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Bacterial diversity in a finished compost and vermicompost: differences revealed by cultivation-independent analyses of PCR-amplified 16S rRNA genes

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Abstract Bacterial communities are important catalysts in the production of composts. Here, it was analysed whether the diversity of bacteria in finished composts is stable and specific for the production process. Single-strand conformation polymorphism (SSCP) based on polymerase chain reaction amplified partial 16S rRNA genes was used to profile and analyse bacterial communities found in total DNA extracted from finished composts. Different batches of compost samples stored over a period of 12 years and a 1-year-old vermicompost were compared to each other. According to digital image analysis, clear differences could be detected between the profiles from compost and vermicompost. Differences between three different periods of compost storage and between replicate vermicompost windrows were only minor. A total of 41 different 16S rRNA genes were identified from the SSCP profiles by DNA sequencing, with the vast majority related to yet-uncultivated bacteria. Sequences retrieved from compost mainly belonged to the phyla *Actinobacteria* and *Firmicutes*. In contrast, vermicompost was dominated by bacteria related to uncultured *Chloroflexi*, *Acidobacteria*, *Bacteroidetes* and *Gemmatimonadetes*. The differences were underscored with specific gene probes and Southern blot hybridizations. The results confirmed that different substrates and composting processes selected for specific bacterial communities in the finished products. The specificity and consistency of the bacterial communities inhabiting the compost materials suggest that cultivation-independent bacterial community analysis is a potentially useful indicator to characterize the quality of finished composts in regard to production processes and effects of storage conditions.

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Introduction

Compost is the end-product of a biological decomposition and stabilization of organic substrates under conditions that allow high temperatures as a result of biologically produced heat. The classical self-heating composting process can be substituted by inoculating earthworms, e.g., *Eisenia foetida* (Annelidae), to produce vermicomposts under mesophilic conditions (Edwards 1995). Typically, composts and vermicomposts can be used to improve horticultural or agricultural soil. The beneficial effect of these products can be twofold, an improvement of soil nutrients and structure to stimulate plant growth (Barker 1997; Albiach et al. 2001) and a suppressive effect on soil indigenous plant pathogens (Bailey and Lazarovits 2003). It has been assumed in several studies that in addition to the immediate substrate effects of the compost and the physical improvement of the soil structure, the introduction of compost-born microorganisms also contributes to the beneficial effects, although the importance of this aspect is still under debate (Kowalchuk et al. 2003; Noble and Coventry 2005).

For the composting processes, the importance of microbial communities is well established (Ryckeboer et al. 2003). Novel cultivation-independent techniques based on the direct analyses of phospholipids fatty acids (Cahyani et al. 2002; Bolta et al. 2003; Steger et al. 2003), quinolines (Tang et al. 2004), or the analyses of rRNA genes encoding for the small subunit ribosomal RNA (for bacteria, 16S rRNA) (Dees and Ghiorse 2001; Peters et al. 2000; Tebbe 2002) have recently dramatically increased knowledge about the contribution of different microorganisms to various compost production phases. In fact, most of those recent studies indicate that many not-yet-cultured microorganisms contribute to the composting processes. On the other hand, the importance of *Bacillus* and relatives for the

hot composting stage, or *Actinobacteria* during compost maturation, which was already indicated by cultivation, was confirmed (Peters et al. 2000; Ryckeboer et al. 2003). In contrast to numerous studies that have analysed the microbiology of the composting processes, the microbiological characterization of finished compost or vermicompost is still in its infancy, and a systematic microbiological analysis of products from composting facilities is still lacking (Hassen et al. 2001; Tang et al. 2003). It can be suspected that various factors will influence the microbial colonization of finished products, i.e., (1) the origin and composition of the initial substrates, (2) the previous process conditions and (3) the substrate quality of the finished product.

The objective of this study was to detect, characterize and compare the most abundant bacteria in finished compost and vermicompost and collect information about the stability and reproducibility of the bacterial community structure in these materials. In order to evaluate the results for a broader practical application, samples of this study were taken from a commercial compost production facility and not from experimental processes under controlled laboratory conditions. The resident bacterial community structures were visualized with single-strand conformation polymorphism (SSCP), an electrophoretic technique that produced profiles according to the diversity of polymerase chain reaction (PCR)-amplified 16S rRNA genes from directly extracted compost DNA (Schwieger and Tebbe 1998; Peters et al. 2000). The effect of storage was analysed with the compost samples generated with similar material under the same process conditions and stored over a period of up to 12 years at room temperature. In addition, the consistency of the resident bacterial communities that occur in comparable production batches was analysed with 1-year-old vermicompost generated in three independent windrows. The major contributors of the SSCP profiles were characterized by sequencing of the most dominant 16S rRNA genes followed by database comparisons (Peters et al. 2000; Dohrmann and Tebbe 2004). The consistency and specificity of the different SSCP profiles was further analysed with gene probes and Southern blot DNA–DNA hybridizations.

Materials and methods

Field site and sampling

Compost and vermicompost samples were collected in 2002 at a composting facility of the Marcopolo Environmental Group (Borgo San Dalmazzo, Cuneo, Piedmont, Italy). The finished compost was produced from well-sorted organic wastes and yard trimmings in the course of 12 years and subsequently stored. Vermicompost was generated from separated solids from pig, cow and chicken manure, mixed with straw and agricultural crop residues, and processed by earthworms (*Eisenia foetida*).

As soon as the compost processes were finished, the material was filled into sacks (65×90 cm) and placed in a storage room. The sacks from each year of production and storage were grouped together and placed on wooden shelves. The product was stored at room temperature. Representative samples were taken from a total of nine different sacks with three sacks stored for 1, 5 and 12 years, respectively, corresponding to a short, intermediate and long period of storage.

The vermicompost was located in an open field in three windrows (40–70 m length, 6–8 m width, 2 m height) produced from the same feedstock. The piles were protected by a thick nylon layer on the outside. Subsamples were collected at 30-cm depth from the top and from both sides of the windrows three times along their perimeter (at the beginning, in the middle and at the end of the windrow). The top and the lateral subsamples were combined in order to have a total of three samples for each windrow. The samples were sealed in plastic bags and directly transported to the laboratory, where they were stored at 4°C for up to 40 days before analysis. Total humidity was determined from 100 g of fresh weight and dried in an oven at 105°C until constant weight.

Extraction of DNA from compost and vermicompost and generation of SSCP profiles

Each collected sample was ground in liquid nitrogen and stored at −70°C until DNA extraction. Total DNA was extracted from subsamples of 100 mg (wet weight) of compost and 200 mg (wet weight) of vermicompost using the BIO101 FastDNA Spin Kit for soil (Qbiogene, Carlsbad, CA). To extract DNA, the frozen samples were treated for 30 s in an MSK cell homogenizer (Braun, Melsungen, Germany) at 4,000 rpm for bacterial cell disruption. The DNA concentration was measured fluorometrically using Pico Green dye (Molecular Probes, Leiden, the Netherlands), and a microtiter plate reader (Fluoroskan II, Labsystems, Helsinki, Finland). PCR products were further purified with the Wizard DNA Clean-Up System (Promega, Mannheim, Germany).

Primers Com1 (forward), and Com2-ph (reverse), both hybridizing to phylogenetically conserved regions within the 16S rRNA genes, were used to amplify an approximately 400-bp fragment encompassing the two phylogenetically highly variable regions, V4 and V5 (Schwieger and Tebbe 1998; Schmalenberger et al. 2001), from the compost and vermicompost DNA, respectively. For a subsequent single-strand digestion, primer Com2-ph was phosphorylated (Schwieger and Tebbe 1998). Reaction mixtures contained 1× PCR buffer with 1.5 mM $MgCl_2$, deoxynucleoside triphosphates (200 μM of each dATP, dCPT, dGTP and dTTP), 0.5 μM of each primer, 2.5 U/100 μl of DNA polymerase (HotStarTaq, Quiagen) and 1 μl of the diluted DNA extract. Cycle conditions for the reactions were initial denaturation at 95°C (15 min),

30 cycles of 94°C (60 s), 50°C (60 s), and 72°C for 70 s with a final extension for 5 min at 72°C. PCR products were purified (Qiaquick PCR Purification Kit, Qiagen) and quantified fluorometrically as described above.

SSCP profiles were generated and visualized as described elsewhere (Schwieger and Tebbe 1998; Tebbe et al. 2001; Dohrmann and Tebbe 2004). The SSCP gels were bordered by marker DNA of partial 16S rRNA genes PCR-amplified with the same primers as described above from *Bacillus licheniformis*, *Rhizobium trifolii*, *Flavobacterium johnsoniae* and *Rhizobium radiobacter* (double band).

Extraction, re-amplification and sequencing of DNA from silver-stained SSCP profiles

Selected bands of the SSCP community profiles were cut out with a sterile razor blade, and the single-stranded DNA of these bands was eluted for 3 h at 37°C and 500 rpm in 50 µl "crush and soak" buffer (0.5 M ammonium acetate, 10 mM Mg^{2+}-acetate, 1 mM EDTA [pH 8.0] and 0.1% sodium dodecyl sulfate) (Sambrook et al. 1989). The eluted DNA was precipitated with ethanol, centrifuged and dried as previously described (Schwieger and Tebbe 1998; Dohrmann and Tebbe, 2004). The precipitated DNA was finally resuspended in 12µl of 10 mM Tris–HCl, pH 8.0. The sequences were recovered by PCR with the Com-primers in a 50-µl volume, and products were purified as previously described. Half of the products were digested to obtain single-stranded DNA to confirm the correct position of re-amplified single bands on the SSCP gels, using the conditions as described above. The re-amplified DNA molecules recovered from bands of community profiles were used for cloning and sequencing (Peters et al. 2000; Schmalenberger et al. 2001; Dohrmann and Tebbe 2004).

DNA sequence analysis

DNA was sequenced in both directions, and consensus sequences were generated as described elsewhere (Schmalenberger et al. 2001). For further analysis, the consensus sequences were loaded into the ARB database (http://www.arb-home.de) (Ludwig et al. 2004). After alignment of the partial 16S rRNA genes, sequences were integrated with the parsimony interactive function into an existing phylogenetic tree consisting of sequences with more than 1,000 nucleotides. The sequences were analysed for chimera using the appropriate tool provided by the RDP database (Version 8, http://35.8.164.52/html/analyses.html). The DNA sequences were analysed for their closest relatives using the Fasta Nucleotide Database Query provided by the European Bioinformatics Institute (http://www.ebi.ac.uk/fasta33/nucleotide.html). All new rRNA gene sequences retrieved in this study have been deposited at the same institute and can be found under the accession numbers AJ973329 to AJ973372.

Southern blotting of SSCP gels and gene probe hybridizations

The Southern blotting procedure of selected SSCP gels was carried out as described elsewhere (Schmalenberger and Tebbe 2003). Specific probes of 18 to 20 base length matching inside the 16S rRNA gene position 538–906 (corresponding to the numbering of *Escherichia coli*) were designed with the ARB software environment (http://www.arb-home.de). The probes, synthesized by MWG-Biotech AG, were labelled with the alkaline phosphatase direct labelling kit (Amersham Pharmacia Biotech) according to the protocol provided by the manufacturer.

Membranes (Hybond N+, Amersham Pharmacia Biotech) were hybridized with 20 ml of hybridization solution and 5 ng/ml of probe into glass tubes in a mini hybridization oven (Oncor Appligene, Illkrich Graffenstaden, France) according to the manufacturer's protocol (Amersham Pharmacia Biotech). Hybridization temperatures were optimized empirically in the range from 40 to 45°C (except probe Com2 that hybridized at 35°C), depending on the gene probe and annealing conditions. The incubation was overnight. Washing procedures were conducted with a primary wash buffer containing urea (2 M) and a secondary wash buffer containing Tris-base (Amersham Pharmacia Biotech). The primary wash buffer (50 ml per tube) was applied for 10 min at hybridization temperature. The washing procedure was repeated once. Subsequently, the doubly concentrated secondary wash buffer was used twice for 5 min and at 10°C below hybridization temperature to reduce the content of probes that hybridized non-specifically with the nylon membranes. Finally, secondary wash buffer was applied twice for 5 min at 15°C below the respective hybridization temperature. For probe Com2, washing conditions were set as follows: primary wash buffer (50 ml per tube) applied twice for 10 min at hybridization temperature and secondary wash buffer applied twice for 5 min at 10°C below hybridization temperature. Membranes were then incubated with 40 µl/cm^2 of the CDP-Star detection reagent for 5 min (Amersham Pharmacia Biotech) and placed into plastic bags. Chemoluminescence was detected with Hyperfilm ECL (Amersham Pharmacia Biotech) incubated for 1 h to overnight, in a film cassette, depending on the expected strength of the hybridization signal.

Statistical analysis

Where appropriate, the results were analysed with analysis of variance between groups (ANOVA) using the software

Mathematica 5 for AMD64 (Wolfram Research Europe Ltd., Long Hanborough, Oxfordshire, UK).

Results

Humidity and DNA contents of the finished compost and vermicompost

The finished compost and vermicompost analysed in this work showed different humidity values (Table 1). The compost products were powdery and dry. The total humidity ranged from 2.9% of the samples of the short period of storage to 6.0% of those of the intermediate. The differences in humidities between the samples from the different storage periods were not significant ($p<0.05$). In comparison to the compost material, the vermicompost texture was more similar to a common agricultural soil, and the humidity values were higher, ranging from 39.6% of windrow C to 53.3% of windrow A. There were no significant differences in the humidities of the vermicompost from the replicate windrows ($p<0.05$).

The DNA concentrations extracted from compost samples of the long, intermediate and short period of storage were 38.5, 29.8 and 24.8 μg/g dry weight, respectively. When tested with ANOVA, the differences were confirmed to be significant ($p<0.05$). The average DNA concentrations retrieved from the vermicompost samples were slightly lower than those from the compost. For the replicate windrows, the average concentrations were not significantly different ($p<0.05$) (Table 1). In order to obtain comparable SSCP profiles from both compost and vermicompost, the amounts of DNA that were used to generate the SSCP profiles were adjusted to each other, representing a total of 160 ng of single-strand DNA for each profile.

Table 1 Humidity and DNA yields extracted from the finished compost and vermicompost of this study

Samples	Mean total humidity (%)	Extraction yields (μg DNA/g dry weight)
Compost		
Long storage (12 years)	3.33±3.46	38.5±10.0
Intermediate (5 years)	6.00±0.67	29.8±6.1
Short storage (1 year)	2.89±2.14	24.8±6.2
Vermicompost		
Windrow A	53.3±2.4	20.3±6.5
Windrow B	47.0±3.7	19.0±0.5
Windrow C	39.6±4.3	21.3±3.6

SSCP profiles of the bacterial communities from compost and vermicompost

SSCP profiles were generated from a total of 18 different samples in this study, i.e. three replicate compost samples from each of the three storage periods and three replicates from each of the three windrows of vermicompost (Fig. 1a). With one exception, the replicates obtained from the same period of storage (compost) or windrow (vermicompost) were more similar to each other than to the experimental variables, i.e. the different storage periods or independent windrows, respectively. Only for the vermicompost, the similarities between the replicate windrows (lanes D and E in Fig. 1b, corresponding to windrows A and B) were so high that they were indistinguishable from those within each windrow. However, the profiles of the third windrow, indicated as F (windrow C), were slightly different from those of the other two windrows.

Digital image analysis revealed that the profiles from vermicompost and compost were clearly different from each other, indicating the existence of different bacterial communities (Fig. 1b). The profiles generated from compost samples consisted of about 20 distinct bands. Surprisingly, the profiles of the long and short storage periods were more similar to each other than to the profiles from the intermediate stage of storage (Fig. 1b). All profiles from compost DNA shared a dominant band in the position indicated as CA2 in Fig. 1a. The intensity of this band seemed to increase from the short (lanes indicated by C) to the intermediate storage period (lanes indicated by B) and to decrease slightly further on to the long period of storage (lanes indicated by A). The major differences between the profiles from the intermediate period of storage to the others were caused by three bands in the upper regions of the profiles (between marker 1 and 2, from top to bottom) and by three other bands in the lower part (in vicinity of marker 3; two of them indicated as CK1 and CF4).

The vermicompost profiles consisted of about 25 distinguishable bands. In contrast to the compost samples, which showed a number of single dominant bands, the intensities of the bands amplified from vermicompost DNA were more similar to each other (Fig. 1a). The upper part of the profiles revealed a series of bands that were variable in their intensities. Two of these bands, VA1 and VA2, more evident than the others, were common in profiles from all the three windrows, but more intense in two replicate samples from Windrows A and B (lanes D and E), respectively. Despite the fact that the SSCP profiles from compost and vermicompost samples could be distinguished from each other, it was difficult, due to the diversity of bands, to attribute specific bands to one of the processes. However, one band (VC1), which was in a position slightly above marker 2, was found at similar intensities in all vermicompost samples, and this band seemed to be absent in the profiles generated from compost DNA.

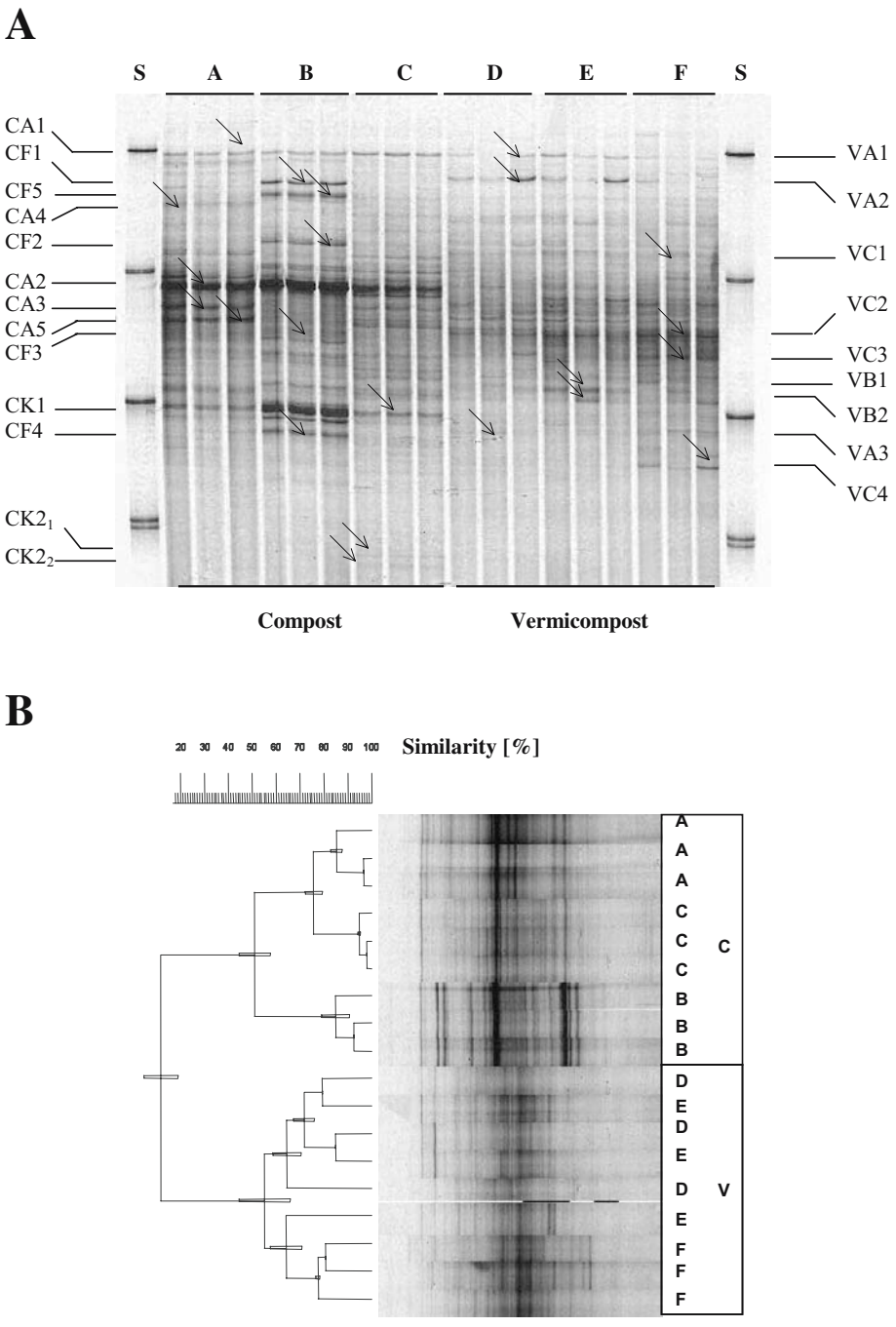

Fig. 1 SSCP profiles of compost (lanes A, B, C) and vermicompost (lanes D, E, F) samples based on PCR-amplified partial 16S rRNA genes. **a** Silver-stained SSCP gels. Compost samples were analysed after 12 years (lane A), 5 years (lane B) and 1 year (lane C) of storage. Vermicompost samples were collected from windrows A (lane D), B (lane E), and C (lane F). Three independent replicates were analysed for each storage period or windrow (*lane S*, *SSCP markers*). Bands indicated with *arrows* and designated at the borders of the gel were selected for DNA sequencing. **b** Similarities of the SSCP profiles of panel **a**. *C* indicates compost and *V* vermicompost samples. For letters A to F, see panel **a**. Calculations of similarities were based on Pearson correlation and tree construction on UPGMA

Identification of SSCP bands

To characterize the quantitatively most important contributors to the SSCP profiles of the DNA amplified from finished compost and vermicompost products, a number of bands of these profiles were isolated, cloned and sequenced. Bands typical for compost or vermicompost as well as bands characteristic for an experimental variable (storage period, composting windrow) or dominant for all profiles were chosen. In total, 22 different bands, indicated in Fig. 1a, were isolated from the different SSCP profiles. In order to confirm that DNA of the chosen bands was specifically isolated, the isolated DNA bands were compared by SSCP to the original profiles. In most cases, the re-amplified products corresponded to the expected positions in the community profiles; only CA4 and CF4 bands were found at a different position and therefore excluded from further analyses. For each of the 20 bands analysed, four to five clones were sequenced. In total, this analysis yielded 41 different sequences. In several instances, two or three different sequences were retrieved from a single band, but in other cases, sequencing indicated that the bands only consisted of a single sequence, e.g., from band CA2, isolated from a profile of

the long storage period; the same sequence was retrieved four times. None of the sequences could be identified as being a chimera.

The nucleotide sequences retrieved from the profiles showed similarities to 16S rRNA gene sequences deposited in the public databases in the range of 90 to 100% (Table 2). Only three sequences were identical to database sequences, and all of them were derived from cultivated isolates. Interestingly, all of these sequences were obtained from compost. In fact, only five of 21 sequences obtained from compost were more closely related to 16S rRNA genes from uncultured than to cultured bacteria. In contrast, the majority of sequences from vermicompost, i.e. 19 of 23, were more closely related to uncultured bacteria.

Table 2 Characterization of 16S rRNA genes extracted from SSCP profiles (see Fig. 1) generated by PCR with DNA of compost and vermicompost as templates

Phylum or subclass	Clone name	Closest relative, GenBank accession number	Similarity to closest relative (%)	Accession number
Chloroflexi	VC2-24	Uncultured bacterium, AB109435	95	AJ973329
	VA3-9	Uncultured bacterium, AJ2976231	91	AJ973330
	VB1-23x	Uncultured bacterium, AJ412677	94	AJ973331
	VB2-17	Uncultured bacterium, AF234150	98	AJ973332
	VC4-36n	Uncultured bacterium, AY250872	94	AJ973333
	CK1-35	Uncultured bacterium, AY150884	97	AJ973334
α-Proteobacteria	VC1-21	Uncultured bacterium, AJ607235	98	AJ973335
	VA3-8	*Maricaulis* sp., AJ227810	91	AJ973336
	VC4-29	Uncultured bacterium, AF234703	99	AJ973337
β-Proteobacteria	CF5-30n	*Alcaligenes* sp., AJ412685	98	AJ973338
	VA2-18x	Uncultured bacterium, AJ608305	98	AJ973339
γ-Proteobacteria	CF1-18	*Lysobacter* sp., AB161359	100	AJ973340
	CK2b-42n	*Methylocaldum gracile*, U89298	99	AJ973341
	CK1-34	Uncultured bacterium, AF424176	93	AJ973342
	VA2-8n	Sulfur-oxidizing bacterium, AF170423	93	AJ973343
	VC1-23n	Uncultured bacterium, AB077347	97	AJ973344
Firmicutes	CA1-2n	*Bacillus oleronius*, AF393508	98	AJ973345
	CF1-13x	Uncultured bacterium, AJ576374	98	AJ973346
	CA1-4	Uncultured bacterium, AB128824	94	AJ973347
	CA5-14	*Thermoactinomyces sanguinis*, AJ251778	97	AJ973348
	CF2-21n	*Bacillus* sp., AJ431332	90	AJ973349
	CK2b-41n	*Tissierella nitritigenes*, X80227	96	AJ973350
	VA1-2a	*Bacillus circulans*, AF540984	99	AJ973351
	VA1-3	*Bacillus* sp., AF463535	98	AJ973352
Actinobacteria	CA1-1n	*Nocardioides aquiterrae*, AF529063	100	AJ973353
	CF3-26	Uncultured bacterium, AY501701	94	AJ973354
	CA2-5	*Streptomyces ferralitis*, AY262826	99	AJ973355
	CA3-9	*Aeromicrobium erythreum*, AF005021	94	AJ973356
	CA3-12	*Streptomyces ferralitis*, AY262826	99	AJ973357
	CA5-14n	*Micromonospora rosaria*, X92631	96	AJ973358
	CF2-24n	Bacterium Ellin325, AF498707	96	AJ973359
	CF3-27	*Mycobacterium* sp., AY162028	99	AJ973360
	CK1-36	*Actinomadura nitritigenes*, AY035999	99	AJ973361
	VC2-25	Uncultured bacterium, AY225655	96	AJ973362
	VC3-26	Uncultured bacterium, AY192284	94	AJ973363
Acidobacteria	VA1-1n	Uncultured bacterium, AY150892	94	AJ973364
	VA2-6n	Uncultured bacterium, AY150892	97	AJ973365
Bacteroidetes	CK2a-37	*Flexibacter japonensis*, AB078055	100	AJ973366
	VB1-12	Uncultured bacterium, AJ431276	95	AJ973367
	VB2-16	Uncultured bacterium, AJ431316	96	AJ973368
	VB2-17n	Uncultured bacterium, AJ431276	94	AJ973369
Gemmatimonadetes	VA3-35x	Uncultured bacterium, AF524020	91	AJ973370
	VC1-20	Uncultured bacterium, AY493975	97	AJ973371
	VC3-28	Uncultured bacterium, AF507712	93	AJ973372

Clones beginning with C were derived from compost DNA, those with V from vermicompost

The sequences extracted from the SSCP profiles could be attributed to a total of seven different bacterial phyla, among them most sequences (66%) fell into the phyla *Actinobacteria*, *Proteobacteria* and *Firmicutes*. Each of the phyla was dominated either by sequences obtained from compost or from vermicompost, except for the *Proteobacteria*, which contained approximately the same number of sequences from both. However, within the *Proteobacteria*, the *Alpha* subclass was clearly dominated by 16S rRNA genes from vermicompost, whereas the *Beta–Gamma* subclass contained more sequences from compost.

The phyla *Firmicutes* and *Actinobacteria* were dominated by sequences from compost, but both groups also contained sequences from vermicompost. In contrast, the phyla *Chloroflexi* (green non-sulfur bacteria), *Bacteroidetes* and *Gemmatimonadetes* contained more sequences from vermicompost than from compost, the latter only represented by 17, 25 and 0% of all sequences, respectively. It should however be noted that the number of sequences from these latter three phyla was relatively small, so that it cannot be excluded that due to the small sampling size, a negative detection may not be as exclusive as suggested by these results.

Comparative analysis of SSCP profiles from compost and vermicompost by means of gene probe hybridizations

To quantify and compare the occurrence of specific rRNA genes in different compost and vermicompost samples, gene probes were developed for Southern blot analyses of the SSCP profiles. Four probes with different specificities were constructed (Table 3). Probe SFER-837 was developed to target the dominant band CA2 that was detectable in all compost samples, but not in the samples from the vermicompost. The sequence retrieved from this band was closely related to the 16S rRNA gene of *Streptomyces ferralitis* (see also Fig. 1a and Table 2). The second probe, LYSO-830 was derived from band CF1, which harboured a DNA sequence identical to a 16S rRNA gene of *Lysobacter* sp. This probe was chosen as a possible indicator for the intermediate period of compost storage (see Fig. 1a). The other two probes were designed from vermicompost specific sequences: HOLO-588, corresponding to band VA2, was derived from an uncultured *Acidobacteriales* sequence related to *Holophaga* sp. This band seemed typical for vermicompost in general. The fourth probe developed in this study, FLEX-854, was specific for an uncultured member from the phylum *Bacteroidetes*, and it targeted band VB2. This band seemed to be typical only for windrow B (see Fig. 1a). In addition to these specifically designed gene probes, the universal probe Com2 was used as a positive control, to confirm that blotted DNA from the SSCP profiles was still present on the membrane after the sequential use of the previously mentioned gene probes. The Southern blot SSCP analyses were accompanied by dot blot DNA–DNA hybridizations that included positive and negative controls (Figs. 2 and 3).

Probe SFER-837 hybridized specifically with the single band from which it was isolated and with all other parallels amplified from DNA extracted from material of the long and the intermediate period of storage (Fig. 2). Surprisingly, the probe did not hybridize to the corresponding band in the profiles generated from material of the short storage period. Thus, the dominant bands that ran in the position of sequence CA2 (Fig. 1) of all storage periods were not composed of the same DNA sequences. As expected, the *S. ferralitis* sequence of band CA2 was not detected in the vermicompost profiles. On the other hand, the hybridization signal obtained with probe LYSO-830, targeting band CF1, was very weak and could only be detected after extended incubation on light-sensitive film (Fig. 3). Nevertheless, the detected signals indicated its specificity for a single *Lysobacter* sp. band in all three replicates of the compost material from the intermediate period of storage.

Probe FLEX-854 neither hybridized efficiently to the band from which it was extracted nor to any other band of the profiles analysed by Southern blotting and gene probe hybridization (data not shown). However, with the other vermicompost specific probe, HOLO-588, it was possible to obtain a positive hybridization signal with the band from which it was isolated (windrow A) and, in addition, with a band at the same position in another SSCP profile, generated with material collected from windrow B (Fig. 3).

Table 3 Characterization of gene probes used in this study

Probe name	Position in the 16S rRNA gene[a]	Target and phylogenetic group	Sequence (5′-3′)	Reference
SFER-837	837–852	*Streptomyces ferralitis*, *Actinobacteria*	GGA CAA CGT GRA ATG TCG	This study
LYSO-830	830–846	*Lysobacter* sp., *Gammaproteobacteria*	CCT AAG TGT CCC CAA CAT	This study
HOLO-588	588–605	*Holophaga* relative, *Acidobacteria*	ACC TTT GAC TTA TCC GGC	This study
FLEX-854	854–871	*Flexibacter* relative, *Bacteroidetes*	ATC GCT TTC GCT TAG CCG	This study
Com2	907–926	Universal	CCG TCA ATT CCT TTG AGT TT	Schwieger and Tebbe 1998

[a]Numbering according to the *Escherichia coli* 16S rRNA gene (Brosius et al. 1981)

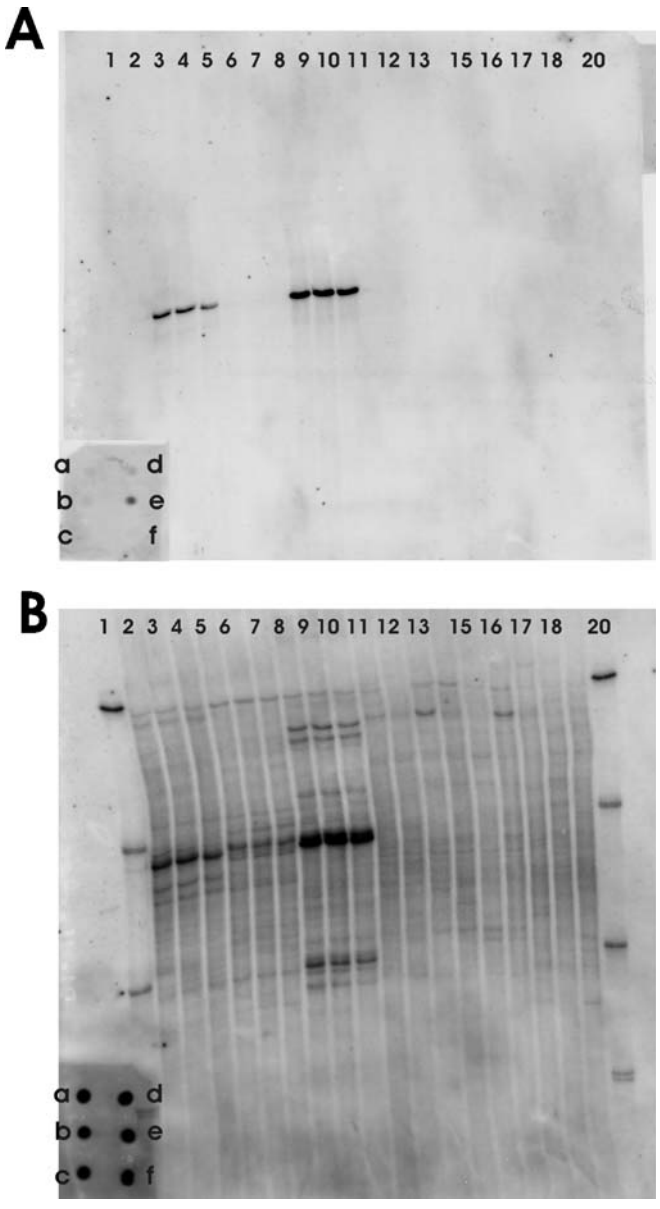

Fig. 2 Southern blot analyses of SSCP profiles. **a** Hybridization with the probe SFER-837, specific for the detection of *Streptomyces ferralitis*. **b** Subsequent hybridization with the universal probe Com2 as the positive control. *Lanes 1* and *20* indicate SSCP markers; *lanes 2–10* compost samples from the long (*2–4*), short (*5–7*) and intermediate (*8–10*) periods of storage; *lanes 11–19* vermicompost samples from windrows A (*11–13*), B (*14–16*) and C (*17–19*). Dot blots shown on the *left lower part* of panels **a** and **b** include partial 16S rRNA genes of controls: *Salmonella typhimurium* (a); band VA2 (b), band VB2 (c), *Escherichia coli* (d), band CA2 (e), and band CF1 (f). For characterization of the 16S rRNA genes from the indicated bands, see Table 2

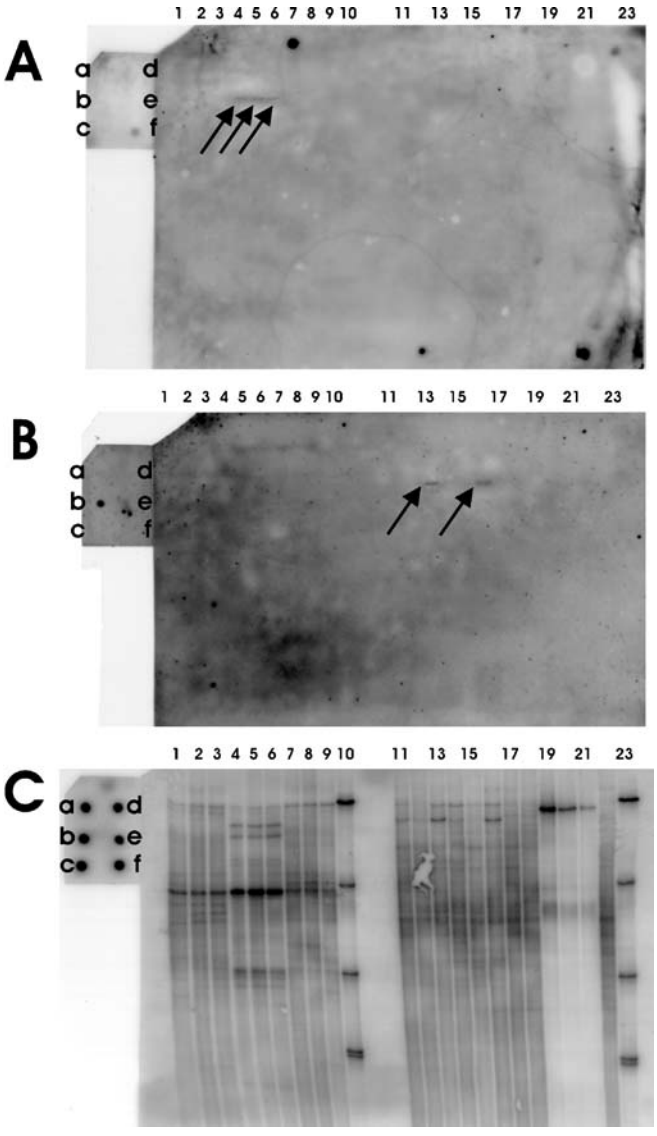

Fig. 3 Southern blot analyses with gene probes **a** LYSO-830, **b** HOLO-588 and **c** Com2, the latter as the positive control. *Lanes 10* and *23* indicate SSCP markers; *lanes 1–9* compost samples from the long (*1–3*), intermediate (*4–6*) and short period (*7–9*) of storage. *Lanes 11–18* and *22* show vermicompost samples from windrows A (*11–13*), B (*14–16*), and C (*17*, *18*, *22*), respectively. *Lanes 19–21* indicate decreasing concentrations of *Salmonella typhimurium* and *Escherichia coli* (top to bottom) partial 16S rRNA genes. For details about the dot blot analyses at the *left side* of each panel, see legend of Fig. 2

The intensity of detection correlated with the intensity of the silver-stained bands (Fig. 1). As this silver-stained band was also present in other vermicompost-derived SSCP profiles, but at lower intensities, the detection in these samples may have been negative due to amounts of target DNA below the limit of detection for this specific probe.

Discussion

In this study, the bacterial diversity of finished compost and vermicompost were compared to each other. As the variability of materials used for both processes were not the same, the results obtained from both materials' products cannot directly be linked to each other. In addition, the diversity of initial organic substrates that are used for both processes is very broad, and thus, the products studied here should not be regarded as representatives for each process, but merely as examples. To indicate the potential of the

bacterial community analyses as a parameter for product evaluation, the material of this study was taken from a commercial composting facility and not from defined laboratory reactors. Despite these differences between both substrates, it was expected that the results of this study were meaningful as the process conditions of composting and vermicomposting are completely different, and it was likely that they were performed by different microbial communities. In composting processes, the self-heating phase with its subsequent maturation is the major biological conversion step, whereas in vermicomposting, the gut passage through worms can be considered as being selective (Tognetti et al. 2005). It should be noted that fungi are also important contributors in the generation of finished composts (Marshall et al. 2003; Anastasi et al. 2004), but in this study, the focus was on bacteria. The differences between both compost and vermicompost as analysed in this study were also reflected in their humidities, with 3 to 6% for compost but 40 to 50% for vermicompost, which were likely to influence the resident bacterial communities in the products. Interestingly, the content of total DNA was slightly higher in the dry compost material than in the humid vermicompost. Possibly, enzyme activities contributing to the decay of cells and DNA were more active in the humid material, and a higher abundance of spores stabilized bacterial cells in the dry material.

The bacterial communities in compost material were characterized by a dominance of *Firmicutes* and *Actinobacteria*. Both groups typically include spore-forming bacteria, which explains both their high abundance in the initial substrates and their thermo-tolerance, allowing them to be active or at least survive the hot composting stage. Most *Firmicutes* detected in this study were closely related to members of the *Bacillus* group. This corroborates results from many cultivation-dependent and several cultivation-independent studies about the importance of the thermophilic aerobic bacilli in classical composting processes (Dees and Ghiorse 2001; Alfreider et al. 2002; Zhang et al. 2002; Schloss et al. 2003; Juteau et al. 2004). Along the same line, bacteria from the group *Actinobacteria*, among them *Streptomyces*, are known to be important during the compost maturation phase (McCarthy and Williams 1992; Peters et al. 2000). By means of the cultivation-independent approach chosen in this study, it was possible to detect close relatives of *Streptomyces* species in the finished compost product.

The vermicompost was mainly colonized by bacteria from the phyla *Chloroflexi*, *Bacteroidetes* and *Gemmatimonadetes*. In addition, bacteria from the subclass *Alphaproteobacteria* and the phylum *Acidobacteria* were exclusively detected in vermicompost. The most striking difference between compost and vermicompost was that the 16S rRNA sequences from vermicompost were mainly related to yet-uncultured bacteria (83% of 23 sequences), whereas for the compost, this percentage was much lower (24%). The phyla *Chloroflexi*, *Acidobacteria*, *Bacteroidetes* and *Gemmatinomadetes* are known to contain many bacteria that are highly abundant in the environment, but most of them are difficult to cultivate (Buckley and Schmidt 2003; Joseph et al. 2003). Even for the subclass *Alphaproteobacteria*, which harbours many cultivated bacteria, none of the three sequences was closely related to a cultured bacterial species. It is too speculative to directly deduce functional properties from the uncultured bacteria detected in this study as the phylogenetic range and functional diversity within each of the phyla are too high (Quaiser et al. 2003; Zhang et al. 2003; Gupta 2004). However, the quantitatively higher diversity detected in vermicompost probably correlates well with a higher functional diversity that is caused by earthworm activities, i.e. digging and feeding. These perturbations modify the physico-chemical conditions and increase the number of microhabitats during the vermicompost process (Dominguez et al. 1997). In this regard, the similarity between vermicompost and native soil is not only evident by its phenotypic properties but also by the inhabiting bacterial communities (Janssen et al. 2002; Furlong et al. 2002; Joseph et al. 2003).

The importance of using a cultivation-independent approach to characterize bacterial community rather than using cultivation is underscored by the fact that more than 50% of the sequences were most closely related to yet-uncultivated bacteria. Despite this clear advantage, the limitation of PCR-based methods to directly detect 16S rRNA genes from environmental samples and from genetic profiles such as those generated by SSCP or denaturing gradient gel electrophoresis (DGGE) should be kept in mind. These limitations relate to an uncertainty about the lysis efficiency of the bacterial cells to release their DNA and the PCR-intrinsic potential biases that have been discussed in detail elsewhere (Farrelly et al. 1995; Suzuki and Giovannoni 1996). In addition to these factors, the limited resolution capacity of gel-based profiling techniques, i.e., SSCP or DGGE, should be considered. In this study, with compost and vermicompost DNA and PCR with primers hybridizing to evolutionary conserved regions, it was typical to observe 20 to 25 bands in each SSCP profile. However, sequencing indicated that more sequences than indicated by single bands contributed to the profiles, and this has also been observed and described in other studies (Sekiguchi et al. 2001; Schmalenberger and Tebbe 2003). Consequently, bands that run in the same position in two profiles are not necessarily caused by the same partial 16S rRNA gene sequence. However, using the gene probes developed in this study, it was possible to identify, for example, a close relative of *S. ferralitis* as a specific inhabitant of the compost products stored for 5 and 12 years at room temperature, respectively. Gene probes were also useful to detect 16S rRNA gene of *Lysobacter* sp. exclusively in material from the intermediate period. In contrast, an uncultured member of the phylum *Acidobacteria* was only found in DNA extracted from vermicompost. For the minor variabilities detected with the gene probes in compost of varying storage periods, however, it

cannot ultimately be decided whether the differences were caused by the storage period or by the intrinsic variation of starting materials in composting facilities investigated here.

Bacterial diversity measurements can only be acceptable indicators for a product quality in the future if the bacterial communities developing in the same products are consistent and stable during subsequent storage of the materials until use. The results of this study indicate that both criteria are fulfilled: the bacterial community structure as revealed by the SSCP profiles of different vermicompost windrows was highly similar, or even indistinguishable, and the storage of finished dry compost over a period of more than a decade did not dramatically affect this parameter. In both aspects, the results of this study can be regarded as an initial support for evaluating the usefulness of such bacterial community analyses for assessing compost quality. Further work should consider, for example, the similarity of different vermicomposting processes to each other, the impact of different initial organic substrates, the results obtained during "bad" composting (e.g., odour emission) or the survival and detection of potential microbial pathogens.

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