

## ORIGINAL ARTICLE

# Development of compost maturity and Actinobacteria populations during full-scale composting of organic household waste

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## Keywords

Actinobacteria-specific primers, large-scale composting, microbial community structure, PCR-denaturing gradient gel electrophoresis, phospholipid fatty acid.

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## Abstract

**Aims:** This study investigates changes in microbiological and physicochemical parameters during large-scale, thermophilic composting of a single batch of municipal organic waste. The inter-relationships between the microbial biomass and community structure as well as several physicochemical parameters and estimates of maturation were evaluated.

**Methods and Results:** Analyses of signature fatty acids with the phospholipid fatty acid and ester-linked methods showed that the total microbial biomass was highest during the early thermophilic phase. The contribution of signature 10Me fatty acids from Actinobacteria indicated a relatively constant proportion around 10% of the microbial community. However, analyses of the Actinobacteria species composition with a PCR-denaturing gradient gel electrophoresis approach targeting 16S rRNA genes demonstrated clear shifts in the community structure.

**Conclusions:** This study demonstrates that compost quality, particularly maturity, is linked to the composition of the microbial community structure, but further studies in other full-scale systems are needed to validate the generality of these findings.

**Significance and Impact of the Study:** The combination of signature lipid and nucleic acid-based analyses greatly expands the specificity and the scope for assessing the microbial community composition in composts. The results presented in this study give new information on how the development of the compost microbial community is connected to curing and maturation in the later stages of composting, and emphasizes the role of Actinobacteria in this respect.

## Introduction

Composting has become an increasingly important strategy for the treatment of municipal organic waste. In order to evaluate the process and the quality of the end product, better knowledge of the microbial community dynamics is needed. As the range of ways to utilize compost products is increasing, the demands on compost quality also increase. It has been demonstrated that compost is applied to agricultural fields as long-term fertilizer (Odlare 2005), to improve soil structure (Jakobsen 1995),

as a substitute for peat in horticulture (Ekland *et al.* 1998), as a suppressive agent against plant pathogens (Hoitink and Boehm 1999) and as a microbial additive to increase enzyme activity (Perucci 1990). Problems associated with immature composts can include malodours, insect swarms, emissions of climate-relevant trace gases and phytotoxicity (Mathur *et al.* 1993). Compost stability is strongly related to microbial activities during the composting process; therefore, several authors have suggested that microbiological parameters can serve as indicators of compost maturity (Eiland *et al.* 2001; Benito *et al.* 2003;

Tiquia 2005). Knowledge about the micro-organisms present in composts, their coexistence and the ways they replace each other during the different stages of the biological degradation process should help to ensure a high quality of the final compost product.

It has been underlined that Actinobacteria play an important role in the later stages of composting (Finstein and Morris 1975; Herrmann and Shann 1997; Ryckeboer *et al.* 2003) and particularly for the degradation of relatively complex, recalcitrant compounds (Goodfellow and Williams 1983). The ability of Actinobacteria to degrade lignocelluloses implies that this group of bacteria has potential to be useful indicators for compost maturity. A previous study in a compost reactor highlighted the importance of temperature as a selective factor for dynamics in the structure and biomass of the actinobacterial community, and this bacterial group constituted a substantial part of the microbial community in the later composting stages (K. Steger, Å. Jarvis, T. Vasara, M. Romantschuk and I. Sundh, unpublished data). In that study, the dominance shifted from members of *Corynebacterium*, *Rhodococcus* and *Streptomyces* in the original material to species of thermo-tolerant Actinobacteria in the cooling phase, e.g. *Saccharomonospora viridis*, *Thermobifida fusca* and *Thermobispora bispora*. Studies in full-scale facilities are needed to clarify whether these changes and the dominance of Actinobacteria in the curing phase also hold for large-scale systems.

A previous comparative study of microbial community structure in pilot-scale and full-scale facilities used analysis of phospholipid fatty acids (PLFA) to characterize the community (Herrmann and Shann 1997). The changes in the fatty acid composition over time were found to be similar in both facilities, but the microbial community within the pilot-scale system progressed more rapidly. Collectively, studies applying various kinds of methods in both pilot- and full-scale processes show dynamic changes in the microbial community structure (Hellmann *et al.* 1997; Klamer and Bååth 1998; Sundh and Rönn 2002). A substantial shift in the community occurs with increasing temperatures, and thermo-tolerant micro-organisms, often Gram-positive bacteria with low G + C content, predominate in the thermophilic phase. During the curing phase, with decreasing temperatures, both mesophilic and thermophilic micro-organisms characterize the community. In large-scale composts, shifts in the microbial community have been investigated by methods like the Biolog assay (Andrews *et al.* 1994; Insam *et al.* 1996), the measurement of enzyme activity (Tiquia *et al.* 2002), PCR-based methods such as denaturing gradient gel electrophoresis (DGGE) (Pedro *et al.* 2001) and single-strand conformation polymorphism (Alfreider *et al.* 2002). Molecular methods have the advantage that they allow

identification of microbial groups associated with shifts in microbial community structure, including uncultivated micro-organisms. Thereby, they yield specific pictures about the microbial diversity in complex systems such as composts.

To our knowledge, previous investigations in full-scale composting systems have all focused on the changes in the overall microbial community and often only in the initial weeks of the process. However, in our study, compost from a large-scale facility was collected from the same composting mass over a period of 57 weeks (400 days). The samples were characterized by physico-chemical analyses, and a rapid maturity test was performed for later stage samples. These results were correlated with changes in microbial biomass and community structure. PLFA analysis was used to obtain quantitative estimates of changes in the Actinobacteria community, whereas PCR-DGGE of 16S rRNA genes was performed with Actinobacteria-specific primers to investigate qualitative changes in diversity within the populations of this particular group. Our hypothesis was that in this large-scale composting process Actinobacteria have the potential to indicate the quality of the final product and, in particular, to act as indicator organisms for compost maturity.

## Materials and methods

### Compost system

Organic waste was composted in the Isåtra treatment plant in Uppland (Sweden). Source-separated organic household waste from several municipalities was collected in easily degradable paper bags and transported to the compost plant. Green waste consisting of shredded residues from parks and gardens was used as a bulking agent and mixed with the organic household waste (1 : 3). The mixture was placed into force-aerated boxes (21 m × 6.50 m) covered with semipermeable membranes. All four boxes at the facility were equipped with countersinks with pipes in the floor to provide forced aeration (the air was non-heat exchanged). After the initial 4-week treatment, the material was transferred to open concrete boxes with forced aeration where decomposition of the material continued for four more weeks. For the final maturing stage, the material was placed in open-air windrows without forced aeration. During this stage, a front loader mixed the material once a month.

### Sampling and physicochemical analyses

The coarseness of the original material (whole potatoes, apples, oranges, etc.) made it difficult to take samples

during the first weeks after the onset of piling. In addition, the harsh winter climate did not allow sampling before the sixth week of composting. Thereafter, samples were taken monthly during the first 7 months and then every other months (9, 11 and 13). The sampling method we used was developed for fairly mixed compost piles by the Swedish Environmental Protection Agency (Naturvårdsverket 1999) and is referred to as the big bucket sampling method. First, a front-loader scoop drove into the pile from both long sides to reach the inner parts of the pile. To obtain a material representative of the whole compost pile, seven subsamples of approx. 10 l were collected from inner and outer parts of the pile, and evenly spread on a plastic sheet. To mix the composite samples, the corners of the sheet were lifted to compile the material, and this procedure was continued for at least two rounds. The mixed sample was then divided into four parts and three-quarters of the material was discarded, while the remaining one-quarter was mixed again. Finally, a sample of about 5 l was transferred to a plastic bag and transported to the laboratory for further analyses. The procedures used for sampling and for preparing the samples for further analyses are described in Fig. 1.

Compost temperatures were measured on each subsample location on the pile using a temperature sensor (Tsuruga, Osaka, Japan) at a depth of 30 cm.

For dry matter determinations, 10 g of the material was dried at 105°C for 24 h and weighed. The ash con-

tent was determined after 12 h at 550°C. The organic matter content was calculated as the difference between dry matter and ash content.

For the pH analysis, 30 ml of deionized water was added to 6 g compost. This water : compost slurry was shaken for 30 min at room temperature. After storage at 20°C overnight, the samples were shaken again for 30 min and allowed to sediment for 30 min, before pH was measured using a reference pH meter.

To determine compost maturity, the commercially available SOLVITA<sup>®</sup> test (Woods End Laboratories, Inc., Mt Vernon, ME, USA) was performed for the later samples (21–57 weeks) according to the manufacturer's instructions. In principle, the test measures the emission of carbon dioxide and ammonia by colour changes on two gel paddles. The Compost Maturity Index was determined using the results from both paddles and a standard index table.

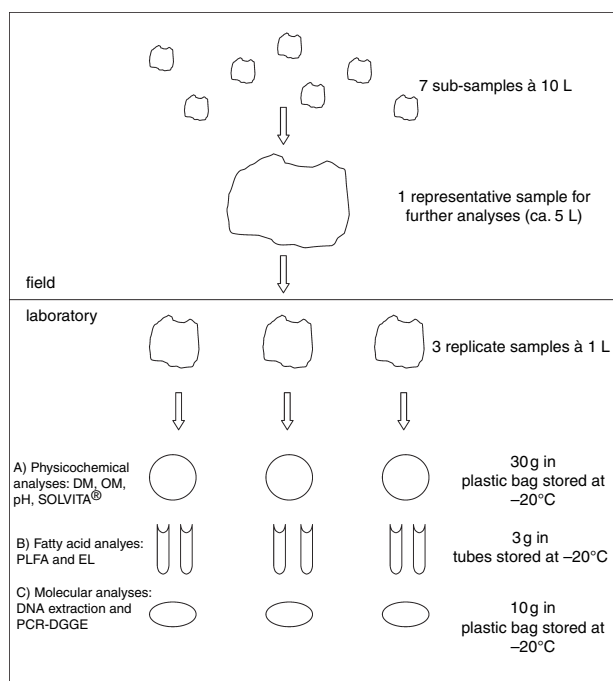
### Lipid analyses

The total microbial biomass and the community composition were investigated by the analysis of PLFA and ester-linked (EL) fatty acids. The PLFA method extracts mainly the fatty acids from intact polar lipids and allows an estimation of the viable microbial biomass. Besides fatty acids from the phospholipids, the EL extraction method additionally includes fatty acids originating from neutral lipids and glycolipids, including those in dead cell material.

For the PLFA extraction, 3 g of compost sample was analysed by the modified one-phase Bligh/Dyer method (Frostegård *et al.* 1991). Lipids were separated using solid-phase extraction with silicic acid columns (Bond-Elut<sup>®</sup> LRC-Si; Varian, Inc., Palo Alto, CA, USA) into neutral, glyco and polar lipids (King *et al.* 1977; Kates 1986). The phospholipids in the polar fraction were converted to fatty acid methyl esters (FAME) by mild alkaline methanolysis according to Dowling *et al.* (1986). However, for the extraction of FAME, a mixture of hexane : chloroform (4 : 1) was used, before the organic fractions were dried and stored at –20°C prior to gas chromatographic (GC) analyses.

The EL method was originally developed to extract fatty acids from soils (Schutter and Dick 2000) and has been slightly modified for compost samples (Steger *et al.* 2003). For the hydrolysis and methylation, 15 ml of freshly prepared 0.2 mol l<sup>-1</sup> KOH in methanol was added to 3 g of compost. After the transformation of EL fatty acids to FAME, the extracted fatty acids were dissolved in heptane and transferred to GC vials for analysis.

FAME were identified and quantified by GC performed on Hewlett Packard 6890 GC-FID and GC-MS



**Figure 1** Schematic outline of the sampling procedure and the preparation of samples for further analyses.

instruments (Hewlett Packard, Palo Alto, CA, USA). The temperature programme has been described previously (Steger *et al.* 2003). In addition, dimethyl disulfide derivatization of FAME for the identification and quantification of monounsaturated fatty acids also followed previous procedures (Steger *et al.* 2003).

The specificity of 10Me18:0 was implied in previous studies, which characterized 10Me18:0 as a taxonomical biomarker for Actinomycetes (Kroppenstedt 1985). Furthermore, 10Me16:0 and 10Me17:0 are also common fatty acids in many Actinobacteria, but much less so in other microbial groups (Kroppenstedt 1992) and therefore, they were also used in this study as biomarkers for this group.

The multivariate method principal components analysis (PCA) was performed to assess the overall patterns of variation in FAME composition over time in the composting process. The analyses were performed with relative concentrations of FAME (mol%), using the SIMCA-P 10.0.4 software package (Umetrics AB, 2002, Umeå, Sweden).

## Molecular analyses

### DNA extraction

DNA was extracted directly from compost samples using the FastDNA<sup>®</sup> Spin Kit for soil (Qbiogene, Carlsbad, CA, USA) as specified by the manufacturer with the following modifications: 0.3 g (fresh weight) of compost samples from each replicate plastic bag (Fig. 1) were transferred to multimix tubes containing 975 µl phosphate buffer and 125 µl MT buffer. Bead beating was performed in a FastPrep<sup>®</sup> FP 120 Instrument (Qbiogene, Carlsbad, CA, USA) for 20 s at speed 5.0. All centrifugation steps were performed at approx. 16 000 g. The DNA was eluted with 50–75 µl of DNA Elution Solution-Ultra Pure Water. DNA extracts of aged compost samples were rather brownish, indicating that the humic compounds were co-purified with DNA. Therefore, from our late samples (37, 47 and 57 weeks), DNA was extracted using the Power Max<sup>™</sup> DNA Kit (MOBIO Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer's instructions. From each replicate plastic bag, approx. 8 g (fresh weight) of compost was used for the extraction. This

method was much more efficient for our samples with their high content of humic substances, and the DNA recovered was suitable for further molecular analyses. DNA concentrations were determined using a Nano-Drop<sup>®</sup> ND-1000 Spectrophotometer (Baylor College of Medicine, Houston, TX, USA). The isolated DNA was stored at –20°C prior to further analysis.

As a positive actinobacterium control for the PCR and DGGE analyses, DNA from *Streptomyces thermodiastaticus* (DSM 41740) was isolated by using the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's instructions for Gram-positive microorganisms.

### PCR-DGGE analyses

For all DNA extracts, part of the actinobacterial 16S rRNA gene was the target in a nested-PCR approach. In the first step, universal bacterial primers fd1 (Weisburg *et al.* 1991) and 926R (Muyzer *et al.* 1995) (Table 1) were used to amplify 10–100 ng of total DNA using 10 pmol of each of the primers. The total reaction volume was 50 µl and each reaction contained 1x PCR buffer, 1 U *Taq* polymerase and each dNTP at 200 µmol l<sup>-1</sup> (Amersham, Uppsala, Sweden). The amplification conditions were 94°C for 5 min, followed by 35 cycles of 40 s of denaturation at 94°C, 40 s for primer annealing at 55°C, 1 min for primer extension at 72°C, and finally 72°C for 7 min. The resulting PCR amplicons (1 µl) were used as templates in DGGE-PCR with primers S-C-Act-235-a-S-20-GC and S-C-Act-878-a-A-19 (Stach *et al.* 2003) (Table 1). The reaction mixtures were prepared as described above. The PCR conditions followed Stach *et al.* (2003). All PCR reactions were conducted in a GeneAmp<sup>®</sup> PCR System 9700 (PE Applied Biosystems, Norwalk, CT, USA). The primers used in this study were synthesized by Invitrogen<sup>™</sup> (Invitrogen Ltd, Paisley, UK).

DGGE was performed according to Muyzer *et al.* (1993) using the Dcode<sup>™</sup> Universal Mutation System (Bio-Rad, Hercules, CA, USA). Similarly sized PCR products were loaded onto vertical polyacrylamide gels containing 7% (v/v) acrylamide–bisacrylamide (37.5 : 1), 0.1% tetramethylethylenediamine (v/v), and 0.46% ammonium

**Table 1** Sequences of specific and universal primers used in the molecular studies

Primer (reference)	16S rDNA target	Primer sequence (5'–3')
fd1 (Weisburg <i>et al.</i> 1991)	Universal	a gag ttg gat cct ggc tca g
926R (Muyzer <i>et al.</i> 1995)	Universal	ccg tca att ctt ttr agt tt
S-C-Act-235-a-S-20 (Stach <i>et al.</i> 2003)	Actinobacteria	cgc ggc cta tca gct tgt tg
S-C-Act-878-a-A-19 (Stach <i>et al.</i> 2003)	Actinobacteria	ccg tac tcc cca ggc ggg g
GC-clamp (attached to the 5'-end of the S-C-Act-235-a-S-20 primer)		cgc ccg ggg cgc gcc ccg ggc ggg gcg ggg gca cgg ggg g

persulfate (w/v). A linear gradient of denaturant was applied from the top (35%) to the bottom (70%) of the gel (100% denaturant was defined to contain 7 mol l<sup>-1</sup> urea and 40% (v/v) formamide). The gels were run in 1x TAE buffer (40 mmol l<sup>-1</sup> Tris-acetate, 1 mmol l<sup>-1</sup> EDTA, pH 8) at a constant voltage of 130 V and temperature of 60°C for 17 h. The gels were stained with SYBR Gold, 10<sup>-4</sup> dilution (Microbial Probes, Eugene, OR, USA) for at least 30 min and the migration patterns were visualized by UV transillumination.

DGGE bands selected for sequence analysis were excised from the gel and placed in 50 µl of elution buffer. DNA was eluted through a freezing-and-thawing procedure: First, samples were stored at -70°C for 16 h, then at room temperature for 1 h, followed by -70°C for 1 h, and finally thawing overnight at +4°C (Throbäck *et al.* 2004). The eluted DNA fragments were re-amplified with S-C-Act-235-a-S-20 and S-C-Act-878-a-A-19 primers (Table 1) under the conditions described above for the second PCR step. The PCR products were purified using the MiniElute PCR Purification Kit (Qiagen GmbH) and then stored at -20°C while awaiting sequence analysis.

#### Sequence analyses

The sequencing reactions were performed using an ABI PRISM<sup>®</sup> BigDye<sup>®</sup> Terminator Cycle Sequencing Ready Reaction Kit v.3.1 and analysed in a Prism 3700 DNA sequencer (Applied Biosystems, Foster City, CA, USA). All selected samples were sequenced in both forward and reverse directions with the above-mentioned primers (Stach *et al.* 2003). All sequence chromatograms were analysed, complemented and assembled with the Staden Package (University of Cambridge, Cambridge, UK), and consensus sequences were retrieved.

These consensus sequences were compared with the 16S rRNA gene sequences from GenBank at the National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov/>) using the basic local alignment

search tool (Altschul *et al.* 1997). For phylogenetic analyses, nucleotide sequences (>500 bp) were aligned using CLUSTAL W software (Thompson *et al.* 1994) with 59 selected 16S rRNA gene sequences from different Actinobacteria phyla, and *Escherichia coli* as outgroup (all retrieved from NCBI). The alignment was manually corrected within SEAVIEW (Galtier *et al.* 1996), and 575 unambiguous aligned sites were selected for phylogenetic analysis.

The optimal maximum likelihood (ML) phylogenetic tree was inferred using PHYML (Guindon and Gascuel 2003) and a general time reversible (GTR) substitution model, with a mixed four-category discrete-gamma model of among-site rate variation plus invariable sites (GTR + Γ + Inv). Bootstrap support values were calculated from 1000 resampled data sets. The tree was drawn using TREEVIEW (Page 1996).

The partial 16S rRNA gene sequences obtained in this study are available in the GenBank (NCBI) database under accession nos DQ639989–DQ640003.

## Results

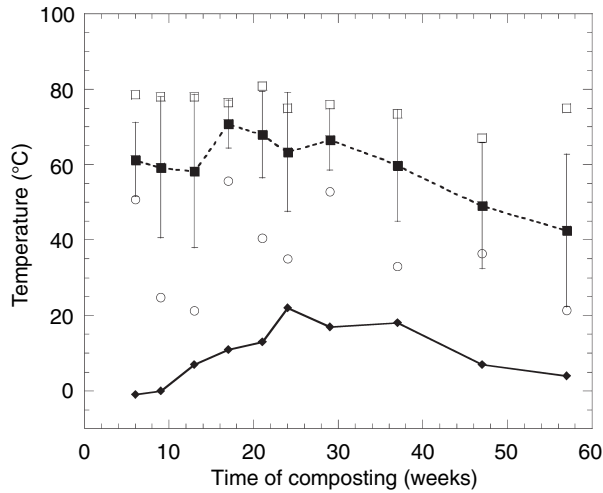
### Compost analyses

The dry matter content of the compost material fluctuated during the process, with the lowest value of 36% at week 9 and the highest value of 63% at week 24 (Table 2). Over the entire sampling period, the organic matter content gradually decreased from 63% to 48% of the dry matter. The pH increased gradually from 5.4 to 8.5 with the only exception being sampling week 9. The first SOLVITA<sup>®</sup> test for compost maturity, performed with the sample of week 21, resulted in a maturity index of 1 which indicated 'raw' compost with a high rate of decomposition. The maturity index improved to an index of 5–6 at week 57, characterizing the material as curing with reduced management requirements.

**Table 2** Physicochemical characteristics of samples at different age from a full-scale organic waste treatment plant (±SD)

Age (weeks)	Dry matter (%)	Organic matter (%)	pH	Maturity index*
6	44.30 ± 0.95	62.55 ± 1.48	5.42 ± 0.08	ND
9	35.63 ± 1.55	59.88 ± 3.94	6.78 ± 0.12	ND
13	45.87 ± 0.68	58.51 ± 2.27	5.93 ± 0.05	ND
17	54.60 ± 0.66	56.42 ± 1.63	6.28 ± 0.03	ND
21	58.77 ± 1.47	55.06 ± 2.66	6.26 ± 0.05	1
24	62.70 ± 1.31	53.68 ± 2.51	6.53 ± 0.01	ND
29	60.78 ± 0.75	54.22 ± 0.63	6.91 ± 0.04	2–3
37	59.93 ± 0.70	52.57 ± 1.59	7.49 ± 0.03	3
47	53.27 ± 0.76	49.82 ± 1.20	7.88 ± 0.02	3
57	49.27 ± 1.51	47.96 ± 2.99	8.49 ± 0.06	5–6

\*SOLVITA<sup>®</sup> Compost Maturity Index considers 1–2: 'raw' compost; 3–6: 'active' compost; 5–6: curing stage; 7–8: 'finished' compost.

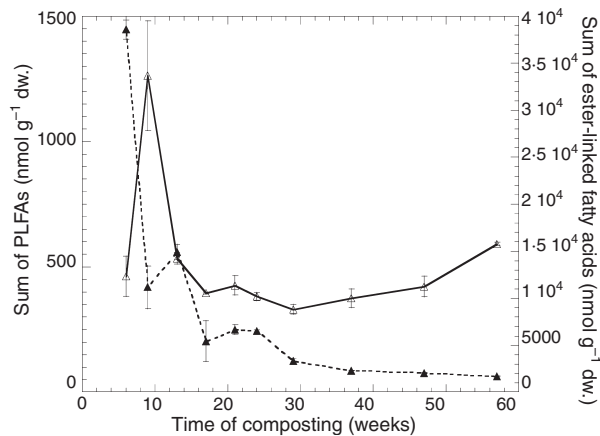


**Figure 2** Temperature profile during full-scale composting of organic household waste (diamonds, air temperature; filled squares, mean temperature in the material; open squares, highest recorded temperature; open circles, lowest recorded temperature).

The thermophilic phase had been reached at the onset of sampling after 6 weeks of processing (Fig. 2). A further slight increase in temperature until week 17 was followed by a slow continuous decrease until the end of measurements at week 57.

### Fatty acid analyses

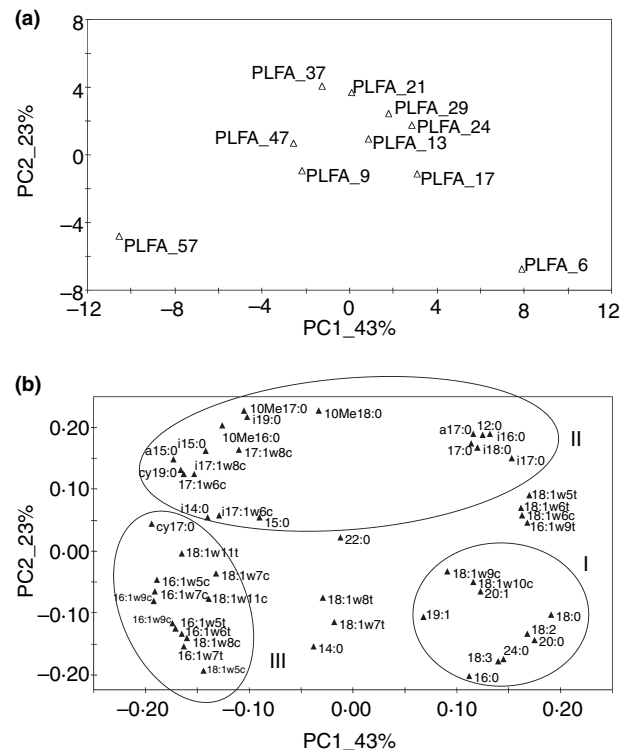
The total concentration of PLFA, an indicator of viable microbial biomass in the material, was about 500 nmol g<sup>-1</sup> dry weight (dw) at the first measurement after 6 weeks of composting (Fig. 3). After 9 weeks, the



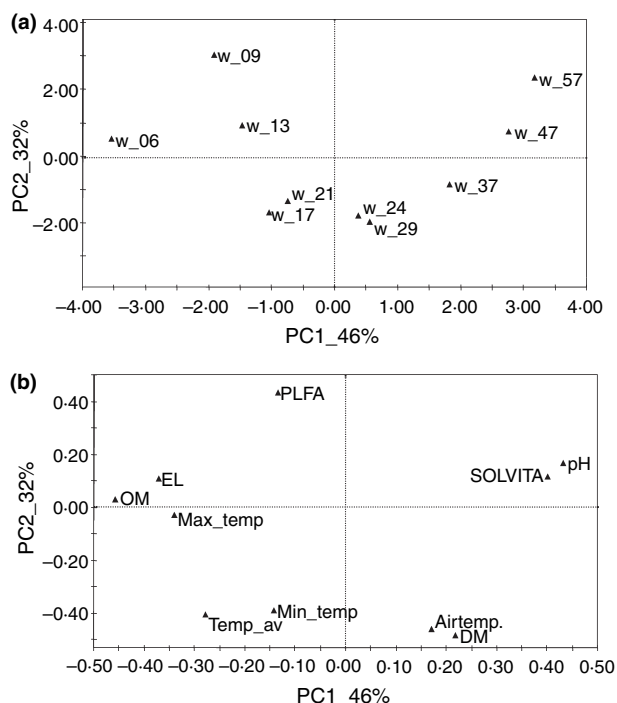
**Figure 3** Total phospholipid fatty acid (PLFA) and ester-linked (EL) fatty acid concentrations during full-scale composting of organic household waste (note different scales of y-axes).  $\blacktriangle$ , PLFA;  $\blacksquare$ , EL.

PLFA concentration was 2.5-fold higher and revealed a peak in microbial biomass at this stage. By week 29, the PLFA concentration had dropped to 350 nmol g<sup>-1</sup> dw. In the following weeks, the PLFA concentration increased again to approx. 650 nmol g<sup>-1</sup> dw at the final measurement at week 57. Compared with the PLFA analysis, the fatty acid concentrations measured with the EL method were substantially higher, initially by 80-fold. A substantial decrease from 39 to 11  $\mu$ mol g<sup>-1</sup> dw occurred between weeks 6 and 9, and by the end of the composting process in week 57, the EL concentration was approx. 2  $\mu$ mol g<sup>-1</sup> dw, threefold higher than with PLFA (Fig. 3).

Multivariate analysis with relative fatty acid concentrations (expressed as mol%) revealed distinct differences between the results of the PLFA and EL analyses (data not shown). However, a similar change in fatty acid composition over time was obvious with both methods. PCA with only the PLFA data revealed a time gradient, primarily along the first axis, but variation within the data was also explained along the second axis (Fig. 4a). The time-dependent change in the PLFA composition was due to a



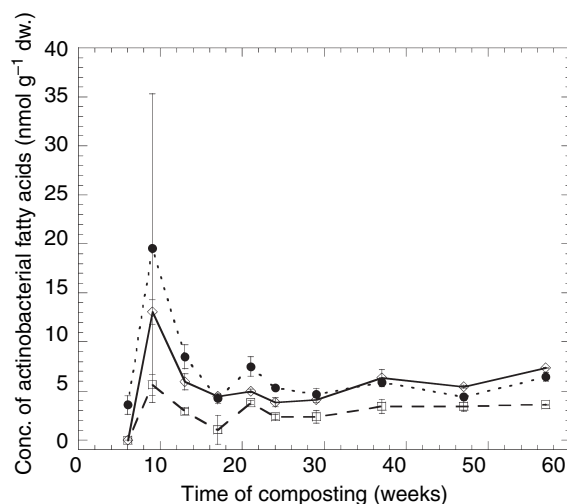
**Figure 4** Principal components analysis of relative phospholipid fatty acid concentrations in compost: (a) score plot (numbers denote sampling week); (b) loading plot with all individual fatty acids (smaller names indicate fatty acids detected in lower concentrations); group I: fatty acids dominating in the beginning; group II: during the hottest period; group III: at the end of composting.



**Figure 5** Principal components analysis of physicochemical parameters measured during the composting process: (a) score plot (numbers denote sampling week); (b) loading plot.

shift from fatty acids typical for eucaryotic cells (e.g. 16:0, 18:3, 18:2, 18:1 $\omega$ 9c, 18:0 and 24:0 – group I) towards iso- and anteiso-branched bacterial fatty acids (e.g. i/a15:0, i16:0, i/a17:0 and i18:0) and 10-methyl branched fatty acids (10Me16:0, 10Me17:0 and 10Me18:0 – group II), which are typical for Gram-positive bacteria and/or thermophiles and Actinobacteria respectively (Fig. 4b). Monounsaturated fatty acids (e.g. 16:1 $\omega$ 5, 16:1 $\omega$ 7 and 18:1 $\omega$ 7 – group III) were most dominant in the later compost stages and indicated the establishment of a new community of Gram-negative bacteria. A similar shift in fatty acid composition was seen in the EL data. Here, the higher proportion of fatty acids derived from microorganisms at the end of composting was accompanied by a shift in their composition towards dominance of bacterial fatty acids (data not shown).

The multivariate analysis with all physicochemical parameters as well as the total PLFA and EL fatty acid concentrations revealed the relationships between optima in the parameters and the different composting phases (Fig. 5). The early stages (weeks 6–13) were characterized by high total concentrations of PLFA and EL fatty acids as well as organic matter content. High temperatures in the compost pile, air temperature and dry matter content were connected with samples from the intermediate stages



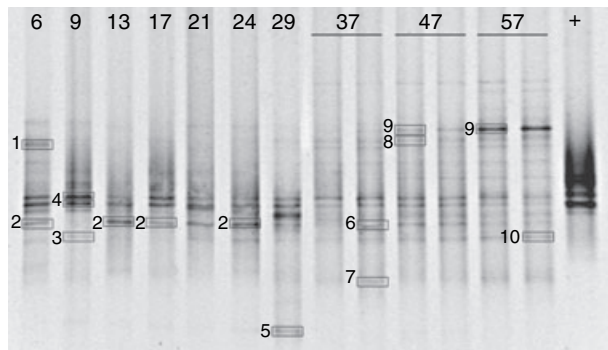
**Figure 6** Total concentrations of 10-methyl branched phospholipid fatty acids typical for Actinobacteria.  $\diamond$ , 10Me16:0;  $\square$ , 10Me17:0;  $\bullet$ , 10Me18:0.

(weeks 17–37), whereas high pH and maturity index were typical for the late-stage samples (weeks 47 and 57).

The concentrations of the 10-methyl branched fatty acids typical for Actinobacteria followed approximately the same pattern of change as the total PLFA concentrations. A peak after 9 weeks of composting was followed by a slight increase in concentrations towards the end of the sampling period (Fig. 6). In general, the concentrations of the three 10-methyl branched fatty acids followed each other closely over time. The contribution of these actinobacterial fatty acids to the total PLFA concentrations was relatively constant at approx. 3% over the entire process.

#### PCR-DGGE and sequence analyses

Time-dependent changes in the structure of the actinobacterial community were revealed by the DGGE analysis of 16S rRNA genes after nested PCR with Actinobacteria-specific primers (Fig. 7). As composting proceeded, actinobacterial communities changed mainly between weeks 6 and 13 and from week 29 onwards. A band that only occurred at week 6 had a sequence that was closely affiliated to the genus *Corynebacterium* (band 1 in Fig. 7). Two major bands at the beginning became weaker during the process and contained sequences that were closely related to *Thermobifida fusca* (band 4). Other bands had sequences that were affiliated to the genera *Saccaropolyspora* (band 2), *Saccharomonospora* (band 3) and *Streptosporangium* (band 5). In the later stages of composting (weeks 37–57), members of the genera *Thermocristum* (band 6), *Actinomadura* (band 7), *Microbacterium* (band 8) and *Streptomyces* (band 10) were also found in the material. A rather weak band at week 47 appeared as the



**Figure 7** Denaturing gradient gel electrophoresis separation of 16S rRNA gene fragments after PCR with Actinobacteria-specific primers. Sampling weeks are indicated on top. Marked bands were excised and sequence analyses revealed affiliation to the following genera: 1, *Corynebacterium*; 2, *Saccharopolyspora*; 3, *Saccharomonospora*; 4, *Thermobifida*; 5, *Streptosporangium*; 6, *Thermocristum*; 7, *Actinomadura*; 8, *Microbacterium*; 9, *Arthrobacter*; 10, *Streptomyces*. (Positive control *Streptomyces thermodiastaticus* is marked as +).

dominant band at week 57 and was identified as *Arthrobacter* (band 9). Comparison of band patterns of replicate samples (weeks 37, 47 and 57) showed that the reproducibility was good. In a few cases, bands obtained from different positions on the gel resulted in similar sequences. Along the same lines, the positive control *Streptomyces thermodiastaticus* gave more than one band in the DGGE (Fig. 7, lane marked with +).

The sequencing of dominant DGGE bands and phylogenetic analysis of the sequences resulted in the ML tree in Fig. 8, which demonstrates that the sequences obtained were most similar to several only distantly related actinobacterial populations.

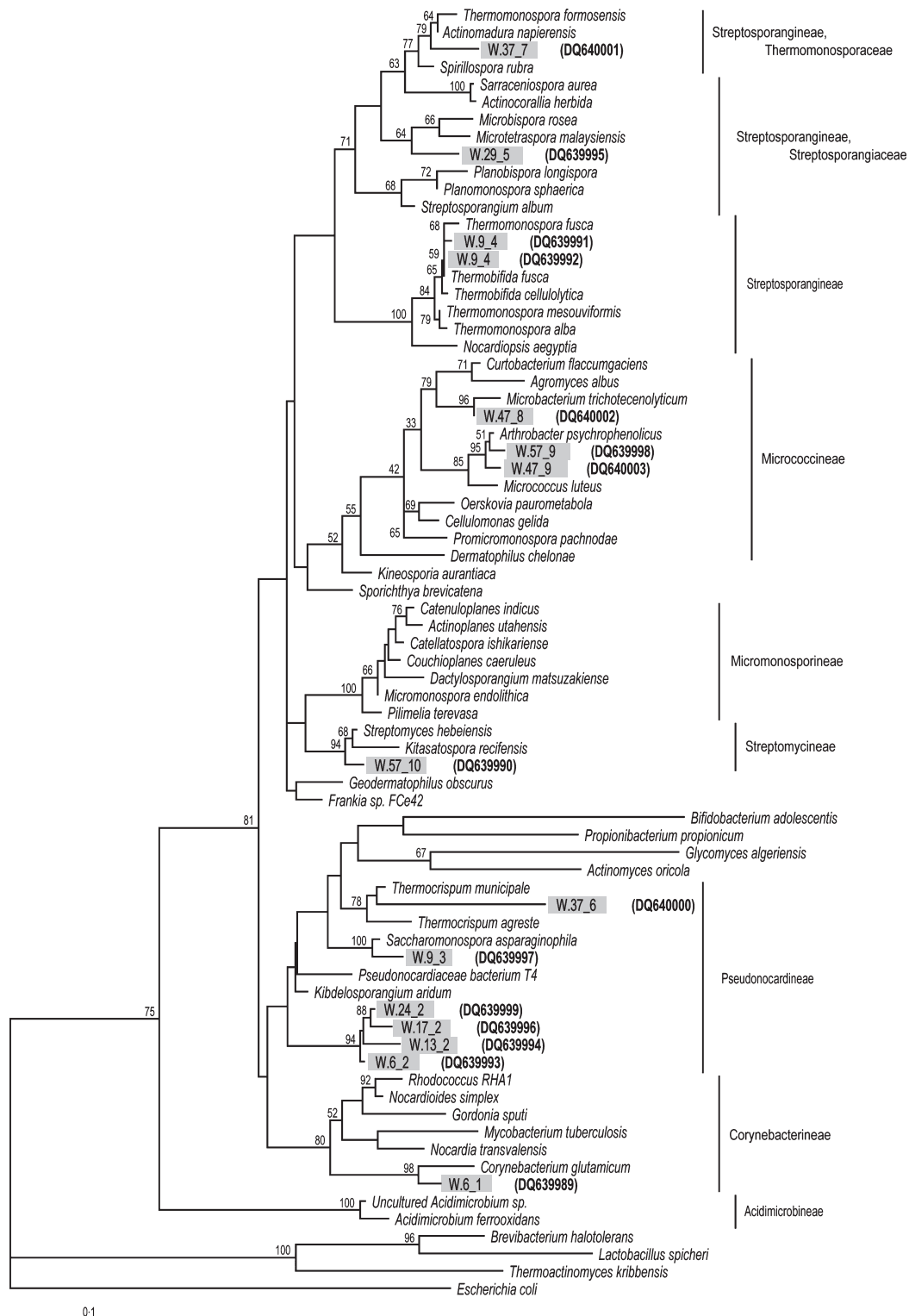
## Discussion

In this study, the decomposition of organic household waste was monitored over a period of 57 weeks in a large-scale plant. The same batch of material was followed throughout all composting stages, which minimizes the impact of different compositions of the raw material. The changes in pH, dry matter and organic matter content were connected to changes in composting management (Table 2). For example, when the material was transferred from force-aerated boxes to open-air windrows without aeration (between weeks 6 and 9), water content and pH increased substantially, implying that the material became more humid without aeration, and possibly that the release of ammonia increased. The continued increase in water content after week 9 was probably a consequence of the continued decomposition process without forced aeration.

At the onset of sampling, the process had already reached the thermophilic stage with temperatures between 50 and 80°C being recorded for all subsamples (Fig. 2). Thermophilic conditions were persistent in the compost material during the following weeks, whereas mesophilic conditions predominated at week 57. The long period of high temperatures most likely ensured sanitization of the material (Epstein 1997), but at the same time, rather high temperatures probably depressed the decomposition rate (Haug 1993). Miller (1993) concluded that the absolute maximum temperature achievable through composting is approx. 82°C. This level was almost reached in some of our subsamples, and process inhibition due to high temperatures seems to be a common problem in full-scale facilities with insufficient control of aeration and temperature parameters (Herrmann and Shann 1997). The large variation in temperature among the subsamples of week 57 indicates that the material was heterogeneous even after a long period of decomposition, and might imply that some material in the heap was still immature. This was supported by the maturity test, which resulted in an index of 5–6, indicating curing material, but not ‘finished’ compost (Table 2). Furthermore, a covariation of Solvita test results with pH was revealed (Fig. 5b) which confirms that higher pH values are characteristic for the late compost stages (Mathur *et al.* 1993).

The development of the total PLFA concentration and thus the microbial biomass was typical for thermophilic-composting processes (Fig. 3), i.e. a peak in PLFA at the early stage followed by a notable decrease and then a slight increase in the later stages (Hellmann *et al.* 1997; Herrmann and Shann 1997; Klamer and Bååth 1998; Sundh and Rönn 2002; Steger *et al.* 2003, 2005). The peak in the early phase can be explained by high microbial activity and growth while the temperature increased. Furthermore, the decrease in microbial biomass over a period of maintained high temperatures can be explained by the fact that in some of the subsamples, temperatures exceeded the range where thermophilic micro-organisms can still maintain their biochemical functions (Haug 1993).

In the hottest samples, the high temperatures probably led to an inactivation or even death of some of the thermophilic micro-organisms. However, when the temperature started to decrease in the material, the PLFA concentration increased slightly, indicating the re-establishment of mesophilic populations. This increase in microbial biomass towards the later compost stages was even more obvious when PLFA concentrations were related to organic material ( $\text{nmol g}^{-1}$  vs data not shown), since inorganic material accumulated during the degradation process. The origin of this new mesophilic community could be bacteria which survived in compartments of lower temperatures during the hottest period or which



**Figure 8** A maximum likelihood tree of phylogenetic relationships of species within the class of Actinobacteria. The scale bar indicates 10% nucleotide substitutions and bootstrap values above 50% are displayed at the nodes. *Escherichia coli* served as an outgroup. (The sequences obtained in this study were shaded in grey; the names indicate sampling week and band number in Fig. 7. Additionally, the accession numbers of the sequences are enclosed in parentheses.)

were added via external contaminations, as the process was performed in open windrows. The new mesophilic community was characterized by the growth of Gram-negative bacteria, as there was a shift in the PLFA composition towards unsaturated fatty acids (Fig. 4b, group III). Prior to this change, the dominance of branched fatty acids (saturated and unsaturated) indicated a dominance of Gram-positive bacteria and/or thermophiles (Fig. 4b, group II). In the beginning of the process, the importance of straight-chain saturated and unsaturated fatty acids implied a community dominated by fungi and partly by Gram-negative bacteria, but it may also reflect the presence of material from food wastes. This larger proportion of fatty acids deriving from the organic material was even more obvious in the results from the EL analysis, which is probably explained by the fact that this method extracts fatty acids not only from polar lipids such as phospholipids, but also from glycolipids and neutral lipids, including those in dead organic matter.

Due to high background in the GC analyses of the 10-methyl branched fatty acids in the EL analyses, these actinobacterial fatty acids were difficult to detect with this method and the analytical system we used. For example, the application of GC columns more suitable to larger sample sizes may have been more efficient in this respect. In contrast to some other studies, we could not detect a substantial growth of Actinobacteria towards the end of composting in our study (Fig. 6). Different studies have reported the persistence of Actinobacteria from the thermophilic to the curing stage where they can dominate the total microbial community (Herrmann and Shann 1997; Tiquia *et al.* 2002; Hiraishi *et al.* 2003). Similarly, in a reactor system (K. Steger, Å. Jarvis, T. Vasara, M. Romantschuk and I. Sundh, unpublished data) we estimated that up to 50% of microbial biomass consisted of Actinobacteria in the later stages of composting. However, making a similar conservative estimate in our full-scale study, Actinobacteria would constitute <10% of the microbial biomass. We have no good explanation for this difference, but perhaps environmental factors, e.g. temperature, oxygen or the substrate composition, might not have favoured the growth of Actinobacteria. The question whether an Actinobacteria proportion of 10% of the microbial biomass is unusually low or rather normal for full-scale systems remains open, which underlines the importance of investigations in several samples from different large-scale composting facilities, as also discussed in earlier studies about, e.g. compost stability (Hue and Liu 1995) or sanitary quality (Christensen *et al.* 2002).

Although the fatty acid analyses indicated a constant proportion of Actinobacteria among the total microbial community, the molecular analyses revealed qualitative

changes in the composition of Actinobacteria during the process (Fig. 7). In the early samples from weeks 6 to 13, when temperatures were already high, thermo-tolerant genera such as *Saccaropolyspora* and *Thermobifida* were found. Despite the rather high temperatures, members of the genus *Corynebacterium* were also present, in line with results from another large-scale study (Andrews *et al.* 1994). The presence of *Corynebacterium* only at week 6 implies that this organism was largely eliminated during the high temperature period. Apart from the genera *Saccaropolyspora* and *Thermobifida*, additional genera of thermo-tolerant Actinobacteria, e.g. *Saccharomonospora* and *Streptosporangium* were detected in the hottest period (up to 80°C) of the process (17–29 weeks). The latter strain has recently been isolated from soil and shown to produce antimicrobial substances (Boudjella *et al.* 2006). However, all these Actinobacteria have the morphological and physiological prerequisites to tolerate such high temperatures, as they can form spores and their growth optimum is often around 50–60°C (Digital Atlas of Actinomycetes; <http://www0.nih.go.jp/saj/DigitalAtlas/index.htm>). Later on, when temperatures started to decrease, new bands appeared that represented other kinds of Actinobacteria, for example, the thermo-tolerant genera *Thermocrispum* and *Actinomadura*. These organisms have previously been found in compost or compost-amended soil respectively (Kornwendisch *et al.* 1995; Ibekwe *et al.* 2001). Furthermore, some bands appearing later in the composting process contained sequences that were affiliated to members of the genera *Microbacterium* and *Arthrobacter*, both belonging to the suborder Micrococci (Fig. 8) and both known to consist of many strains able to degrade persistent and toxic compounds (Rybikina *et al.* 2003; Nordin *et al.* 2005; Manickam *et al.* 2006). Together with the presence of the well-known *Streptomyces* sp., these results imply that the final product may contain Actinobacteria that are able to degrade pollutants, and thereby be useful for bioremediation of contaminated soils (Semple *et al.* 2001), but this needs to be further investigated.

In conclusion, this full-scale composting process showed typical changes in microbial community structure, with a maximum of microbial biomass occurring during the thermophilic phase, which was similar in size with the maximum microbial biomass recorded in other pilot-scale and full-scale studies, irrespective of the kind of organic material that was treated and the techniques that were used. Although the PLFA data suggested that Actinobacteria constituted <10% of the microbial community throughout the entire process, the molecular studies clearly revealed compositional changes within this group. Thermo-tolerant Actinobacteria were mainly found during the complete course of processing, but mesophilic species were detected

at the onset and in the later stages of composting. We interpret the qualitative changes in the Actinobacteria community to indicate the important role of this group of bacteria for the degradation process. The presence of xenobiotic-degrading Actinobacteria is promising for the use of the final product for bioremediation. However, this study underlined the importance of investigations in several supposedly mature samples from different large-scale composting facilities, so as to evaluate Actinobacteria populations as a potential indicator for compost maturity and to validate the generality of these findings.

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