 280, *Hae* III 210, *Hae* III 800, and *Hinf* I 180, and that associated with *Tanycypris* sp. and *Cypretta* sp. was characterized by bacteria with *Hae* III 880, *Sau*3A I 550, *Eco*R I 1400, *Hinf* I 310, *Hinf* I 700, and *Sau*3A I 310, respectively (Fig. 2).

Figure 2 also indicated that the effect of the application of rice straw and compost on the community structure of the bacteria was negligible compared to the effect of the kind of microcrustaceans, although the effect of the application of rice straw and compost was recognized for some of them, as indicated below.

Bacterial communities associated with *Moina* sp.

The effect of the application of rice straw and compost on the RFLP patterns of the bacterial communities

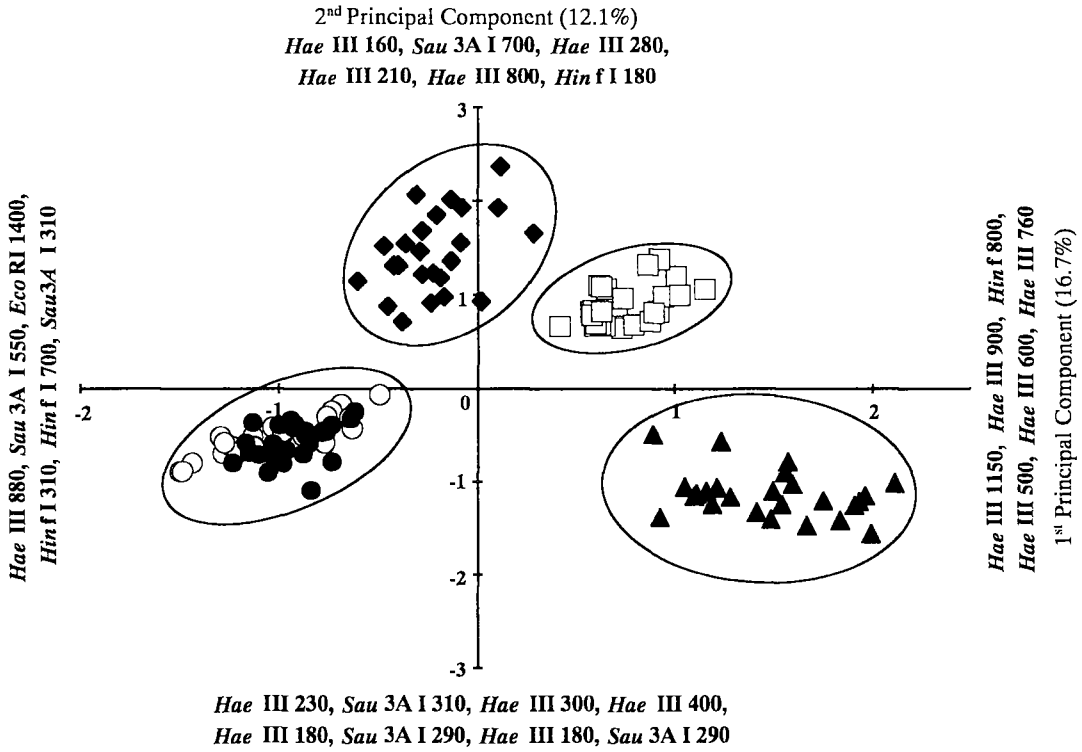

Fig. 2. Principal component analysis of the RFLP patterns of the bacterial communities associated with microcrustaceans in the floodwater of a paddy field microcosm. The RFLP patterns were obtained from the whole samples. DNA fragments on each side show those with high values of eigenvector. ▲, *Moina* sp.; □, *Mesocyclops* sp.; ●, *Tanycypris* sp.; ○, *Cypretta* sp.; and ◆, *Heterocypris* sp., respectively. Plots which form a distinct subcluster in cluster analysis are enclosed with a circle.

associated with *Moina* sp. started to appear four weeks after flooding (Fig. 3). Principal component analysis revealed that the RFLP patterns of the bacterial communities were distributed according to the treatment and sampling weeks, in which the score plots of the bacterial communities in the compost application treatment were distributed separately from those of the other treatments and shifted to the right direction along with the duration of the incubation period. The major fragment sizes of 16S rDNA characteristic of the compost treatment samples were as follows: *Sau*3A I 1000, *Sau*3A I 1350, *Eco*R I 350, *Eco*R I 600, *Eco*R I 400, and *Sau*3A I 240. The effect of NPK only application (control) and rice straw application were similar and characterized by the presence of the following fragment sizes: *Sau*3A I 900, *Sau*3A I 600, *Sau*3A I 210, *Hae* III 880, *Eco*R I 680, and *Sau*3A I 180.

Bacterial communities associated with *Mesocyclops* sp.

The application of rice straw and compost did not affect the RFLP patterns of the bacterial communities associated with *Mesocyclops* sp., for which the duration of the incubation period was more influential (Fig. 4). Principal component analysis and cluster analysis revealed that the RFLP patterns were distributed at three sites; one site for the samples with a short incubation period, one site for the samples with a medium incubation period, and one site for the samples with a long incubation period. During the first three weeks after flooding, *Mesocyclops* sp. was colonized mainly by bacteria with the following fragment sizes: *Hinf* I 210, *Hae* III 230, *Hinf* I 700, *Hinf* I 900, *Hae* III 720, and *Hinf* I 310. Then the predominant bacteria shifted to those with *Sau*3A I 240, *Hinf* 600, *Sau*3A I 370, *Hinf* I 500, *Hinf* I 370, and *Eco*R I 1400 from four to six weeks after flooding, and they were characterized by bacteria with *Hae* III 500, *Sau*3A I 900, *Hinf* I 800, *Hae* III 880, *Hinf* I 1000, and *Hinf* I 500 from eight weeks afterwards.

Bacterial communities associated with *Tanycypris* sp.

Rice straw and compost application did not affect the changes in the bacterial communities associated with *Tanycypris* sp, while the duration of the incubation period affected the associated bacterial communities. Cluster analysis enabled to divide the RFLP patterns into three groups: short incubation period, medium incubation period, and long incubation period. Principal component analysis also revealed that the RFLP patterns were distributed according to the number of weeks of incubation (Fig. 5). During the early weeks after flood-

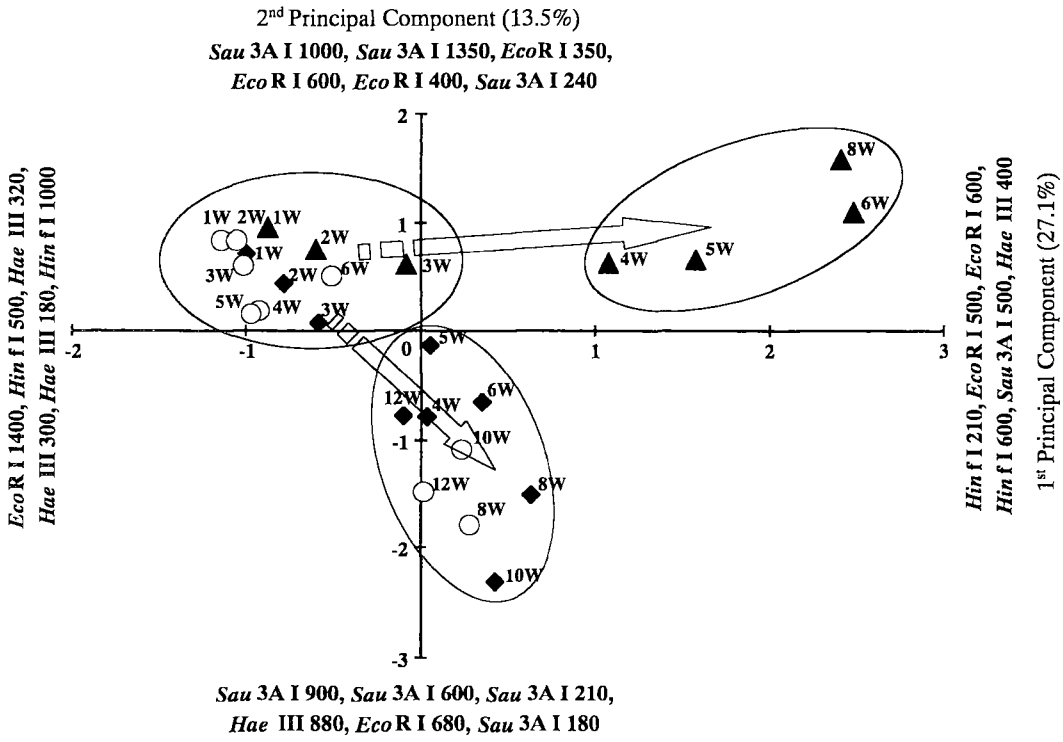

Fig. 3. Principal component analysis of RFLP patterns of the bacterial communities associated with *Moina* sp. in the floodwater of a paddy field microcosm based on the application of organic materials and sampling weeks. DNA fragments on each side show those with high values of eigenvector. ◆, NPK only; ○, NPK + rice straw; ▲, NPK + compost, respectively. Plots which form a distinct subcluster in cluster analysis are enclosed with a circle.

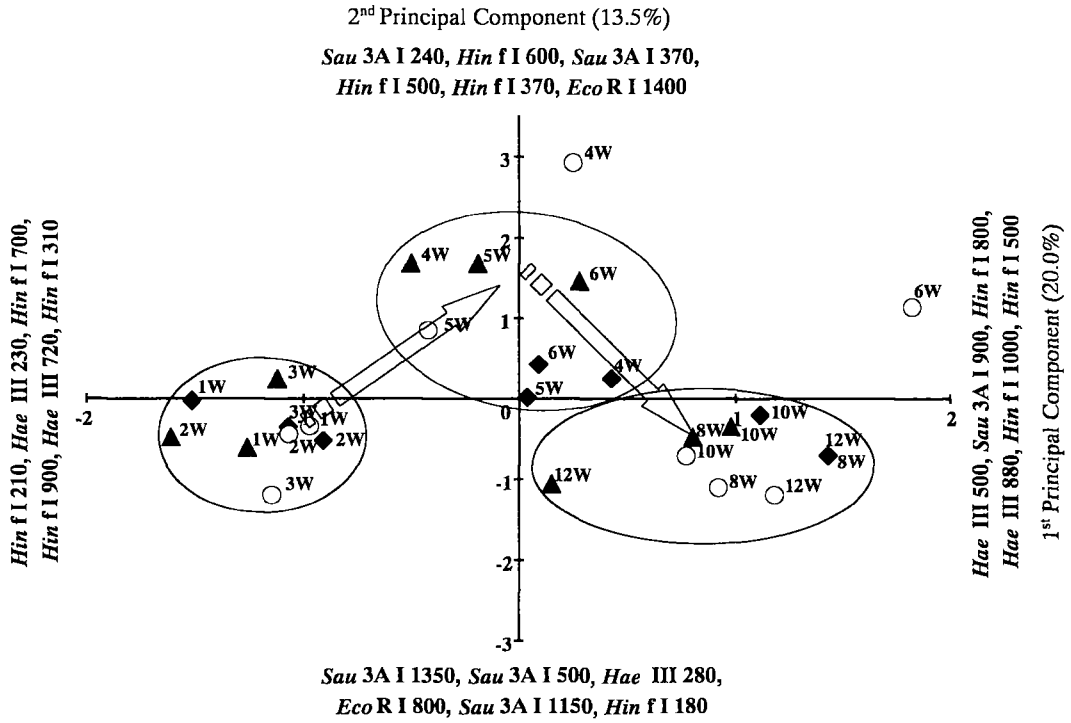

Fig. 4. Principal component analysis of RFLP patterns of the bacterial communities associated with *Mesocyclops* sp. in the floodwater of a paddy field microcosm based on the application of organic materials and sampling weeks. DNA fragments on each side show those with high values of eigenvector. ◆, NPK only; ○, NPK + rice straw; ▲, NPK + compost, respectively. Plots which form a distinct subcluster in cluster analysis are enclosed with a circle.

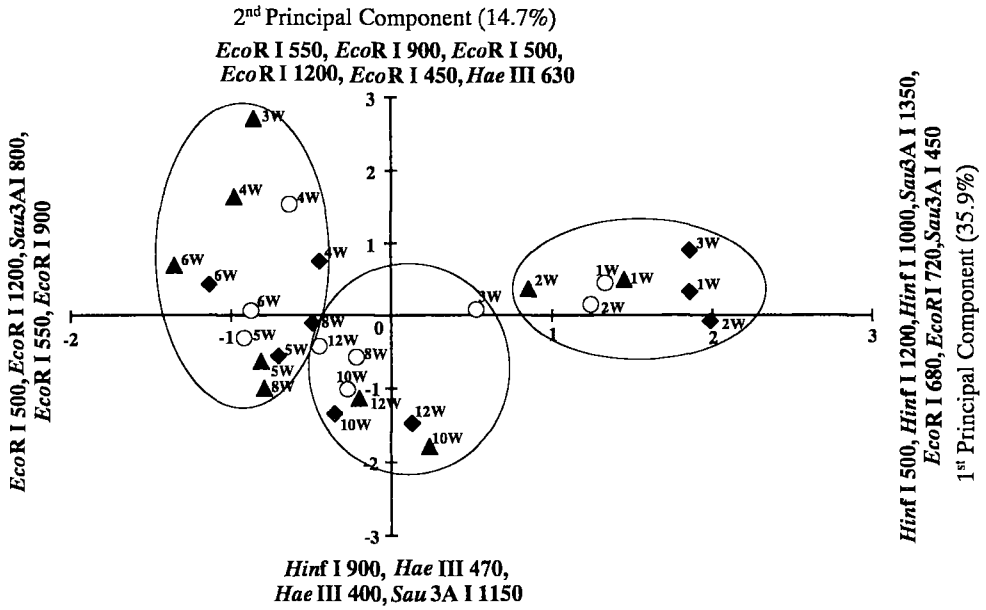

Fig. 5. Principal component analysis of RFLP patterns of the bacterial communities associated with *Tanycypris* sp. in the floodwater of a paddy field microcosm based on the application of organic materials and sampling weeks. DNA fragments on each side show those with high values of eigenvector. ◆, NPK only; ○, NPK + rice straw; ▲, NPK + compost, respectively. Plots which form a distinct subcluster in cluster analysis are enclosed with a circle.

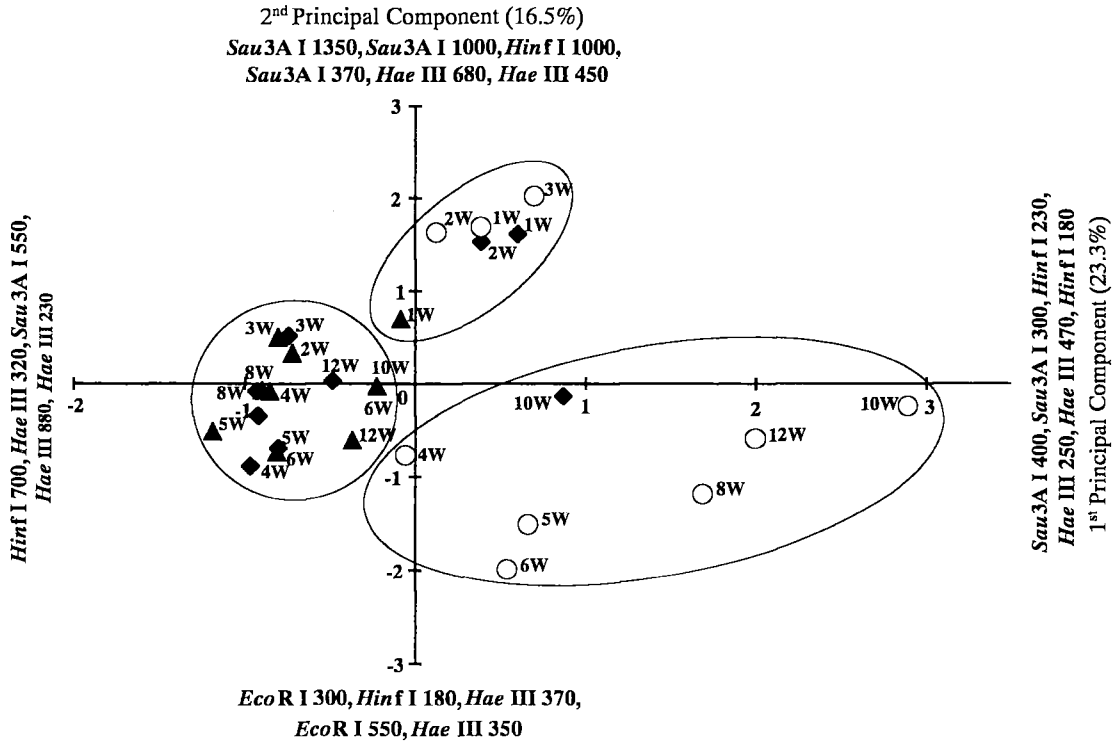

Fig. 6. Principal component analysis of RFLP patterns of the bacterial communities associated with *Cypretta* sp. in the floodwater of a paddy field microcosm based on the application of organic materials and sampling weeks. DNA fragments on each side show those with high values of eigenvector. ◆, NPK only; ○, NPK + rice straw; ▲, NPK + compost, respectively. Plots which form a distinct subcluster in cluster analysis are enclosed with a circle.

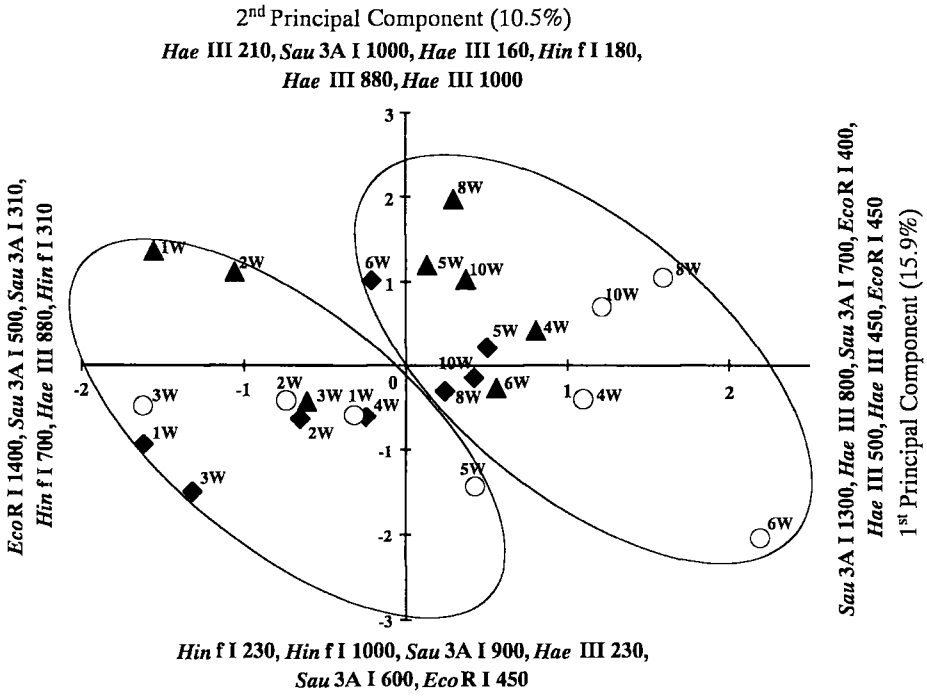

Fig. 7. Principal component analysis of RFLP patterns of the bacterial communities associated with *Heterocypris* sp. in the floodwater of a paddy field microcosm based on the application of organic materials and sampling weeks. DNA fragments on each side show those with high values of eigenvector. ◆, NPK only; ○, NPK + rice straw; ▲, NPK + compost, respectively. Plots which form a distinct subcluster in cluster analysis are enclosed with a circle.

ing, *Tanycypris* sp. was colonized mainly by bacteria with the following fragment sizes: *Hinf* I 500, *Hinf* 1200, *Hinf* I 1000, *Sau*3A I 1350, and *Eco*R I 680 on the right side. From four to six weeks after flooding, the predominant bacteria shifted to those with *Eco*R I 500, *Eco*R I 550, *Eco*R I 900, and *Sau*3A I 800 on the left side, and they switched again from eight to twelve weeks after incubation to bacteria with the following fragment sizes: *Hinf* I 900, *Hae* III 470, *Hae* III 400, and *Sau*3A I 1150.

Bacterial communities associated with *Cypretta* sp.

The effect of the application of rice straw and compost on the RFLP patterns of the bacterial communities associated with *Cypretta* sp. started to appear four weeks after flooding. Cluster analysis enabled to divide the RFLP patterns of the bacterial communities associated with *Cypretta* sp. into three clusters; the first consisted of bacterial communities with a short incubation period, the second of those from the treatment consisting of rice straw application, and the third of those from the treatments consisting of the application of compost and NPK only (control). The second and the third clusters were divided further into several subclusters with small squared distances, mainly based on the duration of the incubation period (data not shown). The first and the second principal component analysis also revealed that the RFLP patterns were distributed according to the treatment and sampling weeks, in which the score plots of the bacterial communities from the treatment with rice straw application indicated the predominance of bacteria having DNA fragments with the following sizes: *Sau*3A I 400, *Sau*3A I 300, *Hinf* I 230, *Eco*R I 300, *Hinf* I 180, and *Hae* III 370. Bacterial communities in the control and compost treatments were distributed in the same cluster and they were characterized by bacteria with the following fragments sizes: *Hinf* I 700, *Hae* III 320, *Sau*3A I 550, *Hae* III 880, and *Hae* III 230. Up to the third week of incubation, the bacterial communities associated with *Cypretta* sp. were characterized by bacteria with following fragment sizes: *Sau*3A I 1350, *Sau*3A I 1000, *Hinf* I 1000, and *Sau*3A I 370 (Fig. 6).

Bacterial communities associated with *Heterocypris* sp.

Rice straw and compost application did not affect the bacterial communities associated with *Heterocypris* sp. (Fig. 7). In contrast, the duration of flooding affected the associated bacterial communities. Both cluster and principal component analysis enabled to divide the bacterial communities into two groups: bacterial communities with an incubation period of less and more than four weeks, respectively.

DISCUSSION

Community structure of the bacteria associated with microcrustaceans. As shown in Fig. 2, the RFLP patterns of the 16S rDNA products from the bacterial communities associated with the respective microcrustaceans were significantly different from each other, indicating the presence of different bacterial communities for the respective microcrustaceans. Microscopic observation showed that bacteria with a different shape colonized the body surface of different microcrustaceans (Taniguchi et al. 1997a, b, 1999).

Aquatic organisms are grouped ecologically into planktonic and benthic members from the main habitat in the aquatic environment. *Moina* sp. was generally recognized to belong to the planktonic group, while *Cypretta* sp. and *Tanycypris* sp. were benthic in the microcosms studied. *Heterocypris* sp., the other Podocopida, was observed both on the soil surface and in the floodwater. Benthic behavior of both *Cypretta* sp. and *Tanycypris* sp. might have contributed to the overlapping of the score plots of the RFLP patterns of the bacterial communities associated with *Tanycypris* sp. and *Cypretta* sp. in the principal component analysis (Fig. 2). In addition, clear separation of the score plots of the RFLP patterns of the bacterial communities associated with *Moina* sp. from those with *Tanycypris* sp. and *Cypretta* sp. seemed partly due to differences in the behavior among them (Fig. 2). Bacterial communities associated with *Tanycypris* sp. and *Cypretta* sp. shared many DNA fragments with the same size, and additional DNA fragments specific to *Cypretta* sp. were observed. As mentioned previously, the bacterial communities associated with *Tanycypris* sp. produced the smallest number of DNA fragments by endonuclease digestion. In a previous study that was conducted in the paddy field from where the soil samples for the present study were collected, the bacterial communities associated with *Tanycypris* sp. produced the smallest number of restriction fragments of 16S rDNA (Niswati et al. 2002). In addition, SEM observation by Taniguchi et al. (1997a) revealed the absence of microorganisms on the body surface of *Tanycypris* sp., whereas morphologically specific bacteria colonized a specific site of the body surface of *Cypretta* sp. On the other hand, *Moina* sp. and *Mesocyclops* sp. were colonized by morphologically different microorganisms (Taniguchi et al. 1997b). Generally, the fragment size and the number of fragments in the present study were similar to those recorded in the previous study in a paddy field (Niswati et al. 2002), although the mild heating was performed for the soil materials in the present study.

Effect of rice straw and compost application

on bacterial communities associated with microcrustaceans. Application of rice straw and compost affected only the microbial communities associated with *Moina* sp. and *Cypretta* sp. (Figs. 3 and 6), while their application did not affect the bacterial communities associated with *Mesocyclops* sp., *Tanycypris* sp., and *Heterocypris* sp. (Figs. 4, 5, and 7), although the community structure of the free-living bacteria in the floodwater changed by the application of chemical fertilizers or organic materials (Okabe et al. 2000; Kimura et al. 2002). This finding indicated that the association between the bacteria and the host microcrustacean was very close and stable, presumably due to symbiotic relations (Chang and Jenkins 2000) and / or the stable microhabitat of specific tissues that were inherent to each microcrustacean.

At three weeks after flooding, the rice straw and compost treatments affected differently the bacterial communities associated with *Moina* sp. (Fig. 3). The changes in the bacterial communities depending on compost application seemed to correspond to the fluctuations in the abundance of *Moina* sp. by the application of organic materials. The application might have changed the physiology of *Moina* sp. and bacteria associated with the species. The high sensitivity of *Moina* sp. has often been used as an indicator of environmental change (Wong 1996). Taniguchi et al. (1997b) and Yamazaki et al. (2001) reported that the fluctuations in the abundance of *Moina* sp. were more conspicuous than those of the other microcrustaceans in the floodwater of Japanese paddy fields. In addition, Amarasinghe et al. (1997) reported in a laboratory experiment that the life cycle and reproduction of Cladocerans were strongly affected by the food supply. Gaiser and Bachmann (1993) also observed that differences in the environmental conditions such as temperature and quality of nutrients in the lake resulted in changes in Cladocera and their epizoic diatoms. Taylor and Aiken (1985) who conducted a laboratory experiment to analyze the growth and reproduction of *Daphnia* under conditions of abundant and limited food supply, found that the growth and reproduction of *Daphnia* were curtailed at a low food concentration.

Bacterial communities associated with *Cypretta* sp. were also affected by rice straw application. The exoskeleton of *Cypretta* sp. that was grown in the rice straw treatment looked more brownish and was different from that of *Cypretta* sp. grown in the compost and the control treatment microcosms. Changes in the soil and / or water conditions associated with the application of rice straw may affect the calcareous shell formation of the Ostracoda (Delorme 1991) and resultant bacterial communities.

Effect of the duration of the period after flooding. The studies presented here were undertaken to examine the effect of the flooding duration on the bacterial communities associated with microcrustaceans. The results showed that the bacterial communities associated with the respective microcrustaceans were generally divided into three groups as follows: bacterial communities with a short incubation period (1–3 weeks), those with a medium incubation period (4–6 weeks), and those with a long incubation period (8–12 weeks). Seasonal variations in the incidence of Cladocerans were reported by Yamazaki et al. (2001), which indicated that the changes in the floodwater environment with time led to fluctuations in the hosts and their associated bacteria, presumably due to the changes in the chemical properties of the floodwater of paddy soils. Okabe et al. (2000) reported that the percentage of distribution of Gram positive and Gram negative bacteria in the floodwater of a paddy field based on PLFAs analysis changed with the time after flooding, and Kimura et al. (2002) pointed out that the RFLP and DGGE patterns of the bacterial communities also changed with the time after flooding.

Conclusion. RFLP analysis in the present study demonstrated that a specific community structure of bacteria associated with microcrustaceans developed in the floodwater of paddy fields for the respective microcrustaceans. Effects of rice straw and compost application on the bacterial communities associated with *Moina* sp. and *Cypretta* sp. and the duration of flooding on the bacterial communities associated with all the microcrustaceans were also observed, although the effects were negligible compared to those of the kind of host microcrustaceans. The RFLP analysis enabled to detect the differences in associated bacterial communities among the host microcrustaceans, and the effects of the duration of the incubation period and the application of rice straw and compost. However, phylogenic information on the bacteria that characterized the host specificity, seasonal variations, and effect of the application of organic materials should be studied in detail by other methods.

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