Culture-Based and Denaturing Gradient Gel Electrophoresis Analysis of the Bacterial Community Structure from the Intestinal Tracts of Earthworms (*Eisenia fetida*)

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The bacterial communities in the intestinal tracts of earthworm were investigated by culture-dependent and -independent approaches. In total, 72 and 55 pure cultures were isolated from the intestinal tracts of earthworms under aerobic and anaerobic conditions, respectively. Aerobic bacteria were classified as *Aeromonas* (40%), *Bacillus* (37%), *Photobacterium* (10%), *Pseudomonas* (7%), and *Shewanella* (6%). Anaerobic bacteria were classified as *Aeromonas* (52%), *Bacillus* (27%), *Shewanella* (12%), *Paenibacillus* (5%), *Clostridium* (2%), and *Cellulosimicrobium* (2%). The dominant microorganisms were *Aeromonas* and *Bacillus* species under both aerobic and anaerobic conditions. In all, 39 DNA fragments were identified by polymerase chain reaction−denaturing gradient gel electrophoresis (PCR−DGGE) analysis. *Aeromonas* sp. was the dominant microorganism in feeds, intestinal tracts, and casts of earthworms. The DGGE band intensity of *Aeromonas* from feeds, intestinal tracts, and casts of earthworms was 12.8%, 14.7%, and 15.1%, respectively. The other strains identified were *Bacillus*, *Clostridium*, *Enterobacter*, *Photobacterium*, *Pseudomonas*, *Shewanella*, *Streptomyces*, uncultured Chloroflexi bacterium, and uncultured bacterium. These results suggest that PCR−DGGE analysis was more efficient than the culture-dependent approach for the investigation of bacterial diversity and the identification of unculturable microorganisms.

Keywords: Denaturing gradient gel electrophoresis, bacterial community, 16S rDNA, earthworms

Earthworms are remarkable workers that have many important roles in nature, including mixing and aerating the soil, improving soil structure and water infiltration, moderating soil pH, bringing up minerals in the soil, making nutrients more available to plants, breaking down plant and animal residue into compost, and increasing beneficial microbial action in the soil [38]. *Eisenia fetida* is an excellent vermicomposting earthworm and is a common earthworm in the agricultural ecosystems of Korea. It can process large amounts of organic matter and can eat food quantities equivalent to its own body weight each day. It can tolerate fluctuations in temperature, acidity, and moisture levels that many worm species cannot [12]. In addition, Aira *et al.* [1] reported that *E. fetida* has the ability to digest organic matter, through degradable enzymes (*e.g.*, amylase, protease, lipase, and cellulase) excreted by microorganisms in the gut of the earthworms.

Less than 1% of all bacterial cells in nature can be grown by culture-based approaches such as culture media [4, 34, 35]. The incubation time is long, and the results may not accurately reflect the population dynamics of microbial communities because of changes in the environmental conditions (*e.g.*, temperature, pH, light) [22]. Recently, studies of bacterial communities have mainly depended on culture-independent approaches based on the analysis of DNA or RNA. These approaches are based on techniques in which total DNA or RNA is directly extracted from microorganisms, including low-abundance microorganisms or unculturable microorganisms [11]. Denaturing gradient gel electrophoresis (DGGE) can be used to separate 16S rDNA sequences with one base-pair differences. DGGE was introduced to microbial ecology by Muyzer *et al.* [25]. DGGE has been used to assess microbial community diversity in sludge [27], sub-seafloor biosphere [29, 42], soil [18], biofilm [15], river [3], wastewater [19, 43], crude oil [21], human intestines [31], insects [33], manure [20], probiotic products [9, 37], cheese [6, 13], milk [5], and fermented sausage [2, 32]. Many researchers have used DGGE, making it a well-established technique for the investigation of microbial community diversity [24].

To understand the beneficial effects of earthworms on soil properties, the role of microorganisms in the intestinal

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tracts of earthworms must be rigorously defined. The intestinal tracts of earthworms host a variety of soil microorganisms [30]. The DGGE technique is quick to perform and well suited for analysis of microbial communities from which it is impossible to obtain viable microorganisms. The aim of this study was to investigate the bacterial community diversity in the intestinal tracts of earthworms and to determine the correlation between the culture-dependent approach and culture-independent approach (DGGE analysis). Furthermore, it might help to isolate microorganisms with beneficial effects on earthworms and to identify a community of low-abundance microorganisms and unculturable microorganisms by DGGE analysis.

MATERIALS AND METHODS

Earthworm Collection and Feeding
Adult *E. fetida* was collected from an earthworm farm in Eumsung, Korea, and cattle manure was collected from a commercial farm in Wonju, Korea. The cattle manure, which was blended with sawdust (1:1, w/w ratio), was composted for 5 weeks. The earthworms were stored in composted cattle manure at $20^{\circ}C$ with 70% relative humidity.

Isolation of Microorganisms
The surface of all 10 earthworms was disinfected with 70% ethanol and sterilized with the flame of an alcohol lamp. The intestinal tracts of the earthworms were isolated in an anaerobic chamber (Forma Scientific Inc., Marietta, OH, USA) by surgical operation and suspended in 9 ml of a sterile 0.85% NaCl solution supplemented with 0.01% Tween 80. After 10-fold serial dilutions of 1 ml of the suspension, the diluents were spread onto brain heart infusion (Difco, Becton Dickinson and Company, Sparks, MA, USA) agar plates, prepared according to the method of Shin *et al*. [36], and cultured at $30^{\circ}C$ for 48 h under aerobic or anaerobic conditions. Aliquots (1 ml) of the suspension were prepared to extract directly the bacterial DNA.

Sampling and DNA Extraction
The feed samples were taken from the composted cattle manure, which had not been incubated by earthworms. The casts of the earthworms were carefully collected on top of the composted cattle manure. Feed and cast samples (ca. 1 g) were suspended in 9 ml of a sterile 0.85% NaCl solution supplemented with 0.01% Tween 80. Aliquots (1 ml) of the suspension of feed, intestinal tract, and cast samples were centrifuged at 4,000 $\times g$ for 10 min, and the resulting pellets were washed with sterile phosphate-buffered saline (pH 7.0). DNA was extracted by using a DNeasy tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturer's directions. The content and purity of the extracted DNA were confirmed by using a biophotometer (Eppendorf, Hamburg, Germany).

Polymerase Chain Reaction
The 16S rDNA fragments were amplified using the following universal PCR primers 341F forward (5'-CCTACGGGAGGCAGCAG-3') and 518R reverse (5'-ATTACCGCGGCTGCTGG-3') [16, 28]. For DGGE analysis of the PCR products, a 40-nucleotide GC-rich sequence

(GC-clamp, 5'-CGCCCGGGGCGCGCCCCGGGCGGGGCGGGG GCACGGGGGG-3') was attached to the 5' end of the primer [41]. The PCR reactions contained 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 4 mM $MgCl_2$; 0.5 units of *Taq* polymerase; 0.4 mM each of dATP, dCTP, dGTP, and dTTP; 20 pmol of each primer; 1 µl of template DNA (20 µg/ml); and sterile water to a final volume of 20 µl. PCR amplification of the 16S rDNA sequences was performed with a Mastercycler gradient (Eppendorf, Hamburg, Germany) with an initial denaturation at $95^{\circ}C$ for 5 min, followed by 35 cycles of denaturation at $94^{\circ}C$ for 45 s, annealing at $52^{\circ}C$ for 45 s, and extension at $72^{\circ}C$ for 1 min, before the final extension at $72^{\circ}C$ for 5 min. The PCR products were confirmed on 2% agarose gels.

DGGE Analysis
The DGGE analysis was performed with a Bio-Rad DCode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA). The denaturing gel electrophoresis conditions were between 40% and 70% denaturing gradient [100% denaturing solution corresponds to 7.0 M urea (Sigma Chemical Co., St. Louis, MO, USA) and 40% (v/v) formamide (Sigma Chemical Co.) and 8% (w/v) polyacrylamide gels [Acryl/Bis 29:1, 40% (w/v) solution; Amresco, Solon, OH, USA]. Electrophoresis was performed in a 0.5× TAE buffer [20 mM Tris, 10 mM Na-acetate, 0.5 mM Na_2EDTA (pH 8.0)] initiated at 20 V for 30 min, and then continuously run at 60 V for 12 h at $60^{\circ}C$. The gels were stained for 20 min in TAE buffer containing 1:10,000 concentrated GreenStar Nucleic Acid Staining (Bioneer Co., Seoul, Korea) and photographed with a UV transilluminator (Korea Bio-Tech Co., Seoul, Korea). The DGGE banding intensities on the gel were analyzed using the Gel-Pro Analyzer software (Media Cybernetics Inc., Bethesda, MD, USA).

Sequence Analysis
The 16S rDNA sequences from the isolated strains and DGGE gels were sequenced by use of a PCR-based technique. To amplify partial 16S rDNA fragments from the isolates, universal primers (27F: 5'-AGAGTTTGATCATGGCTCAG-3'; 1492R: 5'-GGATACCTTGTTA CGACTT-3') were used. The DGGE bands were excised from the gel with a surgical blade (Feather Safety Razor Co. Ltd., Osaka, Japan), and the pieces of acrylamide containing the bands were placed in sterilized Eppendorf tubes. Next, 50 µl of sterilized water was added and the tube contents subjected to passive diffusion overnight at $4^{\circ}C$. The elutent was used as template DNA in PCR using primers 341F (without the GC-clamp) and 518R. The re-amplified PCR products were ligated into a T vector (Invitrogen, Carlsbad, CA, USA), and DNA sequencing was performed with an ABI 377 Genetic Analyzer (Applied Biosystems, Foster, CA, USA). The 16S rDNA sequences from the isolated strains and the 16S rDNA fragments from the DGGE bands were aligned with the reference sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov) using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS

Microbial Diversity by the Culture-Dependent Approach
The plate count obtained for the intestinal tracts of the earthworms was 1×10^5 colony forming units (CFU)/g on the brain heart infusion agar plates (data not shown). In total,

Table 1. Identification of the aerobic microorganisms isolated from the intestinal tracts of earthworms.

No.	Closest relative	GenBank accession no.	Identity (%)	Proportion (colonies)
1	*Aeromonas hydrophila*	AY987754	98	14/72
2	*Aeromonas veronii*	AF418209	99	8/72
3	*Aeromonas punctata*	AY987758	98	7/72
4	*Bacillus pumilus*	DQ683078	97	8/72
5	*Bacillus cereus*	DQ289058	98	15/72
6	*Bacillus subtilis*	AY775778	98	4/72
7	*Pseudomonas mendocina*	DQ641475	97	5/72
8	*Photobacterium ganghwense*	AY960847	97	7/72
9	*Shewanella putrefaciens*	AF136269	98	4/72

72 and 55 pure cultures were isolated from the intestinal tracts of the earthworms under aerobic and anaerobic conditions, respectively. The species of the isolates was determined by 16S rDNA sequence analysis and comparison with sequences in the GenBank databases. The aerobic isolates belonged to five genera; *Aeromonas*, *Bacillus*, *Photobacterium*, *Pseudomonas*, and *Shewanella* (Table 1). The microbial community in the intestinal tracts was composed of 40% *Aeromonas*, 37% *Bacillus*, 10% *Photobacterium*, 7% *Pseudomonas*, and 6% *Shewanella* (Fig. 1A). The anaerobic isolates belonged to six genera; *Aeromonas*, *Bacillus*, *Shewanella*, *Paenibacillus*, *Cellulosimicrobium*, and *Clostridium* (Table 2). The anaerobic microbial community in the intestinal tracts of the earthworms was composed of 52% *Aeromonas*, 27% *Bacillus*, 12% *Shewanella*, 5% *Paenibacillus*, 2% *Cellulosimicrobium*, and 2% *Clostridium* (Fig. 1B). The species of the aerobic isolates were identified as *Aeromonas hydrophila*, *Aeromonas veronii*, *Aeromonas punctata*, *Bacillus pumilus*, *Bacillus cereus*, *Bacillus subtilis*, *Photobacterium ganghwense*, *Pseudomonas mendocina*, and *Shewanella putrefaciens*; these isolates showed more than 97% 16S rDNA sequence similarity with the reference sequences (Table 1). The anaerobic species with more than 97% similarity of the 16S rDNA sequences were *A. hydrophila*, *A. veronii*, *A. punctata*, *B. cereus*, *Cellulosimicrobium cellulans*, *Clostridium subterminale*, *Paenibacillus motobuensis*, and *S. putrefaciens* (Table 2). The population densities determined by the culture-dependent approach showed that *Aeromonas* and *Bacillus* species were the dominant

facultative microorganisms in the intestinal tracts of earthworms under both aerobic and anaerobic conditions.

DGGE Analysis of Bacterial Community Structure
In parallel with the culture-dependent approach, the results from DGGE analysis of PCR-amplified 16S rDNA fragments directly extracted from feeds, intestinal tracts, and casts of earthworms were used to investigate the correlation of the bacterial community structures. The banding patterns on the DGGE gels (Fig. 2) revealed differences in the bacterial community structures. The clone library of DGGE bands was determined by 16S rDNA sequence analysis. In total, 13, 16, and 10 bacterial 16S rDNA clones were sequenced from feeds, intestinal tracts, and casts of earthworms, respectively. In the feeds sample, there were two sharp bands (102, 104), representing the dominant microorganisms (indicated by the intensity of the band), whereas the other bands (101, 103, and 105–112) were at low intensities. In the intestinal tracts sample, four sharp bands (201, 202, 204, and 209) were present, whereas the other bands (203, 205–208, and 210–216) were at low intensities. In the casts sample, three sharp bands (303, 308, and 310), representing the dominant microorganisms, were present, whereas the other bands (301, 302, 304–307, and 309) were at low intensities. The DGGE gel was represented by bands 102 and 201; bands 103, 202, and 301; bands 106 and 205; bands 107 and 207; bands 208 and 306; bands 109 and 210; bands 110 and 211; bands 111 and 309; bands 112 and 213; and bands 215 and 310; all of which were

Table 2. Identification of the anaerobic microorganisms isolated from the intestinal tracts of earthworms.

| No.	Closest relative	GenBank accession no.	Identity (%)	Proportion (colonies)
1	*Aeromonas hydrophila*	AY987754	97	12/55
2	*Aeromonas veronii*	AF418209	98	14/55
3	*Aeromonas punctata*	AY987758	98	3/55
4	*Bacillus cereus*	DQ289058	98	15/55
5	*Cellulosimicrobium cellulans*	AB210965	97	1/55
6	*Clostridium subterminale*	AB294137	98	1/55
7	*Paenibacillus motobuensis*	AY741810	97	3/55
8	*Shewanella putrefaciens*	AF136269	98	6/55
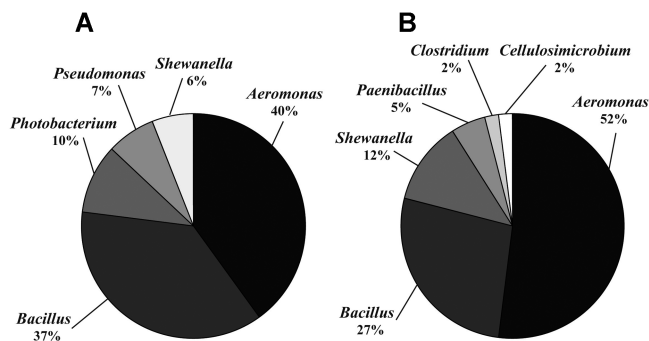
Fig. 1. Proportion of the isolated microorganisms from the intestinal tracts of earthworm.

Dilutions of the intestinal tracts of the earthworms were cultured on brain heart infusion agar plate at 30°C for 48 h under aerobic (**A**) and anaerobic (**B**) conditions. The 16S rDNA sequences from all the isolated strains were sequenced by use of a PCR-based technique. Sequences were aligned with the GenBank reference sequences for 16S rDNA by using the Basic Local Alignment Search Tool (BLAST). Results from the DNA sequence analysis of the isolates are summarized in Table 1 and Table 2.

common to the microorganisms in the feeds, intestinal tracts, and casts samples. Thus, the high-intensity bands seem to represent the dominant microorganisms.

Identification of Sequenced 16S rDNA Fragments from DGGE

The 16S rDNA sequence and their similarity with reference sequences allowed the identification of the species represented by the DGGE bands (Table 3). The clone library contained 13 different 16S rDNA sequences from the feeds of earthworms, 16 sequences from the intestinal tracts, and 10 sequences from the casts. In the feeds, intestinal tracts, and casts samples, bands 102 and 201 had a 97% similarity to *Aeromonas* sp., bands 103, 202, and 301 had 97−98% similarity to an uncultured Chloroflexi bacterium, bands 106 and 205 had 97% similarity to uncultured soil bacterium, bands 107 and 207 had 97% similarity to *Clostridium* sp., bands 208 and 306 had 97% similarity to uncultured γ-proteobacterium, bands 109 and 210 had 97%−98% similarity to *Pseudomonas* sp., bands 110 and 211 had 98% similarity to *Shewanella* sp., bands 111 and 309 had 98% similarity to *Aeromonas* sp. IGCAR 19/07, bands 112 and 213 had 95% similarity to *Streptomyces* sp. CS44, and bands 215 and 310 had 97−98% similarity to *B. pumilus*.

DISCUSSION

Earthworms are well known for modifying the properties of soil and feedings on large quantities of soil and organic residues. In particular, *Eisenia fetida* was breeding with feed including livestock manure, sludge, food garbage, *etc.*,
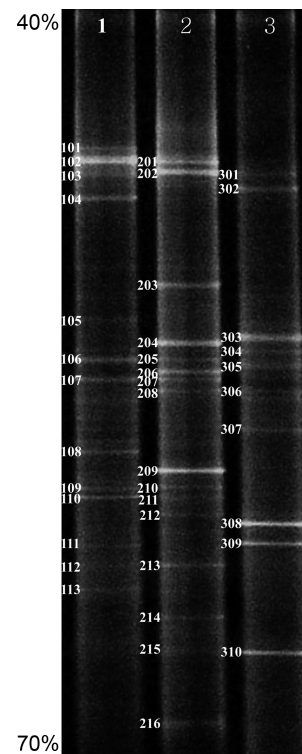

Fig. 2. Denaturing gradient gel electrophoresis analysis of PCR-amplified 16S rDNA fragments from earthworm feeds, earthworm intestines, and earthworm casts.

Lane 1, earthworm feeds; lane 2, earthworm intestinal tracts; and lane 3, earthworm casts. PCR amplification of the 16S rDNA sequences was performed with the primers 341F-GC and 518R for 35 cycles. DGGE analysis was performed between a 40% and 70% denaturing gradient.

in earthworm farms. They also contain a wide variety of microorganisms, which are associated with the decomposition of organic residues and contribute to the beneficial effects of earthworms on soil properties [38]. The main aim of this study was to investigate the bacterial community in the intestinal tracts of earthworms and to examine the correlation between the results of culture-dependent and culture-independent (PCR−DGGE analysis) approaches.

Earlier studies into the interactions between earthworms and bacterial communities have been carried out using traditional culture-based methods [8]. Culture-dependent approaches need to isolate and culture the microorganisms in order to identify their morphological, biochemical, and genetic characteristics. However, this technique has the disadvantages of low selectivity and being time-consuming because of long culture periods; this technique may also not accurately reflect the bacterial community because of changes in the environmental conditions [22]. The use of DGGE analysis in microbial ecology was first introduced by Muyzer *et al.* [25]. This technique is based on differences in the melting behavior of double strands of PCR-amplified 16S rDNA fragments in denaturing gradient gels containing

Table 3. Diversity of the microorganisms identified from the feeds, intestinal tracts, and casts of earthworms by denaturing gradient gel electrophoresis analysis.

Lane	Band number	16S rDNA sequence results	GenBank accession no.	BLAST homology (%)	Band intensity (%)
1	101	Uncultured bacterium	EF153309	98	3.5
	102	*Aeromonas* sp.	AY987767	97	7.7
	103	Uncultured Chloroflexi bacterium	AY542256	97	3.8
	104	Uncultured *Pseudomonas* sp.	AY191342	98	5.9
	105	*Enterobacter* sp.	CP000653	98	2.2
	106	Uncultured soil bacterium	DQ642739	97	3.2
	107	*Clostridium* sp.	AY695835	97	2.4
	108	Uncultured alpha-proteobacterium	AB178095	98	2.4
	109	*Pseudomonas* sp.	AM409194	98	1.8
	110	*Shewanella* sp.	CP000503	98	1.9
	111	*Aeromonas* sp. IGCAR 19/07	EF23546	98	1.9
	112	*Streptomyces* sp. CS44	EF494235	97	2.2
	113	*Aeromonas* sp.	EF428989	97	3.2
2	201	*Aeromonas* sp.	AY987767	97	5.1
	202	Uncultured Chloroflexi bacterium	AY542256	98	4.3
	203	*Clostridium subterminale*	AB294137	97	2.8
	204	*Aeromonas* sp.	AF302145	98	4.6
	205	Uncultured soil bacterium	DQ642739	97	1.8
	206	*Photobacterium* sp.	EF101501	98	2.6
	207	*Clostridium* sp.	AY695835	97	1.7
	208	Uncultured γ-proteobacterium	DQ446068	97	1.8
	209	*Aeromonas punctata*	AY987758	98	5.0
	210	*Pseudomonas* sp.	AM409194	97	1.4
	211	*Shewanella* sp.	CP000503	98	2.0
	212	Uncultured soil bacterium	DQ642735	97	1.7
	213	*Streptomyces* sp. CS44	EF494235	98	3.5
	214	*Bacillus cereus*	DQ289058	97	1.5
	215	*Bacillus pumilus*	DQ683078	97	1.3
	216	*Bacillus subtilis*	AY775778	98	1.6
3	301	Uncultured Chloroflexi bacterium	AY542256	98	2.9
	302	Uncultured bacterium	EF153309	98	2.8
	303	*Aeromonas* sp.	AY987771	97	5.8
	304	*Aeromonas* sp.	AM262154	98	2.4
	305	Uncultured soil bacterium	DQ642739	97	3.5
	306	Uncultured γ-proteobacterium	DQ446068	97	1.7
	307	*Aeromonas salmonicida*	AF108136	97	3.2
	308	*Edwardsiella tarda*	EF371905	97	5.7
	309	*Aeromonas* sp. IGCAR 19/07	EF23546	98	3.7
	310	*Bacillus pumilus*	DQ683078	98	5.9

chemical denaturants (formamide and urea). In addition, GC-clamps (35–40 bp) are used to prevent the PCR products from being completely denatured during electrophoresis.

DGGE can be used to differentiate between sequences of 16S rDNA from different bacteria because the sequences denature at different concentrations of denaturants, resulting in a specific banding pattern. The combination of PCR and DGGE proved to be a useful culture-independent approach for studying the complex bacterial communities [26].

In this study, we investigated the bacterial community in the intestinal tracts of earthworms by both culture-dependent and -independent approaches. The microorganisms isolated

from intestinal tracts of earthworms by traditional culture-based approaches included *Aeromonas*, *Bacillus*, *Photobacterium*, *Pseudomonas*, and *Shewanella*. The most notable finding is that there was no considerable difference in the bacterial community under aerobic and anaerobic conditions (Table 1 and Table 2). It is evident that *Aeromonas* and *Bacillus* species are the dominant microorganisms in the intestinal tracts of earthworms. *Aeromonas* is a Gram-negative, facultative anaerobic rod that morphologically resembles members of the family Enterobacteriaceae. *Bacillus* is a Gram-positive genus of aerobic rod-shaped bacteria that is able to secrete large quantities of enzymes. *Photobacterium* genus is a Gram-negative member of the family Vibrionaceae and has the ability to emit light (bioluminescent). *Pseudomonas* is a genus of gamma-Proteobacteria, Gram-negative, rod-shaped, and polar-flagella bacteria. *Shewanella* is the sole genus of the Shewanellaceae family of marine bacteria. Culture-dependent studies of microorganisms isolated from earthworms have reported that the numbers of bacteria are greater in the intestinal tracts of earthworms than in the soil [7]. Similarly, Toyota and Kimura [40] reported that strains belonging to *A. hydrophila* may be indigenous to the earthworm *E. fetida*. In addition, *A. hydrophila* strains have been isolated from earthworm *Pheretima* species [39]; this might be a species typical of intestinal tracts of some animals. Kim *et al.* [17] reported that a wide variety of microorganisms were isolated from the intestinal tracts of the earthworm *E. fetida* by traditional culture-based methods. These belonged to *Aeromonas*, *Agromyces*, *Bosea*, *Gordonia*, *Klebsiella*, *Microbacterium*, *Nocardia*, *Pseudomonas*, *Rhodococcus*, *Tsukamurella*, *Streptomyces*, and *Bacillus*, which was the dominant microorganism. Shin *et al.* [36] also reported that the microbial populations in earthworm guts include *Clostridium*, *Staphylococcus*, and *Propionibacterium* under anaerobic condition. Furlong *et al.* [10] reported that the cast isolates from the earthworm *Lumbricus rubellus* were dominated by *Aeromonas* subspecies.

In this study, it was possible to provide details about the bacterial community, including the abundance of species in the feeds, intestinal tracts, and casts of earthworms, by using PCR–DGGE. Fasoli *et al.* [9] and Ji *et al.* [14] reported that the use of DGGE with an identification ladder allowed the rapid detection of probiotic bacteria and bacterial pathogens. The 16S rDNA was directly extracted from the samples and was amplified with universal primers (341 forward and 518 reverse), which are usually used for soil microorganisms [16, 28]. A DGGE gel with a 40–70% denaturing gradient was used to obtain greater band separation and quality of the band pattern. The profiles obtained by DGGE analysis revealed the correlation between the bacterial community structures in the feeds, intestinal tracts, and casts of the earthworms. The only significant difference from the DGGE profiles showed that

the intestinal microorganisms had wider bacterial diversity than those in feeds and casts. In the intestinal tracts sample, an intense band was observed, representing the dominant microorganism *Aeromonas* species, and the presence of unculturable microorganisms belonging to an uncultured Chloroflexi bacterium, uncultured soil bacterium, uncultured gamma-proteobacterium, and uncultured bacterium were detected by PCR–DGGE analysis. *Aeromonas*, uncultured Chloroflexi bacterium, and uncultured soil bacterium were identified in the feeds, intestinal tracts, and casts of earthworms, whereas *A. punctata*, *B. cereus*, *B. subtilis*, *Clostridium subterminale*, and *Photobacterium* were only identified in the intestinal tracts. *A. hydrophila* has been described as a common pathogen of fish and amphibians; however, it is frequently isolated from earthworms [23, 39]. The intestinal microorganisms of earthworms are partially derived from feeds; however, almost all of them seemed to exist in the intestinal tracts. In particular, *Aeromonas* sp. represented the strongest band on the DGGE gel and had 12.8%, 14.7%, and 15.1% of the total band intensities in the feeds, intestinal tracts, and casts, respectively. Therefore, the dominant microorganism was closely related to an *Aeromonas* species, and it appeared to be a major contributor in the feeds, intestinal tracts, and casts of earthworms. We observed that the 16S rDNA fragments from the same species had a definite migration position in the DGGE gels, thus validating this as a molecular technique for rapid species identification. The bacterial communities described in this study are similar to those previously described [17, 36, 40]. *Aeromonas* and *Bacillus* species constitute the dominant microorganisms in the intestinal tract of earthworms and are mainly responsible for the organic biodegradable activity that occurs during composting.

In summary, we found that both culture-independent and -dependent approaches detected the same predominant microorganism sequences. In addition, DGGE analysis was able to detect unculturable species and more species than were isolated by the conventional culture-based approach. The PCR–DGGE technique is a reliable and rapid culture-independent approach for the study of the bacterial community. However, traditional culture-based methods and DGGE analysis should not be considered separately, because viable microorganisms can be obtained by the culture-dependent approach.

Acknowledgment

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