Journal of Applied Microbiology 2001, **91**, 668–676

Microcosm enrichment of 1,3-dichloropropene-degrading soil microbial communities in a compost-amended soil

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761/2/01: received 2 February 2001, revised 23 April 2001 and accepted 25 April 2001

A.M. IBEKWE, S.K. PAPIERNIK, J. GAN, S.R.YATES, D.E. CROWLEY AND C.-H. YANG. 2001.

Aims: A microcosm-enrichment approach was used to investigate bacterial populations that may represent 1,3-dichloropropene (1,3-D)-degrading micro-organisms in compost-amended soil.

Methods and Results: After 8 weeks of incubation, with repeated application of 1,3-D, volatilization fluxes were much lower for compost-amended soil (CM) than with the unamended soils, indicating accelerated degradation due to addition of compost, or development of new microbial populations with enhanced degradation capacity. Denaturing gradient gel electrophoresis (DGGE) profiles of the PCR-amplified region of 16S rDNA genes were used to identify dominant bacterial populations in the fumigant-degrading soil. The DGGE results indicated that specific bacterial types had been enriched, and a more diverse fingerprint was observed in the community derived from the compost-amended soil compared with the unamended soil. Fragments from 16 different DGGE bands were cloned, sequenced and compared with published 16S rDNA sequences. Two clones, designated E1 and E4, were unique to all soils to which compost was added, and corresponded to strains of *Pseudomonas* and *Actinomadura*, respectively.

Conclusions: The results show that the addition of compost to soil increases specific microbial populations and results in the accelerated degradation of fumigants.

Significance and Impact of the Study: Application of compost manure to soil can help degrade soil fumigants at a faster rate.

INTRODUCTION

The repeated application of fumigants such as 1,3-dichloropropene (1,3-D) in agricultural soils can result in accelerated degradation of this compound. This fumigant is used as a nematicide for high value crops. A decrease in field performance for 1,3-D following repeated application has been reported (Smelt *et al*. 1989; Ou *et al*. 1995; Ou 1998). This loss in efficacy is associated with an increase in microbial degradation in the adapted soils. Adaptation of such soils to fumigants may be due to the selection of microbial populations with high degradative potentials (Van Dijk 1974; Van Hylckama and Janssen 1992; Verhagen *et al*. 1995).

The stimulation of microbial degradation from adapted soil is difficult to evaluate because of the complexity of the soil environment and the lack of sensitive methods for *in situ* probing of microbial populations. Recently, Gan *et al*. (1998a, b) showed that applying organic amendments can accelerate 1,3-D degradation in surface soil. They observed that the degradation of 1,3-D was significantly enhanced in soils amended with compost manure compared with the unamended soil, and they attributed the increase in degradation to microbial transformation of this compound. Their results confirmed the findings of Hoitink and Fahy 1986, which showed that application of compost to the soil surface stimulated soil microbial activities.

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In studies investigating enhanced degradation, microbial populations have commonly been enriched from samples collected from plots that had been treated with a particular compound for many years. This approach works on the basis that most of the populations that grow on media supplemented with the appropriate compound are potential degraders. In an environment where different chemicals have been applied, different microbial communities may develop that are responsible for the degradation of these compounds. Therefore, the use of techniques that can quickly identify the degrading populations is essential.

Molecular analysis of microbial communities offers a greater potential for the study of adapted bacteria that can be enriched from environmental samples. In agricultural soils that are repeatedly fumigated, it is likely that fumigant degraders may become part of the dominant bacterial populations. PCR-DGGE amplification of 16S rDNA of these communities may generate fingerprints of the most dominant bacterial populations (Santegoeds *et al*. 1996; Jackson *et al*. 1998; Muyzer and Smalla 1998) and may be useful for determining degrading populations that have been enriched. It can also provide information on the specific strains involved in degradation of different compounds.

Over a 6-month period, a field soil was repeatedly treated with 1,3-D in an attempt to stimulate degrading populations. Volatilization was monitored weekly by the use of an automated sequential sampler system (Wang *et al*. 1999) after the injection of 1,3-D into soil columns, and data were analysed by GC. This process continued for 12 weeks and the last injection was carried out at 16 weeks. The entire experiment lasted for 6 months. This was used to monitor degradation from week to week and after 6 months, the rate of degradation was faster than at the beginning. The main goals were to characterize the differences in bacterial populations from organically-amended and unamended soils, and to determine whether the adapted populations that had been enriched, as revealed by DGGE profiles of the 16S rDNA from the dominant bands, are degrading populations.

MATERIALS AND METHODS

Soil, organic amendments and chemicals

The soil used for this study, an Arlington sandy loam (coarse-loamy, mixed, thermic, Hapic Durixeralf) was taken from the top 10 cm at the University of California, Riverside Agricultural Experiment Station. There was no history of fumigant treatment in this plot. The soil had a pH of 7·2 and organic matter content of 1·1%. Moist soil was passed through a 4 mm sieve and stored at room temperature for 48 h before the start of the experiment. Composted steer manure (CM) purchased from a local supplier was used as an organic amendment. The CM contained 25% moisture.

Telone II containing 50% (*Z*) and 45% (*E*) isomers was provided by DowElanco (Indianapolis, IN, USA). The 1,3-D standard containing 48% (Z) isomer and 50% (E) isomers was purchased from ChemService (West Chester, PA, USA).

Microcosm experiment

Microcosms consisted of duplicate glass columns of about 3905 cm^3 containing 3·6 kg soil and compost manure (4 : 1 w/w) (dry wt); compost and soil were incubated at room temperature. The columns were packed to about 75% of their capacity with amended soil, with or without CM. The same column system had been used previously for measuring 1,3-D volatilization and was found to be very reliable (Gan *et al*. 1998a). To keep the soil enrichment moist, moistened air was flushed through the headspace of each column. 1,3-D was injected (100 μl) as pure chemical into the soil column once a week for the first 12 weeks of the study at a depth of about 30 cm. Volatilization of 1,3-D from the soil was monitored with a constant airflow of 150 ml min^{-1} that swept 1,3-D volatilization off the soil surface into activated C sampling tubes (ORBO-32, Supelco Inc., Bellefonte, PA, USA) installed at the outlet. The sampling tubes were replaced every 4 h. The 1,3-D recovered in these tubes was analysed by gas chromatography (GC, HP 6890, Hewlett Packard, San Fernando, CA, USA) after the charcoal granules had been extracted with ethyl acetate in closed headspace vials. Volatilization was monitored in 4 h increments for 4 days each week for the first 12 weeks of the experiment. During the last 12 weeks, columns were injected twice (weeks 16 and 20) and samples were collected for two weeks to ensure that all fumigants had degraded. Volatilization fluxes of 1,3-D were obtained by dividing the amount of 1,3-D recovered in the sampling tubes by the sampling interval. Cumulative volatilization losses, in percentage of applied chemical, were estimated by integrating volatilization fluxes over the total sampling period.

Degradation of 1,3-D in compost-amended soil and enrichment culture

After 24 weeks, the columns were stored at 8°C for 1 week, mixed, and small portions taken for DNA extraction, plate counts and 1,3-D degradation in the compost-amended soil. Basal mineral salt medium (Janssen *et al*. 1987) was used for the enrichment and enumeration of 1,3-D-degrading microbial populations (Verhagen *et al*. 1995), with 1,3-D as the sole carbon source. Flasks containing basal mineral salt medium with 1,3-D and 10 mg yeast extract l^{-1} were inoculated with soil obtained from microcosms receiving 1,3-D application. After inoculation, dilutions of the

cultures were plated onto solid basal mineral salt media. The plates were incubated at 28°C for 7 days and colonies tested for degrading capacity.

To evaluate the rate of 1,3-D degradation in compost-amended and unamended soils after 6 months of incubation, soil samples were pooled from duplicate treatment jars and mixed for analysis. Aliquots (13 g dry wt) of the above samples were placed in 21 ml headspace vials (Supelco Inc.) with 5 μl acetone solution containing 100 μg μl^{-1} 1,3-D. Half of the pooled samples were autoclaved for determination of chemical degradation. The treated vials were sealed with aluminium caps and Teflon-faced butyl rubber septa, and placed in an incubator at 25°C. Three replicate vials from each treatment were removed at 0, 8, 24, 96, 144 and 192 h, and stored at –20°C. At the end of the experiment, 1,3-D was extracted as described by Gan *et al.* (1998a). Degradation of 1,3-D in minimal media after 8 days of incubation was also determined as above.

DNA extraction, PCR primers and DGGE analysis of soil

Total bacterial community DNA for DGGE analysis was extracted from each treatment column. About 500 mg of soil was placed in FastPrep tubes (BIO 101, Vista, CA, USA) containing lysing matrix. Isolation of total DNA was accomplished with a FastPrep DNA isolation kit according to the manufacturer's protocol (BIO 101). PCR was first performed using about 50 ng of template DNA with the primers, PRBA338f and PRUN518r, located at the V3 region of the 16S rRNA genes of bacterioplankton (Øvreas *et al.* 1997). PRBA 338f consists of a region that is conserved among the domain bacteria, and PRUN 518r is located at a universal conserved region. Ready-To-Go PCR beads (Amersham-Pharmacia Biotech, Piscataway, NJ, USA) were used for PCR amplification. PCR mixtures for bacterial 6S rDNA amplification contained 10 pmol of the primers, 4 μg bovine serum albumin and sterile distilled water in a final volume of 25 μl. The PCR cycles used for amplification were as follows: 92°C for 2 min followed by 30 cycles of 92°C for 1 min, 55°C for 30 s, 72°C for 1 min, and a single final extension at 72°C for 6 min.

DGGE was performed with 8% (w/v) acrylamide gels containing a linear chemical gradient ranging from 30 to 70% denaturant, with 100% defined as 7 mol l^{-1} urea and 40% formamide. Gels were run for 3 h at 200 V with a Dcode^{TM} Universal Mutation System (Bio-Rad Laboratories, Hercules, CA, USA). DNA was visualized after ethidium bromide staining by u.v. transillumination and pictures were taken with a Polaroid camera.

To determine the complexity of microbial communities from 1,3-D-amended and unamended soils, a sevenfold dilution series of the soil samples was conducted in minimal salt media. Samples were mixed for 1 h and 500 μl portions of samples were used for DNA extraction as described above. PCR amplification was done for samples from 10^{-1} to 10^{-7} dilution, followed by DGGE.

Major bands were excised for identification of bacterial species from both the original samples and the diluted samples. Bands were placed into sterilized vials with 20 μl sterilized distilled water and kept overnight at 4°C to allow the DNA to passively diffuse out of the gel strips. Eluted rDNA (10 μl) was used as DNA template with the universal bacterial primers described above. The sizes of the PCR products were checked on a 1·5% agarose gel. The DNA was eluted and cloned into a pGEM-T Easy vector (Promega, Madison, WI, USA), and transformed into *Escherichia coli* JM109. Isolation of plasmids from *E. coli* was performed using standard protocols from the Qiagen Plasmid Mini Kit (Valencia, CA, USA). The 200 bp rDNA inserts in pGEM–T were sequenced with the ABI PRISM Dye Terminator Cycle Sequencing Kit with AmpliTag DNA Polymerase, FS (Perkin Elmer).

Phylogenetic analysis

Sequence analysis used the blast searches (National Center for Biotechnology Information: http://www.ncbi.nlm.nih.gov). The 16S rDNA sequence from the fumigant-enhanced microbial communities were aligned with parts from complete 16S rDNA sequences from closely-related bacteria obtained from Gene Bank.

RESULTS AND DISCUSSION

Characteristic of the enrichment community that degrades 1,3-D

Gas chromatographic analysis showed that after 8 weeks of enrichment, volatilization fluxes (μg h^{-1}) were much lower in the compost-amended soils compared with the unamended soils for the (Z) and (E) isomers of 1, 3-D (Fig. 1). Fluxes were monitored for 96 h each week for the first 8 weeks after injection of fumigant. The average cumulative volatilization losses in terms of percentage of applied 1,3-D showed that the amount of 1,3-D recovered from the amended soil was much lower than that from the unamended soil (Fig. 2a,b). This was in agreement with a previous report by Gan *et al.* (1998a) that showed lower volatilization fluxes for compost-amended soil compared with unamended soil. Low volatilization means high degradation rates in these samples. Analysis of week by week cumulative volatilization losses as a percentage of 1,3-D applied provided a full understanding of the contribution of organic amendment to the increased rate of degradation. After 8 weeks, degradation of 1,3-D was significantly enhanced

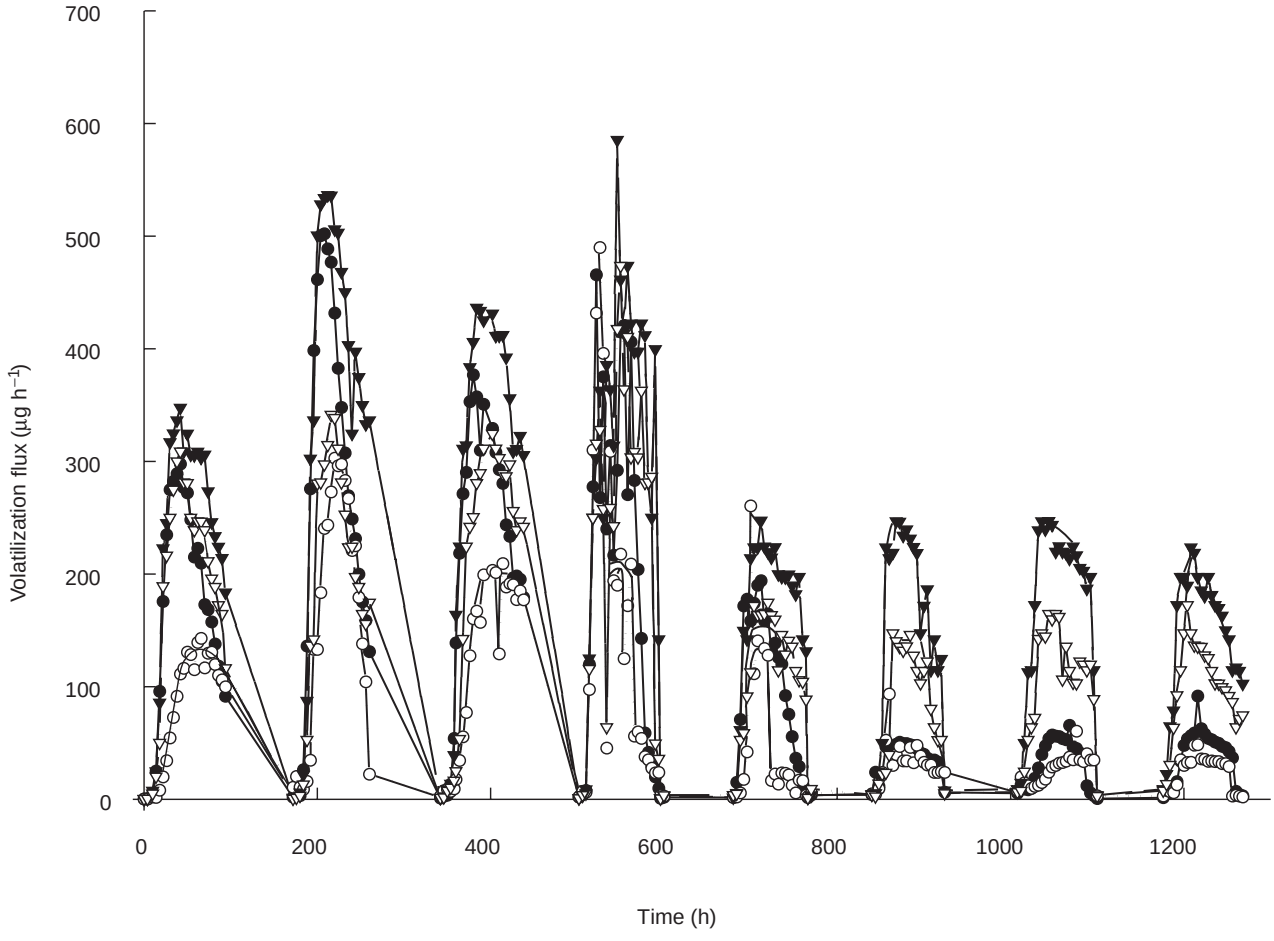


Fig. 1 Volatilization flux (μg h^{-1}) of 1,3-dichloropropene isomers from columns packed with the Arlington sandy loam, with and without organic amendment, after Telone-11 injection at 30 cm depth. Point differences indicate 4 h time intervals between sampling, and the data points indicated in the graph are for an 8-week sampling period. Cis = *Z* isomer and trans = *E* isomer. (●), cis; (○), trans; (■), C-cis; (□), C-trans

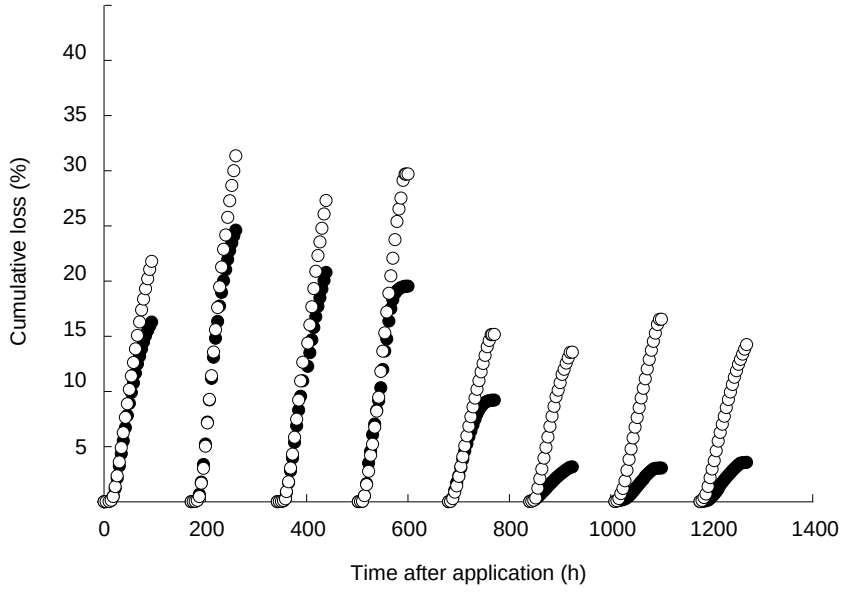


Fig. 2 Cumulative volatilization losses in percentage of applied 1,3-D. (a) *Z* isomers from amended and unamended soils, and (b) *E* isomer from amended and unamended soils. (●), Amended soil, cis 1,3-D; (○), unamended soil, cis 1,3-D

compared with the first 4 weeks. At the end of the experiment, about 8% of applied (Z) and 5% of (E) 1,3-D was lost from the amended soils, compared with 20% and 15%, respectively, for the unamended soils.

The degradation rate of both (Z) and (E) isomers of 1,3-D in amended soil after 6 months showed first-order kinetics, with the constant K 0·14 or half-life ($t_{½}$) of 2 days (Fig. 3a,b). This suggests that 1,3-D will be volatilized into the air by gas-phase diffusion within 2 days at this rate of degradation. Degradation of 1,3-D in the unamended soil was substantially reduced, with a first-order $t_{½}$ of 5·7 days for the (Z) isomer and 4·9 days for the (E) isomer. When both the amended and the unamended soils were autoclaved, the half-life for the (Z) isomer increased to 6 days for the amended soil and 14 days for the unamended soil. For the (E) isomer, the half-life increased to 5·3 days for the amended and 8·75 days for the unamended soil. It took an additional 3 days to degrade both isomers of 1,3-D without organic amendment, suggesting the contribution of organic amendment to the degradation of 1,3-D. Seven more days were required for the degradation of 1,3-D in the sterilized unamended soil (E) isomer, and 12 days for the (Z) isomer, suggesting that a longer time is required for chemical degradation to remove 1,3-D from the environment.

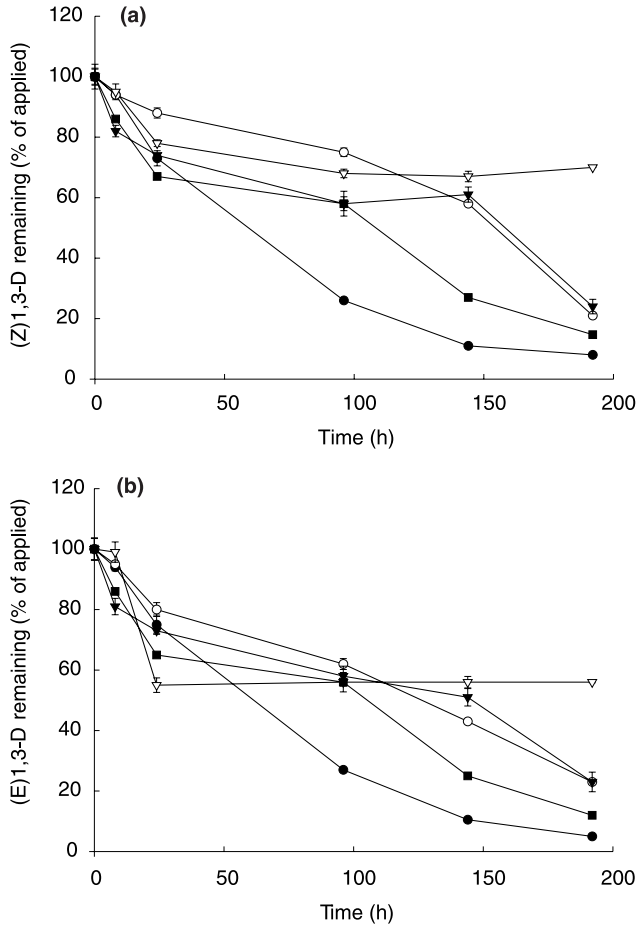


Fig. 3 Degradation rates of 1,3-D isomers in compost-amended and unamended Arlington sandy loam soil and enrichment culture. (a) *Z* isomer (b) *E* isomer. (●), Amended soil; (○), unamended soil; (▼), mixed colonies; (■), unamended sterilized soil; (■), sterilized amended soil

Since the focus of this study was to investigate bacterial populations that may be representative of long-term fumigant-adapted microbial communities involved in the degradation process, the cultivable populations that can grow on basal mineral medium with 1,3-D as the sole carbon source were initially observed. The enrichment culture with 1,3-D as the sole carbon source was used to study degradation of this compound. Also, sterilized soils, and soils with and without amendment, were used to compare degradation with the enrichment culture. The enrichment culture was unable to degrade the (Z) ($t_{½}$ of 3·7 days) and (E) ($t_{½}$ 3·4 days) isomers of 1,3-D as fast as the amended soil after 4 days of incubation (Fig. 3a,b). It is interesting to note that the enrichment culture was more effective in transforming the (E) isomer (80%) than the (Z) isomer (65%).

Total degradation of 1,3-D was higher in the amended soil than the unamended soil and significantly lower in the sterile soils. The 4 day incubation study confirmed the observations in Figs 1 and 2 that the degradation capacity of these samples was predominantly of biological origin. The enrichment culture was plated on solid basal mineral media with 1,3-D as the sole carbon source, and separate colonies were tested to determine their capacity to degrade 1,3-D. None of the tested colonies, either individually or in a combination of two or three, was capable of degrading 1,3-D. This shows a plating bias towards selecting 1,3-D-degrading populations. This is why there is no report of isolation of 1,3-D-degrading populations from soil that has not been repeatedly treated with the compound for many years. In fact, most of the reports of 1,3-D-degrading isolates are from soils that have been treated with the compound for about 5–10 years. Verhagen *et al.* 1995 demonstrated that repeated treatment of soil with 1,3-D for about 10 years resulted in accelerated microbial degradation of this compound. They isolated 15 bacterial strains with 1,3-D-degrading capacity. Our results clearly demonstrate that compost-amended soils can significantly enhance degradation of 1,3-D from soil with no history of 1,3-D application. However, this process is carried out by a consortium of micro-organisms that is very difficult to isolate. One possible reason for this may be that during the first year of 1,3-D treatment of agricultural soils, most of the biological degradation is a result of direct metabolism, co-metabolism or fortuitous metabolism.

Comparison of 1,3-D-degrading microbial communities by 16S rDNA DGGE

DGGE patterns of amended and unamended soil, with and without 1,3-D (Fig. 4a), were analysed to determine differences in banding patterns which may reflect differences in microbial communities adapted to the fumigant and also associated with degradation of this fumigant. Comparison of the bacterial communities showed the banding patterns to be very similar between 1,3-D-amended soils (lanes 4 and 5). However, the banding pattern from the control soil with 1,3-D (lane 3) was different from the amended soil. Differences in banding patterns were also observed in the amended soil without fumigant (lane 2) when compared with those with fumigants. This showed that some species in the fumigant-treated soil became more prevalent. Seven major bands were detected in the 1,3-D-treated soils (E1, E2, E3, E4, E5, E6 and E8), and these bands represented detectable strains of the microbial communities that had been formed during the six-month period of the experiment. The significance of this may be that the bacterial strains from these bands may degrade 1,3-D. Bands E1, E2, E3, E5, E6, E7, E8, E9 and E14 were dominant bands in the two 1,3-D-treated soils (Fig. 4a, and see Table 1 for names of different clones and their accession numbers). Bands E9 and E12 were unique to the umamended soil with fumigant, and these clones were mainly unidentified species.

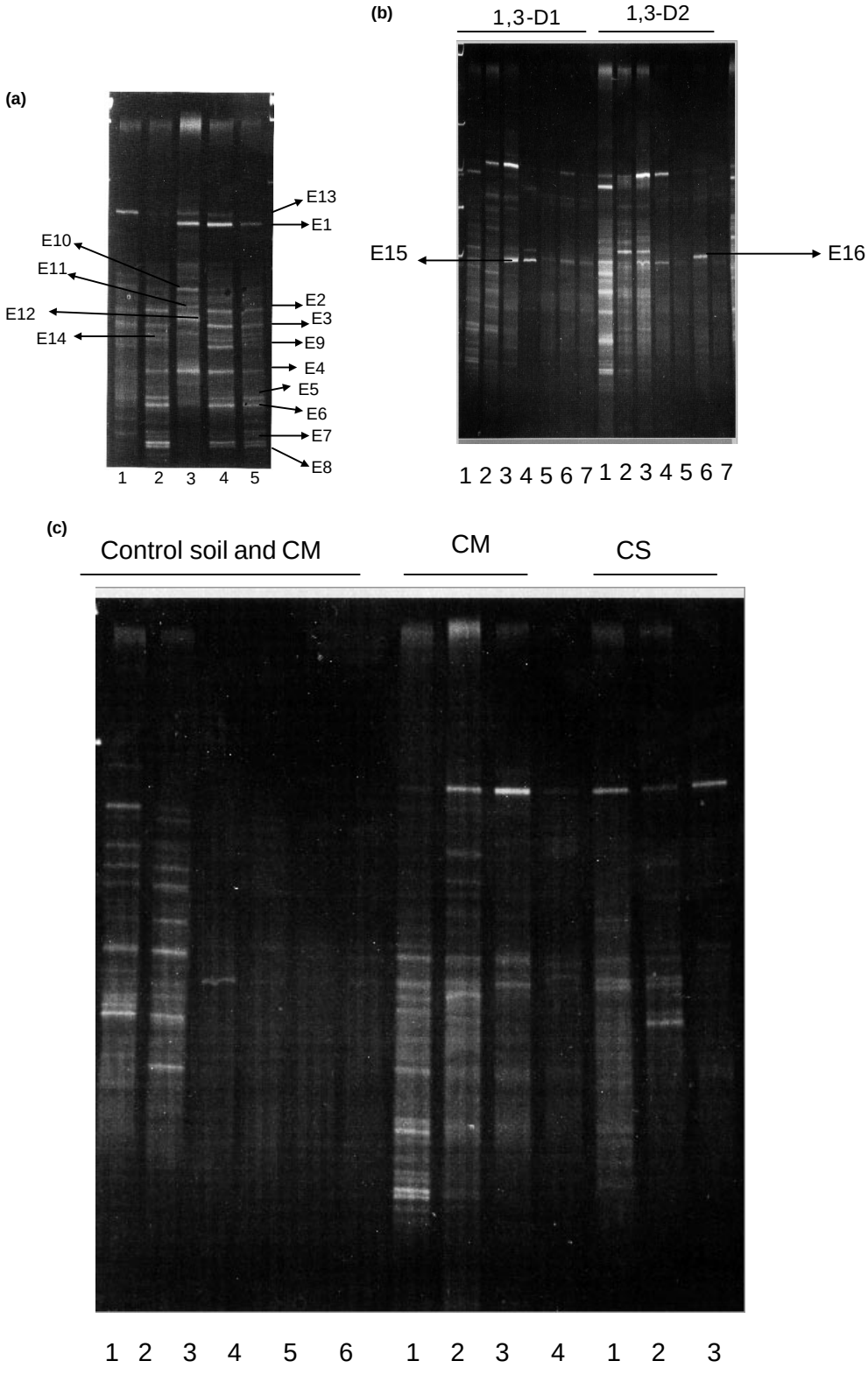
The technique of amplifying the 16S rDNA gene from the soil community avoids the bias imposed by selection but introduces other unknown factors. Eichner *et al.* (1999) showed that the number and intensity of bands do not equal the number and abundance of species within the microbial community, due to features of 16S rDNA-based phylogeny and bias inherent to PCR amplification from complex template mixtures. The reasons for most of the limitations are that DGGE banding patterns are subjected to PCR bias due to DNA extraction methods, potential preferential amplification and the formation of chimera (Wintzingerode *et al.* 1997). Other problems may result from one organism producing more than one DGGE band because of multiple, heterogeneous rRNA operons (Cilia *et al.* 1996; Nübel *et al.* 1996; Rainey *et al.* 1996). Also, for some phylogenetic groups of bacteria, 16S rDNA sequences do not allow discrimination between species, so one DGGE band may represent several species with identical rDNA sequences (Vallaey *et al.* 1997).

In the second analysis, 1,3-D-fumigated soils amended with compost manure and non-fumigated samples were serially-diluted in minimal media to 10^{-7}. DGGE analysis was done on these samples after DNA extraction. Compost manure only and control soils were also diluted to 10^{-4}; the soils were enriched with 1,3-D as the sole carbon and the same analysis was carried out. This was done to uncover species that may not be revealed by the banding pattern due to the complexity of the samples. The main bands of 16S rDNA amplified from compost-amended 1,3-D-enriched soil were still visible after diluting 10^{-3}. Two new bands appeared at 10^{-3} and were excised as clone E15 and E16 (Fig. 4b). Band E16 re-appeared at higher dilutions (10^{-6}), which indicates that these sequences were less efficiently amplified in the highly complex soil samples. No new bands were recovered from compost manure and the control soils at higher dilutions (Fig. 4c). Greater numbers of dominant bands were present in the compost sample than in the control soil. The uncovering of new banding patterns is typical of what happens in microbial communities when equally-abundant species are diluted and the new communities are recovered in less complex communities. Serial dilution was used here to reveal less-abundant species for a proper understanding of the complexity of the enriched communities.

Banding patterns in the amended soil without 1,3-D (Fig. 4c), and after serial dilution as revealed by PCR-DGGE of 16S rDNA of the major microbial communities, showed that this soil was different from the non-amended and compost soils. This clearly shows that the introduction of an organic amendment into agricultural soils adds not only nutrients and other components to the soil that may enhance the structure of the soil, but also new microbial populations. In areas where fumigants are applied, the addition of an organic amendment with the application of fumigants may increase the total quality of that soil (Hoitink and Fahy 1986). Hoitink and Fahy showed that when organic wastes are composted, successional changes in microbial populations and community structure occurs. In the present experiment (Fig. 4a,c), soil without organic amendment (control) (lane 1) had 14 dominant bands, soil with organic amendment (lane 2) had 21 dominant bands, control soil with 1,3-D (lane 3) had 17 bands, and 1,3-D-amended soils (lane 4 and 5) had 19 and 18 bands, respectively. The presence of bands E2, E3, E5, E6, E7, E8 and E9 in 1,3-D-amended soil, but not in the control soil, suggested that more bacterial strains became adapted to 1,3-D. The results in this study suggest that some micro-organisms in soils treated with compost manure can adapt to 1,3-D and form microbial consortia that can degrade this compound.

Analysis of DGGE bands

To investigate the dominant organisms in the compost-amended fumigant-treated soils, fragments from 16 selected DGGE bands were sequenced and subjected to BLAST Gene Bank analysis as shown in Table 1. According to the BLAST description, all our clones were members of the gamma subdivision of Proteobacteria and Gram-positive


(a)
E13
E1
E10
E11
E12
E14
E2
E3
E9
E4
E5
E6
E7
E8
1 2 3 4 5
(b)
1,3-D1
1,3-D2
E15
E16
1 2 3 4 5 6 7 1 2 3 4 5 6 7
(c)
Control soil and CM
CM
CS
1 2 3 4 5 6 1 2 3 4 1 2 3

◀

Fig. 4 DGGE analysis of bacterial communities from compost-amended and control soils. Amplified products were separated on a gradient of 30–70% denaturant. All labelled bands were excised from the gel, re-amplified and subjected to sequence analysis. These re-amplification products were cloned, and the clones were screened as described in the text. (a) Community structures after six months of enrichment. Lane 1: Arlington sandy loam without organic amendment; lane 2: with organic amendment; lane 3: without organic amendment but 1,3-D; lanes 4 and 5: with organic amendment and 1,3-D. (b) Community structures for duplicate jars of 1,3-D after serial dilution with phosphate buffer from 10^{-1} to 10^{-7}. The numbers 1–7 indicate 10^{-1}–10^{-7}. (c) Community structures after serial dilution. No new bands were developed from these treatments. The banding patterns of organic-amended (CM) and control samples were different from each other, with the CM treatment with the control soil having more diverse banding patterns

bacteria, with a few clones classified as unidentified soil bacteria. Bands E3, E4 and E15 were Gram-positive bacteria found in 1,3-D-amended soil. The partial sequence from clone E4 found in this group showed it to be present in the four soils with 1,3-D and organic amendment. This suggests the dominance of *Actinomadura* species in compost-amended samples. This species was first isolated from tropical rain forest soils by Wang *et al*. (1999). Bands E8, E1, E14 and E16 were dominated by *Cytophaga* and *Pseudomonas* species. Clone E1 had a 100% similarity index with *Ps. reactans* and this clone was found in all the fumigant-treated soils. *Pseudomonas* sp. may be one of the most abundant bacterial species in soil, and there are many reports of the ability of this species degrade 1,3-D (Lebbink *et al.* 1989; Verhagen *et al*. 1995). Fifteen bacterial strains with the capacity to degrade 1,3-D (of which four were *Pseudomonas* sp.) were isolated from enrichment cultures grown from adapted soils (Verhagen *et al*. 1995). One of the strains, *Ps. cichorii* 170, was shown to degrade 1,3-D completely to 3-chloroallyl alcohol (Poelarends *et al*. 1998). These authors noted that genetic characterization of the genes involved in the complete metabolism of 1,3-D was identical to the *dhaA* of the Gram-positive bacterium *Rhodococcus rhodochrous* NCIMH13064 and the *dhiA* genes from *Rhodococcus* sp. strain m15–3 (Bosma *et al*. 1999). In the present study, clone E15 was identified as *R. ruber*, with 99% similarity to the 16S rDNA sequence of this bacterium. This clone was identified after serial dilution of our samples. *Mycobacterium* sp. (clones E3 and E5), *Saccharomonospora viridis* (clone E2) and *Nocardiopsis* sp. (clone E6) were identified as major bands in treatments with 1,3-D, suggesting that these bacteria may be involved in the degradation of 1,3-D.

Table 1 Micro-organisms identified from predominant 16S rDNA DGGE bands obtained from compost-amended and unamended 1,3-dichloropropene-fumigated soil

BLAST Gene Data Base match	Sequence similarity (%)	Gel position	Accession no.
Pseudomonas sp. P71410	100	E1	AF2141136
Saccharomonospora viridis	98	E2	Z38021
Mycobacterium sp. CH1	97	E3	X93033
Actinomudura sp.	91	E4	AF131331
Mycobacterium sp. CH1	100	E5	AF054278
Nocardiopsis sp. IM-3791	97	E6	AF131451
Pseudomonas balearica	99	E7	FO54936
Capnocytophaga cynodegmi	95	E8	X97245
Uncultured soil bacterium wr1128	95	E9	AJ233588
Actinomudura sp. IM-8475	97	E10	AF131332
Streptomyces sp.	99	E11	AF131518
Uncultured soil bacterium JP11-5	92	E12	AB028096
Uncultured soil bacterium DW-19	94	E13	AF128792
Pseudomonas sp. P71410	95	E14	AF214136
Rhodococcus ruber	99	E15	X80625
Pseudomonas sp. P71410	100	E16	AF214136

The importance of this information is that once a community is established, it is possible that it will continue to use 1,3-D as the sole carbon source for growth and other metabolic activities, resulting in the accelerated degradation of this compound. The major advantage of this approach is the ability to identify and classify fumigant-adapted or -degrading microbial populations without the requirement for isolation of pure culture. As it is well established that the isolation of degrading populations from soil can be a very long process, DGGE can be used as a quick method for screening soils for the major isolates. With this information, different culturing media can be used to select the strains that may be involved in degradation. From the present experiments, most of the strains in Table 1 that became dominant after 6 months of microcosm enrichment were of the closely-related *Pseudomonas* and *Actinomadura*. In most cases, classical enrichment tends to yield *Pseudomonas* strains and related Gram-negative bacteria because of their high growth rates. In this study, Gram-positive bacteria became the predominant bacterial populations after prolonged enrichment in the microcosm.

REFERENCES

Bosma, T., Kruizinga, E., de Bruin, E.J., Poelarends, G.P. and Janssen, B.D. (1999) Utilization of trihalogenated propanes by *Agrobacterium radiobacter* AD1 through heterologous expression of the haloalkane dehalogenase from *Rhodococcus* sp. Strain m15–3. *Applied and Environmental Microbiology* **65**, 4575–4581.

Cilia, V., Lafay, B. and Christen, R. (1996) Sequence heterogeneities among 16S ribosomal RNA sequences, and their effect on phylogenetic analyses at the species level. *Molecular Biology and Evolution* **13**, 451–461.

Eichner, C.A., Erb, R.W., Timmis, K.N. and Wagner-Döbler, I. (1999) Thermal gradient gel electrophoresis analysis of bioprotection from pollutant shocks in the activated sludge microbial community. *Applied and Environmental Microbiology* **65**, 102–109.

Gan, J., Yates, S.R., Crowley, D. and Becker, J.O. (1998a) Acceleration of 1-3-Dichloropropene degradation by organic amendments and potential application for emissions reduction. *Journal of Environmental Quality* **27**, 408–414.

Gan, J., Yates, S.R., Wang, D. and Ernst, F.F. (1998b) Effect of application methods on 1,3-Dichloropropene volatilization from soil under controlled conditions. *Journal of Environmental Quality* **27**, 432–438.

Hoitink, H.A.J. and Fahy, P.C. (1986) Basis for the control of soilborne plant pathogens with composts. *Annual Review of Phytopathology* **24**, 93–114.

Jackson, C.R., Roden, E.E. and Churchill, P.F. (1998) Changes in bacterial species composition in enrichment cultures with various dilutions of inoculum as monitored by Denaturing Gradient Gel Electrophoresis. *Applied and Environmental Microbiology* **64**, 5046–5048.

Janssen, D.B., Keuning, S. and Witholt, B. (1987) Involvement of a quinoprotein alcohol dehydrogenase and an NAD-dependent aldehyde dehydrogenase in 2-chloroethanol metabolism in *Xanthobacter autotrophicus* GJ10. *Journal of General Microbiology* **133**, 85–92.

Lebbink, G., Proper, B. and Nipshagen, A. (1989) Accelerated degradation of 1,3-dichloropropene. *Acta Horticulture* **225**, 361–371.

Muyzer, G. and Smalla, K. (1998) Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie van Leeuwenhoek Journal of Microbiology* **73**, 127–141.

Nübel, U., Engelen, B., Felske, A. et al. (1996) Sequence heterogeneties of genes encoding 16S rRNAs in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. *Journal of Bacteriology* **178**, 5636–5643.

Ou, L.-T. (1998) Enhanced degradation of the volatile fumigant-nematicides 1,3-D and methyl bromide in soil. *Journal of Nematology* **30**, 56–64.

Ou, L.-T., Chung, K.-Y., Thomas, J.E., Obreza, T.A. and Dickson, D.W. (1995) Degradation of 1,3-Dichloropropene (1,3-D) in soils with different histories of field applications of 1,3-D. *Journal of Nematology* **27**, 127–242.

Øvreas, L., Forney, L., Daae, F.L. and Torsvik, V. (1997) Distribution of bacterioplankton in meromictic lake Sælenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Applied and Environmental Microbiology* **63**, 3369–3373.

Poelarends, G.J., Marga, W., Larkin, M.J., van Elsas, J.D. and Janssen, D.B. (1998) Degradation of 1,3–Dichloropropene by *Pseudomonas cichorii* 170. *Applied and Environmental Microbiology* **64**, 2931–2936.

Rainey, F.A., Ward-Rainey, N.L., Janssen, P.H., Hippe, H. and Stackebrandt, E. (1996) *Clostridium paradoxum* DSM 7308T contains multiple 16S rRNA genes with heterogeneous intervening sequences. *Microbiology* **142**, 2087–2095.

Santegoeds, C.M., Nold, S.C. and Ward, D.M. (1996) Denaturing gradient gel electrophoresis used to monitor the enrichment culture of aerobic chemoorganotrophic bacteria from a hot spring cyanobacterial mat. *Applied and Environmental Microbiology* **62**, 3922–3928.

Smelt, J.H., Teunissen, W., Crum, S.J.H. and Leistra, M. (1989) Accelerated transformation of 1,3-Dichloropropene in loamy soils. *Netherlands Journal of of Agricultural Science* **37**, 173–183.

Vallaeys, T., Topp, E., Muyzer, G. et al. (1997) Evaluation of denaturing gradient gel electrophoresis in the detection of 16S rDNA sequence variation in rhizobia and methanotrophs. *FEMS Microbiology and Ecology* **24**, 279–285.

Van Dijk, H. (1974) Degradation of 1,3-dichloropropenes in the soil. *Agriculture-Ecosystems* **1**, 193–204.

Van Hylckama Vlieg, J.E.T. and Janssen, D.B. (1992) Bacterial degradation of 3-chloroacrylic acid and the characterization of cis- and trans-specific dehalogenases. *Biodegradation* **2**, 139–150.

Verhagen, C., Smit, E., Janssen, D.B. and van Elsas, J.D. (1995) Bacterial dichloropropene degradation in soil; screening of soils and involvement of plasmids carrying the dhlA gene. *Soil Biology and Biochemistry* **27**, 1547–1557.

Wang, D., Ernst, F.F. and Yates, S.R. (1999) Automated sequential sampler for collection of highly volatile atmospheric contaminants. *Journal of Environmental Quality* **28**, 345–349.

Wang, Y., Zhang, Z.S., Ruan, J.S., Wang, Y.M. and Ali, S.M. (1999) Investigation of actinomycete diversity in the tropical rainforests of Singapore. *Journal of Indian Microbiology and Biotechnology* **23**, 178–187.

Wintzingerode, F.V., Göbel, U.B. and Stackebrandt, E. (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiology Review* **21**, 213–229.