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Phenotypic diversity and antibiotic resistance in soil bacterial communities

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The community structure in two different agricultural soils has been investigated. Phenotypic diversity was assessed by applying BIOLOG-profiles on a total of 208 bacterial isolates. Diversity indices were calculated from cluster analysis of the BIOLOG data. The bacterial isolates were also evaluated for resistance towards six different antibiotics, mercury resistance and the presence of plasmids. The presence of tetracycline-resistant determinants class A to E among Gram-negative bacteria was analysed with DNA probes. The distribution of tetracycline resistance markers among colonies growing on non-selective and tetracycline-selective plates were compared. The phenotypic approach demonstrated some difference in the diversity within the two soils. The frequency of antibiotic resistance isolates was high in both soils, whereas the frequency of mercury resistance differed significantly. We found no correlation between plasmid profiles and antibiotic resistance patterns. We found all the tetracycline resistance determinants except class B, indicating that the diversity of the tetracycline resistance determinants was complex in populations of resident soil bacteria under no apparent selective pressure for the genes in question.

Keywords: agricultural soil; bacterial diversity; antimicrobial and mercury resistance; plasmids; tetracycline resistance determinants (class A to E)

Introduction

The occurrence of antibiotic-resistant and heavy metal-resistant bacteria in soil has been recognised as an important indicator of pollution. Environments influenced by anthropogenic activities and pollution are subjected to a vast amount of toxic xenobiotic compounds that challenge the integrity of the indigenous flora. Typically, populations develop that sustain microbial processes in the presence of the inhibitory toxicants [3,19]. A high incidence of resistant bacteria has been reported from soil environments [8], and several studies have focused on the association of plasmids with antibiotic and heavy metal resistance [13,25,31,32]. Bacterial resistance to tetracycline and to mercury compounds is the most widely found and the best understood plasmid-mediated resistance [9,15,18,26].

Heavy metal contamination has been shown to affect the species composition of microbial communities in soil [8]. Disturbances caused by heavy metals to microbial biomass and activity are known to be reflected in decreased litter decomposition and subsequently less efficient soil nutrient cycling [29]. Several studies have also demonstrated stress-induced changes in specific parts of soil microbial communities. Changes in microbial communities resulting from agricultural practices can have profound impacts on ecosystem dynamics. In ecological studies, analysis of biological communities may consider community structure eg species diversity and function. Therefore research is needed to increase our understanding of the diversity and function of soil microbial communities.

We have examined bacterial diversity together with the antibiotic and mercury resistance patterns in bacterial populations isolated from two different agrciultural soils. The aims of this study were four-fold: 1) to determine the phenotypic diversity within the culturable part of the indigenous bacterial community using the BIOLOG test system; 2) to assess antibiotic and mercury resistance patterns, and the plasmid profiles within the isolates; 3) to use tetracycline resistance genes as markers for comparing their distribution in the plate count population growing on non-selective media (R2A-plates) with the population of bacteria retrieved on tetracycline-selective R2A-plates; and 4) to assess the difference between the occurrence of tetracycline-resistant determinants in the bacterial isolates and DNA extracted directly from the soil.

Materials and methods

Soil sampling and characterisation

Two different agricultural soils, sampled south of Bergen, Norway were investigated. None of the chosen sites had any record of intentional exposure to mercury or antimicrobial agents. Samples were collected from a sandy soil and an organic soil on April 1, 1993. Five subsamples from each site were collected, mixed and sieved (mesh size 2 mm), and kept in black polyethylene bags at 4°C. Chemical and biological data from the soils used in this study are listed in Table 1.

Isolation of bacterial strains

A 10-g (wet weight) soil sample was homogenised at low speed (3 × 1 min) in a sterile Waring blender with 90 ml sterile-filtered, cold Winogradsky salt solution [21] diluted 1 : 20. One hundred microlitres of serial ten-fold dilutions were plated on R2A-medium (Difco, Detroit, MI, USA) and colony-forming units (CFU) were counted. The media

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Table 1 Chemical and biological characteristics of the two agricultural soils

Soil	pH	% Dry weight[a]	% Organic matter[b]	Total bacterial count[c]	Colony-forming units[c]
Sandy soil	5.5	73	10	2.1×10^{10}	1.9×10^{7}
Organic soil	6.1	43	53	1.8×10^{10}	1.8×10^{7}

[a]Determined as percent of wet weight.
[b]Determined as percent of dry weight.
[c]Determined per gram of wet weight.

included 5 mg ml^{-1} cycloheximide to inhibit growth of eukaryotes. The plates were incubated at 15°C, and colonies were enumerated every day until maximal plate counts were obtained. Then 140 colonies (from each of the two soil samples) from the 10^{-4} dilution were picked randomly, and subcultured on the same medium three times. Some cultures which still seemed to be impure, were discarded. One hundred and four isolates from each of the soils were stored in R2A-medium containing 15% glycerol at –80°C. The soil isolates were Gram stained. Plating studies were also used to obtain and quantify tetracycline-resistant bacteria. Samples were plated on R2A media with 5 μg ml^{-1} or 25 μg ml^{-1} tetracycline, and screened for total viable populations and tetracycline-resistant bacteria.

Phenotypic characterisation

The 208 bacterial isolates were characterised by using the GN MicroPlate (BIOLOG Inc, Hayward, CA, USA) system for growth on 95 different carbon sources. Modifications of the prescribed protocol for preparing bacterial cells for BIOLOG analysis were performed. The manufacturer recommends incubation at 30°C for environmental isolates, but we chose a lower temperature. The colonies were isolated and purified at 15°C, but since all of the isolates grew at 20°C, we used this incubation temperature throughout the experiment. The plates were incubated at 48 h, and cells were transferred with a sterile, cotton tipped swab to 20 ml of 0.85% NaCl. This suspension was used to inoculate BIOLOG GN plates as described by the manufacturer. The data were converted to binary data, and cluster analysis was performed on scores from 95 tests for all 208 isolates. The resistance pattern for the isolates was not included in the cluster analysis. A dendrogram was constructed from the distance matrix using unweighted average linkage (UPGMA). Cluster analysis of data and generation of dendrograms for BIOLOG were performed using software developed by Svein Norland (Department of Microbiology, University of Bergen, Norway). Proximity was measured by the simple matching coefficient, and clusters were grouped by the complete link (farthest neighbour) method. The Shannon index, H′ [23] was calculated (log 2) on the basis of biotypes defined in the cluster analysis. The equitability J [20], was also calculated.

Antibiotic resistance

The isolates were tested for susceptibility to different drugs by the disc diffusion test, as recommended by the disc manufacturer (Oxoid, Basingstoke, Hants, UK) with some modifications. A thin lawn of bacteria in R2A-medium was spread on R2A plates, and discs with one of the following drugs were placed on the lawn: ampicillin (AMP, 10 μg), streptomycin (S, 25 μg), kanamycin (K, 30 μg), Chloramphenicol (C, 30 μg), sulphametoxasol (RL, 25 μg) and tetracycline (TE, 30 μg). The bacteria were incubated at 20°C. Inhibition zones were measured after 2 days incubation, as the diameter of the zones without visible bacterial growth including the disc diameter (6 mm). Bacteria were considered resistant to the particular antibiotic agents when the diameter of the inhibition zone on R2A-medium was $\leq$ 12 mm. As a verification to the activity of the antibacterial agents effect on R2A-medium, *Escherichia coli* K12 were included as a sensitive control.

Mercury resistance

The assay for mercury resistance was performed by growing isolates in R2A-liquid media in 5×5 well plates. The inoculum was taken directly from frozen cultures (–80°C) and grown for 11 h. Droplets from the fresh cultures were then replicated to R2A plates for control and LB plates [22] containing 25 μg Hg ml^{-1} (as $HgCl_2$).

Plasmid screening

The isolates were screened for plasmids by using the method of Kado and Liu [12] with the following modifications: cultures were grown on R2A plates for 2 days at 20°C; cells on a 1-cm^2 area of the plate were collected from the agar surface; the cells were suspended in 100 μl TE 10 : 1 buffer (10 mM Tris, 1 mM EDTA, pH adjusted to 8.0) in an Eppendorf tube, 300 μl lysis solution (0.07 M Na_3PO_4, 0.03 M Na_2HPO_4, 2% sodium dodecyl sulphate [SDS]) was added, and the suspension was incubated for 15 min at 55°C. The lysate was deproteinised with an equal volume of phenol : chloroform (1 : 1). The phases were mixed by inverting the tubes and separated by centrifugation at 12 000 $\times$ *g* (Hettich microliter centrifuge) at room temperature for 5 min. The upper phase was transferred to a new tube, and deproteinised once more with 200 μl chloroform : isoamylalcohol (24 : 1). The water phase was transferred to a fresh tube, and de-aerated in a vacuum centrifuge (Speed Vac Concentrator, model SVC-100H). DNA samples were mixed with gel loading buffer (0.25% bromcresole purple in 50% glycerol, 0.05 M Tris-acetate, pH 7.9) and electrophoresed on a 0.7% agarose gel in $1 \times$ TAE buffer (40 mM Tris-acetate, 2 mM Na_2-EDTA, pH 7.9) at 120 V for 3 h. After electrophoresis the gel was stained with ethidium bromide (1 μg ml^{-1}).

Preparation and labelling of DNA probes

The plasmids carrying the five tetracycline probe fragments classes A to E are listed in Table 2. Plasmids were prepared using the Qiagen kit for midi-plasmid preparations (Qiagen Inc, Chatworth, GA, USA). Restriction enzymes were used as directed by the manufacturer's instruction (Boehringer GmbH, Mannheim, Germany). The purified restriction DNA fragments used as probes were labelled with digoxygenine (DIG) *in vitro* using a non-radioactive random-primed DNA labelling kit (Boehringer GmbH) as described by the manufacturer.

Table 2 DNA probes constructed and used in hybridisation experiments

tet-Resistance determinants	*E. coli* strain	Restriction fragment used as DNA probe	Reference
TetA	JM83	750-bp *Sma*I fragment of pSL18	[16]
TetB	HB101	1275 *Hin*cII fragment of pRT29	[7]
TetC	DO-7	1400-bp *Ava*I-*Hin*dIII fragment of pBR322	[2]
TetD	C600	3050-bp *Hin*dIII-*Pst*I fragment of pSL 106	[16]
TetE	HB101	2500-bp *Cla*I-*Pvu*I fragment of pSL1504	[16]

Isolation of total DNA

DNA was isolated as described by Holben [11] using a direct lysis protocol. The bacteria were lysed *in situ* by adding SDS and then homogenised by using bead beating with subsequent precipitation and purified by CsCl-ethidium bromide centrifugation.

Characterisation of tetracycline-resistance determinants by hybridisation

Plasmids were isolated using the modified method of Kado and Liu [12]. They were transferred from the agarose gels to nylon membranes by Southern blotting [24]. Colony and Southern blot experiments were performed with positively charged nylon membranes (Hybond N^+; Amersham Corp) according to the supplier's instructions. Hybridisation and detection were performed with the (DIG)-dUTP-labelled probes and Detection Kit (Non-Radioactive) according to the manufacturer's instructions (Boehringer Mannheim).

Results

Bacterial isolates

BIOLOG: Substrate utilisation profiles from the BIOLOG data for the two different soil communities showed that bacterial diversity in the organic soil was higher than in the sandy soil. Analysis of the data revealed 13 biotypes at 80% similarity in the sandy soil while the organic soil showed 17 biotypes (Table 3). The difference in diversity as given by diversity indices of the isolated populations is shown in Table 3. The highest diversity was found in the organic soil with a H′ of 2.94 whereas the sandy soil gave a H′ of 2.49. In both of the soils it seems to be a rather low equitability (J), indicating an uneven distribution of biotypes in the populations of isolated bacteria.

Table 3 Diversity of agricultural soil bacteria. Substrate diversity was determined by BIOLOG. Number of biotypes, Shannon index (H′; logarithmic base = 2), Equitability ($J = H'/H'_{max}$)

Soil	Biotypes	H′	J
Sandy soil	13	2.49	0.69
Organic soil	17	2.94	0.72

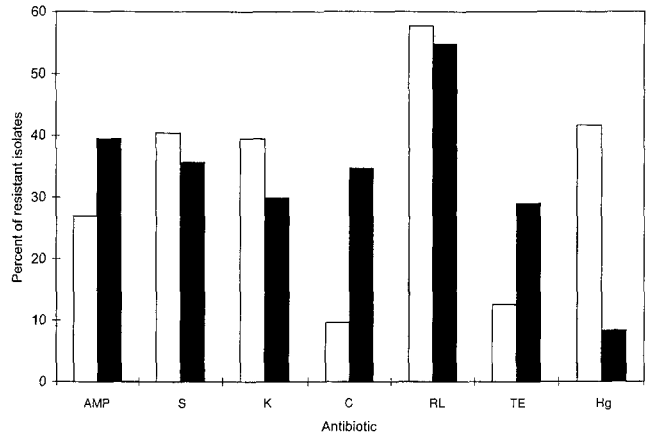

Figure 1 Distribution of antibiotic and mercury resistance of bacterial isolates from two different agricultural soils. Sandy soil (□), organic soil (■). AMP ampicillin; S streptomycin; K kanamycin; C chloramphenicol; RL sulphametoxasol; TE tetracycline; Hg mercury.

Antibiotic and mercury resistance: The frequency of antibiotic-resistant isolates was high in both populations: 72% in the organic soil and 74% in the sandy soil. The highest frequencies of resistance were found against sulfametoxasol. This was seen in both soils. In the organic soil the frequency of resistance against chloramphenicol and tetracycline was higher than in the sandy soil (Figure 1). In the sandy soil the frequency of multiply-resistant isolates (defined as resistant to three or more antibiotics) was slightly higher (39%) than in the organic soil (34%); 28% of the organic soil isolates and only 6% of the sandy soil isolates were resistant to all the six antibiotics tested (Figure 2). Eight percent of the organic soil isolates and 42% of the sandy soil isolates were resistant to mercury (Figure 1). The percentages of mercury-resistant isolates which were also resistant to antibiotics were 75% and 70% from organic and sandy soil, respectively. In the sandy soil the frequency of mercury-resistant isolates was high, and they had an even distribution in proportion to the numbers

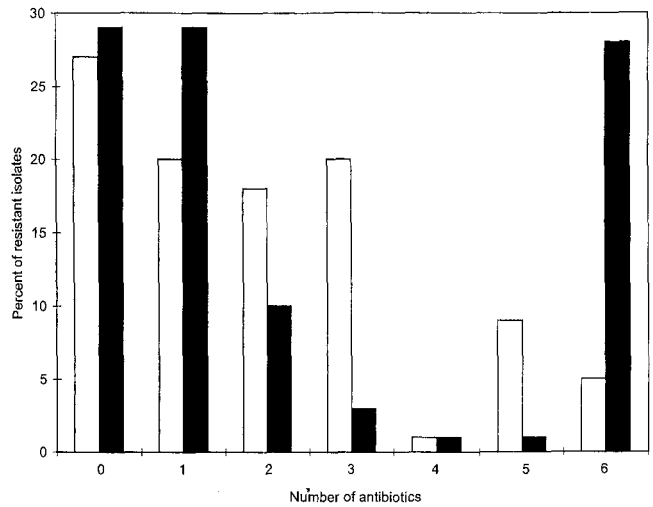

Figure 2 Distribution of soil bacterial isolates from two agricultural soils regarding the number of antibiotics they are resistant to. Sandy soil (□), organic soil (■).

Table 4 Frequency of mercury-resistant isolates correlated to number of antibiotics they resist

Number of antibiotics	Percent distribution of Hg-resistant isolates in	
	Sandy soil	Organic soil
0	30	25
1	14	25
2	17	0
3	10	25
4	3	0
5	19	0
6	7	25

of antibiotics they resist. In the organic soil, mercury resistance was found among those isolates which were resistant to one, three and six antibiotics (Table 4).

Gram reaction: The frequency of Gram-positive bacteria was 9% in the organic soil and 16% in the sandy soil. None of the Gram-positive bacteria expressed phenotypic resistance towards tetracycline. Among the Gram-negative bacteria the frequency of tetracycline-resistant isolates was 30% in the organic soil and 12% in the sandy soil. Tetracycline-resistant isolates from both soils were distributed into different groups belonging to the Proteobacteria (alpha-, beta-, gamma- and delta group) and to Cytophaga/Flexibacter/Bacteroides group (unpublished results).

Plasmid DNA isolation: With the plasmid screening methods used, 20% of the organic soil isolates and 15% of the sandy soil isolates harboured plasmids. The numbers of plasmids per isolate varied from one to four with one or two most frequently seen. All plasmids were of a molecular size larger than 20 MD, except for one isolate which harboured two plasmids that were less than 2 MD. We found a rather low correlation between antibiotic resistance and plasmid profiles. This was also true for mercury resistance and plasmid profiles.

Hybridisation analyses: Colony hybridisation experiments with the 208 bacterial isolates revealed that six of the isolates from organic soil and seven from the sandy soil hybridised with at least one of the class A to E probes (Table 5). Only two isolates from the organic soil and four isolates from the sandy soil expressed phenotypic resistance against tetracycline. The class A probe hybridised with two isolates from the organic soil, but none of the sandy soil isolates hybridised to this determinant alone. Three isolates from the sandy soil and one isolate from the organic soil contained both the class A and the class E determinant. In the sandy soil two isolates exhibited the class C determinant; one was resistant to tetracycline while the other was sensitive. One isolate hybridised to the class D probe, and this was also resistant to tetracycline. These two determinants were found among isolates from organic soil (Table 5).

Table 5 Distribution of tetracycline-resistance determinant classes in soil bacterial isolates from two agricultural soils

Class	Number of isolates from:			
	Organic soil		Sandy Soil	
	Sensitive	Resistant	Sensitive	Resistant
A	1	1	0	0
E	2	1	1	0
AE	1	0	1	2
C	0	0	1	1
D	0	0	0	1

Distribution of tetracycline resistance genes among colonies growing on unselective and selective media

A plating study was performed to see if there was any association between the distribution of tetracycline-resistance determinants among colonies growing on non-selective and selective media. Table 6 shows the frequency of tetracycline-resistant colony-forming units from the two soil samples. From the sandy soil colonies grew only on R2A-medium supplemented with 5 μg, but not with 25 μg TE ml^{-1}. The frequencies of colonies which hybridised with tetracycline-resistance determinants were higher in the sandy soil than in the organic soil. The distribution of tetracycline-resistance determinants from the plating study are presented in Table 7. In both soils the class A, C and E determinants were detected. The class D determinant was also present in colonies from the sandy soil sample. Twenty-one randomly picked colonies that hybridised with the probes from non-selective and selective R2A were subjected to plasmid screening. Only two of these harboured plasmids. One colony hybridised with the class D probe while the other hybridised with the class C probe. None of these resistance determinants were localised on plasmids as determined by Southern blot hybridisation.

Community DNA

Total DNA isolated after direct lysis of bacteria in soil, gave positive hybridisation signals with the class C and class E probes in both soils. With the class C probe the detection limits were 0.5 μg DNA in the organic soil and 1 μg DNA in the sandy soil. The class E probe hybridised with DNA from both soils at a detection limit of 2 μg DNA. None of the other probes hybridised to the total DNA extracted from the community.

Table 6 Total number of CFU on R2A plates supplemented with different concentrations of tetracycline (TE), and the frequency of tetracycline-resistant bacteria in the soil samples

Soil	CFU per gram soil on medium containing:			
	No TE	5 μg ml^{-1} TE	25 μg ml^{-1} TE	% Resistance
Sandy soil	1.1×10^6	4.5×10^4	ND[a]	4%
Organic soil	1.5×10^6	1.9×10^4	1.5×10^4	1%

[a]ND, not detected.

Table 7 Distribution of tetracycline-resistant determinants in the two different soil samples, with different concentrations of TC supplemented to the medium. From the sandy soil no colonies grew on the medium with 25 μg ml^{-1}

Soil[a]	TC (μg ml^{-1})	No. of isolates	% Of isolates carrying the tetracycline-resistance determinant:					
			tetA	tetB	tetC	tetD	tetE	other
SS	0	105	2	0	3	1	4	–
SS	5	45	9	0	2	2	11	76
OS	0	150	5	0	1	0	0	–
OS	5	19	21	0	74	0	5	0
OS	25	15	13	0	13	0	7	67

[a]SS, sandy soil; OS, organic soil.

Discussion

This study demonstrates that bacteria encoding high levels of resistance to mercury and antibacterial agents can be isolated from regular agricultural soils which have not been intentionally exposed to such agents. This may be important when we take into account that this soil has not been extensively polluted. For the last decade the organic soil was not used as a crop field, but only as grass land. The sandy soil has been repeatedly used for cultivation of cereals, vegetables and potatoes. The bacterial counts were similar to those found in pristine forest soils [28,33]

A possible explanation for the high frequencies of antibiotic resistance in both soils could be that antimicrobial agents are produced by microbes within the soil, which exert selective pressure among the soil microbial community. Some colonies displayed zones of inhibition preventing the growth of neighbouring bacteria. These colonies may represent naturally occurring antibiotic-producing bacteria. The high frequency of drug and mercury resistance could be explained by genetic linkage on plasmids. However, we detected no plasmids in many of the multiply-resistant isolates. Plasmids may be present, but we were not able to detect them with our protocol. Only one method of plasmid isolation was attempted. Therefore, the frequency of plasmid-containing bacteria can be underestimated, especially considering the difficulties in extracting mega plasmids from native soil bacteria. Another explanation could be that some of the resistance traits are linked to the chromosome. Antibiotic-producing bacteria have chromosomal resistance genes to protect themselves. Bacteria which are only occasionally exposed to antibiotics tend to harbour the resistance genes on plasmids. The majority of plasmids from both our sites were large enough to carry genes for conjugal transfer, suggesting the possibility of such transfer in this environment. A low correlation between the presence of plasmids and antibiotic resistance has been reported [4,5,10,35]. Perhaps the resistance determinants are located on transposons integrated on the chromosome. Some of the isolates did not express phenotypic resistance although they hybridised with the class A and class E probe. This can be explained by instability of the transposons due to gene rearrangements. Deletions have been observed within class E resistance determinants isolated from marine sediments [35]. Within 21 sediment isolates, nine had deletions within the *tet*-determinant, although they still hybridised with the class E determinant probe [34]. A number of tetracycline determinants have been isolated from Gram-positive sources (eg class K, L and M) and class M has been reported as the most widely distributed determinant among bacteria. In our study only a minor fraction of the isolated bacteria were Gram-positive, and none of these expressed phenotypic resistance.

We were not able to identify the isolates by correlating the BIOLOG data to the database. However the distribution of tetracycline-resistant isolates was spread among several different biotypes. In the organic soil, the majority of the teracycline-resistant isolates were in the Cytophaga/Flexibacter/Bacteroides group, and also among the alpha-, beta- and delta Proteobacteria. In the sandy soil the major part of the resistant isolates was found in the alpha Proteobacteria, but also among the gamma-, and delta Proteobacteria and the Cytophaga/Flexibacter/Bacteroides group.

Several studies document the occurrence of metal-resistant bacteria in both pristine and polluted environments [5,8,10,13,25]. A study performed by Summers *et al* [27], led to the conclusion that enrichment of Hg-resistant strains can result in simultaneous enrichment for genetically linked antibiotic-resistance genes. This is in agreement with our findings. Seventy-five per cent of the organic soil isolates and 70% of the sandy soil isolates that were resistant to mercury were also resistant to one or more antibiotics. However, in the isolates with concomitant resistance to mercury and tetracycline, the tetracycline-resistance genes could not be localised on plasmids.

Soil bacteria may be a major reservoir from which genes encoding resistance to antimicrobial agents can be spread to opportunistic pathogens of man and domestic animals. Therefore an important question is whether the environmental isolates carry resistance determinants related to the best known resistance mechanisms found in clinical isolates. Several investigations of the composition of tetracycline-resistant determinants among Gram-negative bacteria have been performed [1,2,14,16,17,30,35]. The distribution of the class A to E determinants among the bacterial isolates from the two soils showed some differences. In isolates from the organic soil, class A, E, and class AE determinants were found. Isolates from the sandy soil on the other hand showed a more even distribution and higher diversity containing class C and D determinants in addition to class E and class AE. In our study class B was the only tetracycline-resistance determinant tested for that was not detected in any of the soils. Some of the tetracycline-resist-

ant isolates from both of the soils did not hybridise to any of the probes, and may represent other types of tetracycline-resistance determinants.

In the plating study we found only resistance to low concentrations of tetracycline in the sandy soil. All the determinants except class B were relatively equally distributed both in the total plate count population (ie without added tetracycline) and in the population of resistant bacteria. In the organic soil most of the resistant bacteria were resistant to high concentrations (25 μg ml^{-1}) of tetracycline. One important finding was that the majority of the high-level resistant colonies harboured tetracycline-resistance determinants other than class A to E (Table 6). At low tetracycline concentrations (5 μg ml^{-1}) class A, C, and E were the only resistance determinants present, and the class C determinant dominated. Colonies from organisms in sandy soil showed only resistance to 5 μg ml^{-1} tetracycline contrary to the low-level resistant colonies from organic soil, the majority did not harbour any of the tetracycline determinants tested for.

Only bacteria isolated on plates were examined for antibiotic and mercury resistance and plasmid content, and they represent only a small proportion of the active population present in soil [33]. The numbers of CFU able to grow on R2A-agar represent less than 0.1% of the total counts. The distribution of specific tetracycline-resistance determinants in the isolates was therefore compared with that of determinants present in total DNA extracted directly from the soil. The dominance of class C in organic soil was confirmed by hybridisation with total DNA from the soil. The class C probe showed the lowest detection limit with DNA from organic soil, whereas the limit was twice as high with DNA from the sandy soil, indicating less dominance. The relatively equal frequencies of the class E determinant in the two soils was also confirmed. In contrast to the soil bacterial isolates, class A and class D were not detected in the total DNA, probably because the fraction of bacteria carrying these determinants in the total population was below the detection limit for the method. In the isolates the class A determinant was found more frequently than class E in organic soil and more frequently than class C in sandy soil. This indicates a particularly strong selection for bacteria with a class A determinant on agar plates compared to those with class C and class E.

The organic content was five times higher in the organic soil than in the sandy soil, and we found a higher phenotypic diversity in the bacterial isolates from organic soil than from sandy soil. Analysis of the isolates indicated that organisms from the two soils had approximately the same overall antibiotic resistance level. On the other hand the frequency of resistance to mercury was five times higher in isolates from sandy soil than from organic soil. This suggests that there might have been a greater selective pressure on the sandy soil community compared to the organic soil community, probably because the sandy soil has been used as a crop field. The diversity indices also show less diversity in the sandy soil. This is in agreement with previous reports that externally induced perturbation of the community decreases the diversity [3,29].

In conclusion, our investigations demonstrate that high frequencies of bacteria resistant to mercury and antibacterial agents were found in agricultural soils subjected to normal management. Our data show that resistance genes occur commonly in soil bacteria and indicate that a substantial number are probably chromosomally located. Both bacterial biomass and phenotypic diversity were somewhat higher in organic soil as compared to sandy soil. The equitability indicated an uneven distribution of the biotypes in both populations, sandy soil having a slightly higher dominant population than the organic soil. The diversity of tetracycline-resistance determinants however was highest in sandy soil. Thus, there need not be any relationship between the diversity in a specific trait like antibiotic resistance, and the overall phenotypic diversity in a bacterial population. This emphasises the importance of combining different approaches when studying diversity in natural microbial communities.

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References

1 Andersen SR and R-A Sandaa. 1994. Distribution of teracycline resistance determinants among gram-negative bacteria isolated from polluted and unpolluted marine sediments. Appl Environ Microbiol 60: 908–912.
2 Aoki T, T Satoh and T Kitao. 1987. New tetracycline resistance determinant on R plasmids from *Vibrio angillarum*. Antimicrob Agents Chemother 31: 1446–1449.
3 Barkay T. 1987. Adaptation of aquatic microbial communities to $Hg2^{+}$. Appl Environ Microbiol 31: 1446–1449.
4 Baya AM, PR Brayton, VL Brown, DJ Grimes, E Russek Cohen and RR Colwell. 1986. Coincident plasmids and antimicrobial resistance in marine bacteria isolated from polluted and un-polluted Atlantic ocean samples. Appl Environ Microbiol 51: 1285–1292.
5 Belliveau BH, ME Starodub and JT Trevors. 1991. Occurrence of antibiotic and metal resistance and plasmids in *Bacillus* strains isolated from marine sediment. Can J Microbiol 37: 513–520.
6 Reference deleted in proof.
7 Curiale MS and SB Levy. 1982. Two complementation groups mediate tetracycline resistance determined by Tn10. J Bacteriol 151: 209–215.
8 Doelman P. 1985. Resistance of soil microbial communities to heavy metals. In: Microbial Communities in Soil (Jensen V, A Kjøller and LH Sørensen, eds), pp 369–385, Elsevier Applied Science, London.
9 Foster TJ. 1983. Plasmid determined resistance to antimicrobial drugs and toxic metal ions in bacteria. Microbiol Rev 47: 361–409.
10 Hermansson M, GW Jones and S Kjelleberg. 1987. Frequency of antibiotic and heavy metal resistance, pigmentation, and plasmids in bacteria of the marine air-water interface. Appl Environ Microbiol 53: 2338–2342.
11 Holben WE 1994. Direct lysis protocol. In: Methods of Soil Analysis (Bigham JM, ed), pp 733–736, Soil Science Society of America, Inc.
12 Kado CI and S-T Liu. 1981. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol 145: 1365–1373.
13 Kelly WJ and DC Reanney. 1984. Mercury resistance among soil bacteria: ecology and transferability of genes encoding resistance. Soil Biol Biochem 16: 1–8.
14 Lee C, BE Langlois and KA Dawson. 1993. Detection of tetracycline resistance determinants in pig isolates from three herds with different histories of antimicrobial agent exposure. Appl Environ Microbiol 59: 1467–1472.
15 Levy SB. 1984. Resistance to the tetracyclines. In: Antimicrobial Drug Resistance (Bryan LE, ed), pp 91–240, Academic Press, New York.
16 Marshall B, C Tachibana and SB Levy. 1983. Frequency of tetracycline resistance determinant classes among lactose-fermenting coliforms. Antimicrob Agents Chemother 24: 835–840.
17 Marshall B, S Morriddey, P Flynn and SB Levy. 1986. A new tetracyc-

line resistance determinant, class E, isolated from Enterobacteriaceae. Gene 50: 111–117.
18 Mendez B, C Tachibana and SB Levy. 1980. Heterogeneity of tetracycline resistance determinants. Plasmid 3: 99–108.
19 Pennanen T, A Frostgård, H Fritze and E Brååth. 1996. Phospholipid fatty acid composition and heavy metal tolerance of soil microbial communities along two heavy metal-polluted gradients in coniferous forest. Appl Environ Microbiol 62: 420–428.
20 Pielou EC. 1966. The measurement of diversity in different types of biological collections. J Theoret Biol 13: 131–144.
21 Pochon J. Manuel Technique d'Analyse Microbiologique du Sol. Masson et Cie, Paris.
22 Sambrook JE, F Fritch and T Maniatis. 1989. Molecular Cloning. Cold Spring Harbor Laboratory Press, New York.
23 Shannon CE. 1948. A mathematical theory of communication. Bell Syst Technol 27: 379–423.
24 Southern EM. 1975. Detection of sequences among DNA separated by gel electrophoresis. J Mol Biol 98: 503–517.
25 Stoppel RD and HG Schlegel. 1995. Nickel-resistant bacteria from anthropogenically nickel-polluted and naturally nickel-percolated ecosystems. Appl Environ Microbiol 61: 2276–2285.
26 Summers A and T Barkay. 1989. Metal resistance genes in the environment. In: Gene Transfer in the Environment (Levy B and RV Miller, eds), pp 287–308, McGraw-Hill Publishing, USA.
27 Summers AO, J Wireman, MJ Vimy, FL Lorscheider, B Marshall, S Levy, S Benett and L Billard. 1993. Mercury released from dental 'silver' fillings provokes an increase in mercury- and antibiotic-resistant bacteria in oral and intestinal floras of primates. Antimicrob Agents Chemother 37: 825–834.
28 Sørheim R, VL Torsvik and J Goksøyr. 1989. Phenotypic divergences between populations of soil bacteria isolated on different media. Antimicrob Agents Chemother 36: 611–615.
29 Sørheim R. 1993. Approaches to the study of phenotypical and genotypical diversity in mixed populations of bacteria. Dr scient Thesis, University of Bergen, Norway.
30 Sørum H, MC Roberts and JH Crosa. 1992. Identification and cloning of a tetracycline resistance gene from the fish pathogen *Vibrio salmonicida*. Antimicrob Agents Chemother 36: 611–615.
31 Top E, I De Smel, W Verstraete, R. Dijkmans and M Mergeay. 1994. Exogenous isolation of mobilizing plasmids from polluted soils and sludges. Appl Environ Microbiol 60: 831–839.
32 Toranzo AE, P Combarro, ML Lemos and JL Barja. 1984. Plasmid coding for transferable drug resistance in bacteria isolated from cultured rainbow trout. Appl Environ Microbiol 48: 872–877.
33 Torsvik V, J Goksoyr and FL Daae. 1990. High diversity in DNA of soil bacteria. Appl Environ Microbiol 56: 782–787.
34 Wulff S. 1994. Comparison of *tet*-E plasmids isolated from sediment bacteria. Cand scient Thesis, University of Bergen, Norway.
35 Øvreås L. 1991. Characterization of plasmids from a marine fish farm sediment. Cand scient Thesis, University of Bergen, Norway.