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Total bacterial diversity in soil and sediment communities—a review

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Advances in microbial methods have demonstrated that microorganisms globally are the dominating organisms both concerning biomass and diversity. Their functional and genetic potential may exceed that of higher organisms. Studies of bacterial diversity have been hampered by their dependence on phenotypic characterization of bacterial isolates. Molecular techniques have provided the tools for analyzing the entire bacterial community including those which we are not able to grow in the laboratory. Reassociation analysis of DNA isolated directly from the bacteria in pristine soil and marine sediment samples revealed that such environments contained in the order of 10000 bacterial types. The diversity of the total bacterial community was approximately 170 times higher than the diversity of the collection of bacterial isolates from the same soil. The culturing conditions therefore select for a small and probably skewed fraction of the organisms present in the environment. Environmental stress and agricultural management reduce the bacterial diversity. With the reassociation technique it was demonstrated that in heavily polluted fish farm sediments the diversity was reduced by a factor of 200 as compared to pristine sediments. Here we discuss some molecular mechanisms and environmental factors controlling the bacterial diversity in soil and sediments.

Keywords: bacterial communities; diversity; soil; sediment; perturbation

Introduction

About two decades ago methods were developed for direct enumeration of bacteria in environmental samples using fluorescence microscopy. It was then recognized that one gram of soil could contain more than 10^{10} bacterial cells. This was an increase in bacterial count by a factor of 100–1000 compared to colony counts [13].

We became interested in the problem of the disagreement between colony and microscopic counts when studying bacterial communities in soil from Western Norway. A method based on fractionated centrifugation, was developed for the separation of bacteria from soil [13]. When respiration in the bacterial fraction was measured, the activity level could only be explained by assuming that practically all the bacteria counted in the microscope were metabolically active. The amount of DNA in the bacterial fraction showed that the DNA content per microscopically counted cell was about the same as the DNA content per cell in stationary cultures of *E. coli* [45]. From these investigations it was concluded that all the bacteria counted in the fluorescence microscope belonged to the living bacterial community, and that culturing techniques included only 0.1–1% of the active bacteria in our samples.

In the microscope the soil bacteria look like a fairly uniform group, comprising a few dominating morphotypes, most of them being coccoid or rod-shaped, quite similar to those growing on the agar plates. The question then arose whether the population of colony-forming bacteria were representative for the soil bacterial community, or if they were a selected fraction of the community. Another question was how many bacterial types there were in the soil. Were there a few dominating as indicated by the number of morphotypes, or did each morphotype comprise a high diversity? The answer to these questions could be obtained by analyzing the DNA isolated from the soil bacteria [46]. This DNA contains the genetic information from nearly all the bacteria in the soil, in amounts corresponding to their relative abundance. The problem was to find methods for analyzing and interpreting the information contained in the nucleic acids.

Microbial diversity has become an important issue due to the importance of microorganisms in energy and matter transformation. Knowledge about bacterial community structure and diversity is essential to understand the relationship between environmental factors and ecosystem functions. Such knowledge can be used to assess the effect on ecosystems of environmental stress and perturbations like pollution, agricultural exploitation and global changes. It is also realized that the functional and genetic potential of the microorganisms may exceed that of higher organisms, and provide a valuable source for novel products and technologies. Despite their importance, at the present we do not even know the magnitude of the bacterial diversity in most ecosystems.

The measure of bacterial diversity describes the qualitative variation among these organisms in a community or an assemblage. Diversity can also be regarded as the amount of information or 'species' richness in a community [5]. More commonly the diversity concept in addition to the richness component also includes an evenness component, which take into account how the information is distributed among the individuals in a community. In microbial ecology, diversity can be determined using phenotypic or geno-

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typic approaches, and it can be expressed as species- or group-diversity, physiological diversity, or genetic diversity.

Phenotypic diversity

Traditionally, biodiversity is based upon the species as a unit. Species diversity can be expressed as species richness, which is an index (single number) expressing the ratio between the number of species and the number of individuals in a collection [32]. Many diversity indices, like the Shannon index [38], take into account both species richness and species abundance.

A problem when applying species indices as measures of bacterial diversity is that the species concept for bacteria is obscure, and no universally accepted species definition exists for them [31, 41]. Another problem is that environmental bacterial isolates are often difficult to assign to taxa. The species problem can be circumvented by replacing classical identification with numerical taxonomy, and clustering the isolates into phenotypes or biotypes. The biotypes do not have any phylogenetic significance, but are operational units that can be used to characterize and compare populations and communities.

To avoid the species problem, alternative diversity measurements have been proposed. Several authors have applied physiological and functional diversity indices that express the distribution of physiological traits in populations or communities. Examples of such indices are functional evenness [50], substrate versatility [28], and tolerance towards adverse conditions [20]. Although physiological testing can only explore a small part of the total information found in the DNA, it may be useful in some investigations. If the tests are related to important ecosystem processes [11], the information they confer should be relevant to key functional groups in the community.

Functional diversity can also be expressed as the numbers of different functional or ecological groups (eg numbers of guilds or ecotypes). Investigation of biochemical and physiological traits of bacteria growing in the laboratory is used to assess the functional diversity in a community, but the measurements provide little information about the species composition and variation within the functional groups. Information about the factors contributing to the diversity can be obtained by using multivariate statistical analysis [36, 43]. Methods such as principal component analysis and correspondence analysis [34] have been used to describe the most predominant characteristics of bacterial communities and relate them to habitat characteristics.

Genetic diversity

A fundamental problem when using the traditional species- and physiological diversity approaches are their dependence on cultivation and phenotypic characterization of bacterial isolates. As we have demonstrated, most of the bacteria in soil do not grow under laboratory conditions, although they are metabolically active [13]. An additional problem is that soil bacteria often give a high percentage of negative scores when applying catabolic and biochemical test kits, due to low gene expression under the conditions used. This implies that only a minor part of the genetic information in a population or a community is explored when applying traditional methods for diversity measurements. To circumvent the culturing problem there has been a shift from the analysis of isolates to total community analysis. For such analysis molecular techniques and especially nucleic acid analysis have been of great importance. While phenotypic diversity is related to the expression of genes under a given set of conditions, genotypic diversity measures the genetic potentials in microbial communities independent of the environmental conditions.

The development of new molecular methods has provided the tools for analyzing the information in DNA. By applying different methods, DNA analysis can be performed at different precision levels. Highest precision or resolution is obtained by applying 'nucleic acid sequencing', whereas PCR (polymerase chain reaction) fingerprinting techniques give somewhat lower resolution. Molecular fingerprinting of ribosomal sequences (ARDRA: amplified ribosomal DNA restriction analysis) [51] or of repetitive sequences (rep-PCR) [10, 26] are used to characterize bacterial isolates, thereby eliminating the problem of low gene expression met by phenotypic testing. These methods can also be used to assess the diversity of clone libraries of DNA extracted directly from environmental samples, thereby circumventing the culturing problem.

Sequencing of rRNA genes provides information about phylogenetic relationships between bacteria [33, 60]. Sequence analysis of rRNA libraries can give information about the presence of groups of bacteria. The resolution level is not high enough to define species, as strains which show significantly less than 70% DNA : DNA homology may have nearly identical 16S rRNA sequence similarity [41]. However, rRNA sequencing can differentiate at species, genus, and high taxon levels. At present such sequencing cannot provide information about the extent and abundance of species in natural communities.

The gross structure or taxon composition of bacterial communities can be determined by applying phylogenetic probes. Such probes can be designed towards taxon groups at different levels higher than species, and be used to map the members of communities either by direct *in situ* hybridization or by hybridization to DNA isolated from the community [2, 3, 17].

Denaturing or thermal gradient gel electrophoresis (DGGE/TGGE) [30] provides information about the variation in base composition (mole %guanine + cytosine: %G + C) of rRNA genes or functional genes. The genes are PCR-amplified and the products are separated due to their different melting points in denaturing gradient gels. These techniques can be used on community DNA and provide community profiles or fingerprints of specific genes. These methods can be used to assess the diversity within simple communities and within limited parts of complex communities, such as defined sets of genes or subsets of rRNAs. The methods do not however yield information about the total amount of information in DNA from microbial communities.

Determination of total genetic diversity

The information in DNA from complex communities can be analyzed by two techniques providing an expression of the overall diversity. These are the base composition (% G + C) of total DNA, and the measurement of reassociation rate of denatured (single-stranded) DNA. The reassociation rate is a measure of the total genetic complexity or heterogeneity in community DNA.

The base composition profiles show the distribution of DNA molecules according to their % G + C content, and reflect the community composition. It can be determined by thermal denaturation of DNA [27]. The analysis is normally carried out in 0.5 or 1× SSC (standard saline citrate) and the increase in absorbency with increasing temperature is measured spectrophotometrically. In order to obtain high resolution profiles the melting rate should be low, 0.2–0.5°C min^{-1}. A community profile can be obtained by calculating the first derivative of the melting curve.

Recently a technique has been developed [19, 21] whereby base composition profiles can be obtained by isopycnic centrifugation of bisbenzimide-DNA complexes in a CsCl gradient. Bisbenzimide binds preferentially to AT pairs and will decrease the buoyant density of DNA. Thus community DNA can be separated according to the base composition in a density gradient. The advantage of this method is that fractions of DNA with different base composition can be obtained. DNA in each fraction can then be further analyzed by high resolution techniques. The diversity of functional genes or rRNA genes in each fraction can be analyzed by molecular fingerprinting techniques, sequencing, or probing with gene probes.

As known from studies of pure cultures, similarity in mole % G + C cannot be used to assess the relationship between bacteria. Bacterial populations with a narrow range in base composition may still be diverse, but on the other hand will a broad and uniform base composition range indicate high diversity? Determination of the sequence complexity of DNA as measured by reassociation provides more precise assessment of the total diversity. Free living bacteria from soil and sediments have genome size within the same range of magnitude (ie $1.5–8 \times 10^6$ bp), and therefore approximately the same complexity. The complexity can be determined by measuring the rate of reassociation of single-stranded DNA under appropriate conditions. In solutions the renaturation of denatured homologous DNA follows a second order reaction kinetics, where the rate is proportional to the square of the concentrations of homologous DNA strands. The theory for reassociation kinetics was developed by Britten and Kohne [9] and used to study the genome sizes and organization in eukaryotic organisms. They calculated the fraction of reassociated DNA (Co-C/Co) and plotted that against Cot (Cot plot), where Co and C are the molar concentration of nucleotides in single-stranded DNA at the beginning of the reassociation, and at the time t, respectively. The reaction rate constant was expressed as $1/Cot_{1/2}$ where $t_{1/2}$ is the time in seconds for half completed reaction (50% reassociation). Under defined conditions (temperature and monovalent cation concentration), $Cot_{1/2}$ is proportional to the complexity (heterogeneity) of the DNA. $Cot_{1/2}$ is an ideal quantity which expresses the number of base pairs in non-homologous DNA, and is equivalent to the genome size of a single-copy genome [9, 57]. The $Cot_{1/2}$ values are determined relative to a reference DNA with known heterogeneity like *E. coli* DNA (genome size: 4.1×10^6 bp, 2.71×10^9 daltons) or calf thymus DNA.

Reassociation measurements can be applied to DNA from a single bacterium, from mixtures of bacterial isolates, and from bacterial communities [47, 48]. DNA from bacterial communities is a mixture of DNA from an unknown number of different bacteria. The reassociation does not follow an ideal second order reaction curve but has a somewhat flatter slope. The different DNA types are present in different proportions, and the degree of homology varies, therefore the curve is composed of many second order reaction curves with different reaction rates. In this case the $Cot_{1/2}$ does not have any precise kinetic meaning, but nevertheless it can provide information about how many different bacterial genomes the dominating part of the DNA originates from. If the DNA is derived from complex bacterial communities the concentration of homologous DNA fragments is low. Reassociation experiments with such complex DNA are very difficult to perform as they have to run for 1–2 weeks.

$Cot_{1/2}$ can be used as a diversity index, and like the Shannon index it includes both the amounts of information in the DNA and how this information is distributed (how much is found in few DNA types and how much is distributed among many types). Because it responds to both the species richness and evenness, this means that two communities with quite different structure can have identical $Cot_{1/2}$.

Measurement of bacterial genetic diversity by DNA reassociation requires very pure DNA free from eukaryotic DNA and impurities like humic acids, which may inhibit the reaction rate. The bacteria have to be separated from eukaryotic organisms and soil particles before the DNA extraction. This can be done by a fractionated centrifugation technique [13]. Extracellular DNA should be removed from the bacterial fraction by washing with pyrophosphate or hexametaphosphate prior to lysis of the bacteria. Ultrapure DNA with high molecular weight can be isolated from the soil bacterial fraction by enzymatic lysis and purification on hydroxyapatite column [46]. The recovery of bacterial DNA from the soil is normally 15–20%. This is lower than that obtained when DNA is isolated directly from the soil. It can be argued that the fractionation procedure is biased. DNA from the bacterial fraction however, has a very high heterogeneity, indicating that DNA from a large variety of different bacterial types in the soil is included.

The extent of bacterial diversity in pristine soil and sediments

The extent of bacterial diversity in pristine soil and sediments has been investigated using genetic approaches [48, 49]. Thermal denaturation and reassociation have been applied to analyze DNA extracted from the bacterial fractions of a beech forest soil and marine sediments. Thermal denaturation showed that the soil DNA had a less steep melting profile than sediment DNA. The average G + C content of the soil bacterial DNA was 57–59 mole % [48].

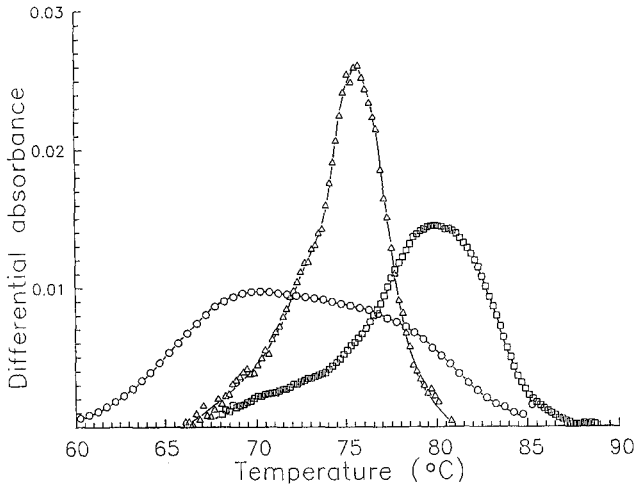

Figure 1 The melting profiles (1st derivative of the melting curves) for DNA from the bacterial fraction of pristine sediment (○), beech forest soil (□), and from *E. coli* (△).

In comparison the base composition of 200 randomly picked bacterial isolates from the same soil ranged from 57 to 63 mole %G + C [47]. The thermal profile of sediment bacterial DNA was very broad compared to soil bacterial DNA, with a plateau ranging over 10°C (Figure 1). This corresponds to a G + C range of 35–55%. The melting profile therefore indicates that the sediment bacterial population consists of several groups of bacteria with entirely different base composition, whereas the soil bacterial population was dominated by bacteria with a high mole % G + C. From these data one may conclude that the diversity of soil bacteria is lower than that of sediment bacteria.

The reassociation rates of the DNA from the pristine beech soil (Figure 2) and marine sediments were both

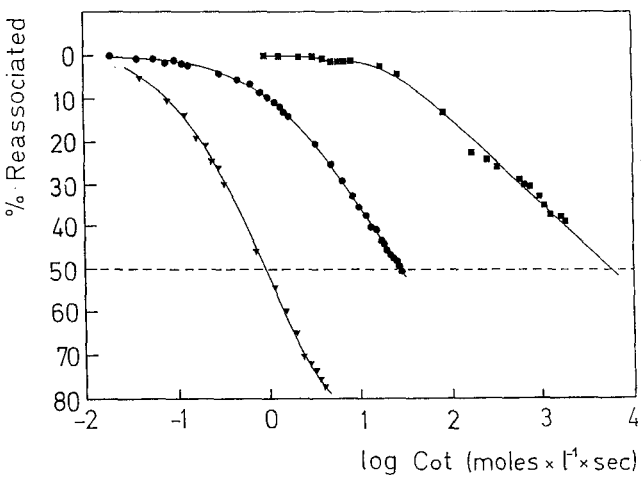

Figure 2 Reassociation (Cot plot) of DNA from the bacterial fraction of beech forest soil (■), 206 bacterial isolates from the same soil (●), and *E. coli* B (▼) in 4× SSC (standard saline citrate) and 30% DMSO (dimethylsulphoxide). The DNA was shared to about 420000 daltons and reassociated at 53°C. Abscissa: Log initial concentration of single-stranded DNA (Co in moles nucleotides L^{-1}) multiplied by time in seconds. Ordinate: Percent reassociated DNA.

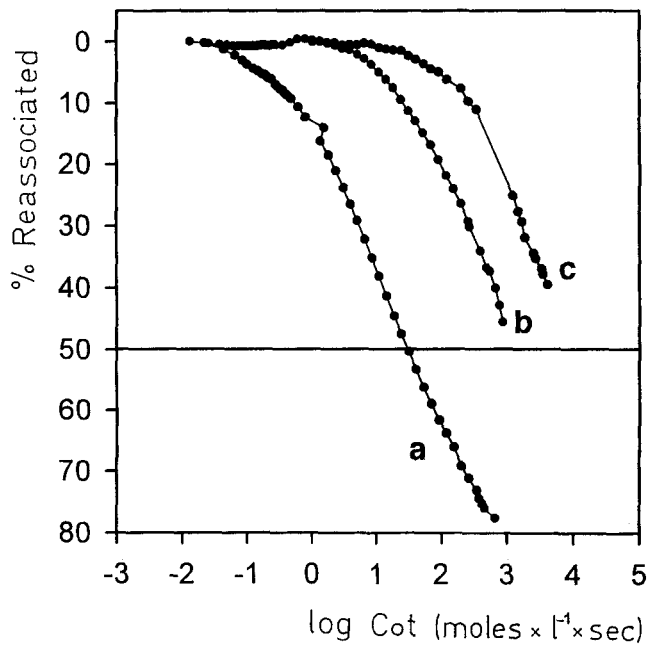

Figure 3 Reassociation (Cot plot) of DNA from the bacterial fraction of fish farm sediment (a), sediment from an abandoned fish farm (b), and pristine marine sediment (c), in 6× SSC (standard saline citrate) and 30% DMSO (dimethylsulphoxide). The DNA was shared to about 420000 daltons and reassociated at 50°C. Abscissa: Log initial concentration of single-stranded DNA (Co in moles nucleotides L^{-1}) multiplied by time in seconds. Ordinate: Percent reassociated DNA.

extremely low (Figure 3). The $Cot_{1/2}$ value of DNA from beech soil was approximately 4700 moles s^{-1} L^{-1}, while that of sediment approached 9000 (Table 1). This is equivalent to DNA complexities of 2.7×10^{10} and 5×10^{10} base pairs. When using the genome size of *E. coli* (4.1×10^6 bp) [15] as a unit, the DNA heterogeneity in soil bacterial community corresponded to approximately 6000 entirely different genomes whereas that in sediments corresponds to approximately 12000 genomes. We found that the average genome size of the soil bacterial isolates was 1.7 times higher than that of *E. coli* [47], thus the DNA heterogeneity

Table 1 Bacterial diversity in different soils and sediments as determined by DNA reassociation

Environment	$Cot_{1/2}$[a]	No. of genomes[b]
E. coli	0.72	1
Forest soil, total community	4500–4700	6000
Forest soil, plate count community[c]	28	30
Agricultural sandy soil, total community	800	1000
Agricultural organic soil, total community	1600	2000
Pristine sediment, total community	9000	12000
Fish farm sediment, total community	40–50	50–70
Abandoned fish farm sediment, total community	1300	1800

[a]In 6× SSC, 30% DMSO.
[b]Equivalent to *E. coli* genome; 4.1×10^6 bp.
[c]DNA from a mixture of 206 isolates.

in soil and sediment corresponds to 4000 and 7000 'standard' soil bacterial genomes respectively.

To estimate the number of species from the observed DNA heterogeneity, the extent of genetic diversity within bacterial species has to be known. The intraspecies genetic diversity probably varies considerably and may be difficult to determine. Most bacteria have a clonal population structure and consist of different clonal groups with little genetic cohesion. In other bacterial species like some *Rhizobium* species [40] and *Burkholderia cepacia* [59], frequent gene transfer and recombination have been observed. Such recombination may prevent the development of a clonal population structure and ensure higher genetic cohesion.

As the diversity of a species is unknown, we have used the number of 'standard' genomes to estimate a minimum and maximum number of species in the environmental samples. The minimum number is obtained if we assume no phylogenetic relationship between species. Accordingly the minimum number of species in forest soil must be somewhere between 4000 and 6000. This is obviously too low, because it must be assumed that in a sample of environmental bacteria, the genomes of some of them will have some homology. $Cot_{1/2}$ will therefore underestimate the number of bacteria in an assemblage. It has been proposed that the bacterial strains within a species should have at least 70% DNA : DNA homology and –5°C decrease in melting temperature [56]. If the 70% homology level is used to circumscribe a species, and we assume that the remaining 30% are completely heterologous, then the number of species in the forest soil would be somewhere between 12000 and 18000. Such rough estimates of the minimum and maximum number of species can only give indications about the order of magnitude of the number of bacterial 'species'.

Our observations that natural environments contain a great variety of bacterial types are in agreement with other studies of uncultured microorganisms using molecular methods. Several investigators have analyzed PCR-amplified rRNA genes in DNA isolated directly from environmental samples. In all the environments investigated, even those where a limited diversity could be expected, novel bacterial groups have been detected. Most of the ribosomal DNA sequences recovered from environmental samples were not related to cultured bacteria even at the genus level. The conclusions of these investigations are that the majority of bacteria in the environment are new species that are unknown to microbiologists, and that the microbial diversity must be much higher than that observed by studying cultured bacteria [16, 24, 42, 54]. Similar investigations also indicate that the biodiversity within known bacterial groups is much greater than previously thought. Studies of the biodiversity within groups of bacteria (planctomycetes and nocardia) in soil, water, and activated sludge showed that new isolates and 16S rRNA clones were not related to previously described members of the group. For some of these groups that are difficult to grow, there are now more published 16S rRNA sequences from uncultured than from cultured members [24, 37, 55].

With the reassociation technique an immense complexity in DNA from soil and sediments has been observed, indicating that the number of species may be in the order of 10000. The DNA was extracted from 100 g of soil or sediment, and the question is whether 10000 species can coexist in such a sample of soil or sediment. In the soil the total number of bacteria was about 1.5×10^{10} per g [48]. If we assume an even species distribution, each species would consist of about 1.5×10^6 cells per g. Using the immunofluorescence technique it has been demonstrated that the population sizes of three specific bacteria in a similar type of organic soil were in the range of 10^6–10^7 per g [39]. The average plate counts for the three bacteria were about 0.5% of the immunofluorescence counts. Even if 99.5% of the cells in soil are in a non-culturable state it is still an ample population size for species sustainment. Rare species, present in frequencies far below the average, can still have a fairly high population size.

Bacteria normally occur in microcolonies in the soil. If we assume that on average there are 100 cells per microcolony, then there would be more than 10000 microcolonies per species in one gram of soil. Such numbers are conceivable when taking into account the immense amount of bacteria in soil and their small size. If the average bacterial cell volume is 0.1 μm^3, then the bacteria in 1 g of soil will occupy approximately 1.5 mm^3 (1.5 μl).

A factor that should be considered when assessing the bacterial diversity in natural environments is the scale effect. Spatial variability is important for the diversity, and such variability may occur at different scale in different environments. Therefore the relationship between community composition and scale may differ. In ecology there is a general perception that communities are composed of a few dominating and many rare species. In the pristine soil and sediments we did not observe any dominating species. This may be due to the sampling scale. Bacterial communities may be located in microhabitats in soil and sediments. In our investigations we were sampling 100 g of soil or sediment. Therefore the diversity as we measure it, is probably equivalent to γ-diversity, the diversity of species over a range of habitats in a region [31].

Diversity of cultured bacteria compared to total bacterial diversity: are isolated bacteria representative of the bacterial community?

The quesiton whether the cultured bacteria were representative of the total bacterial community, or if they were a distinct subpopulation was elucidated by studying bacteria in beech forest soil [47]. Soil samples were plated on a standard plating medium, approximately 200 bacterial colonies were randomly picked, and the bacteria isolated. DNA was isolated from a mixture of the bacterial isolates, and the DNA complexity was determined (Figure 2). $Cot_{1/2}$ of DNA from the isolated bacteria was approximately 28 moles s^{-1} L^{-1} (Table 1), which equals a genome size of 1.4×10^8 bp. This corresponds to a 'population genome' size approximately 20 times that of an average soil bacterium genome. This is used as a minimum estimated number of species. If the 70% homology level is used as a limit for the species, then the observed $Cot_{1/2}$ would correspond to 67 species with equal distribution. The diversity of the same 200 isolates determined by phenotypic characterization and clustering at 80% similarity level, gave 41 biotypes. If the

'population genome' was divided equally between the biotypes, then the biotypes would have approximately 50% DNA : DNA homology. By plotting the $Cot_{1/2}$ values against the number of isolates used in the DNA mixture, the $Cot_{1/2}$ increased until it approached a maximum value when about 90 isolates were mixed. Ninety isolates were therefore enough to cover the entire diversity of the bacteria isolated on the standard medium used. The $Cot_{1/2}$ of DNA derived from the total bacterial community of the same soil was approximately 170 times higher than that of the mixture of isolated bacteria [48]. We therefore concluded that the population of bacterial isolates was not representative of the total community in the soil, but comprised only a small fraction of the bacteria present.

The limitation and bias of the culturing techniques have been demonstrated when sequences of 16S rRNA clones derived directly from DNA of natural samples have been compared with those from bacterial isolates. In most cases there is no close relationship between rRNA sequences of isolates and of rRNA clones from environmental DNA [37, 55]. Direct whole cell hybridization with rRNA-based phylogenetic probes also demonstrates that the culturing conditions yield only a small and probably skewed fraction of the organisms present in the environment [53]. There are several possible explanations for the discrepancy between the total bacterial diversity and the diversity of the culturable bacteria. Limiting factors for growth in the laboratory are: substrate spectrum, substrate concentrations, growth conditions, production of toxic compounds, inability to grow on solid surfaces, and incapability of massive growth. In natural environments like soil and sediments most of the bacteria are adapted to grow in some type of gradients and in close interactions with other bacteria; they may therefore be unable to grow as pure cultures in the laboratory.

The effect of perturbation and stress on bacterial diversity

We have studied the effect of perturbations and stress on the total bacterial community in soil and sediments. Two agricultural soils in western Norway near Bergen, have been investigated. One was a sandy soil from a field, frequently tilled and subjected to crop rotations. The other was an organic soil from a field that had been permanent grazing land for decades and which was infrequently tilled. Reassociation analysis of DNA from the soil bacterial fractions gave $Cot_{1/2}$ values of approximately 800 moles s^{-1} L^{-1} for the sandy soil and 1600 moles s^{-1} L^{-1} for the organic soil (Table 1). These values correspond to genome sizes 1000 and 2000 times larger than the *E. coli* genome, and show that the genetic diversity of the bacterial community in the organic soil was twice as high as that in the sandy soil. A possible explanation for the difference in diversity of these soils could be that the sandy soil had been tilled once a year for several years. The perturbations together with crop rotations probably would yield successions and instability of bacterial populations. Unstable communities are characterized by dominance of a few organisms and hence a low diversity. The organic soil had not been perturbed to the same degree as the sandy soil, and its vegetation was more permanent and diverse. This implies that the bacterial community was more mature and stable than that of the sandy soil. Another explanation could be that organic soils normally have higher diversity than sandy soils, due to their higher physico-chemical complexity. The bacterial diversity in the agricultural soils was 2–5 times lower than that of the forest soil. The latter soil probably had a well developed and stable climax community.

In another investigation the bacterial diversity in sediments from an operating fish farm and an abandoned fish farm was compared [49]. In the operating fish farm the sediment had accumulated organic wastes due to deposits of feed pellets and fecal material. The abandoned fish farm had not been in use for 4 years. The sediment had still a high organic content, but the wastes had been transformed into amorphous humus-like organic matter. DNA was isolated from the bacterial fraction of the top 10 cm of the sediments. The DNA complexity was determined by reassociation (Figure 3). The $Cot_{1/2}$ of DNA from the operating fish farm sediments was 40–50 moles s^{-1} L^{-1}, and that from the abandoned fish farm was 1300 moles s^{-1} L^{-1} (Table 1). This corresponds to about 60 and 1800 entirely different genomes. In comparison DNA from the bacterial fraction of the pristine sediment had a $Cot_{1/2}$ that was about 200 times higher than that of the operating fish farm. The organic pollution therefore dramatically reduced the bacterial diversity. After the fish farm was abandoned the bacterial diversity had increased and was 32 times higher than in the operating fish farm, but it was still seven times lower than in the pristine sediment. The total number of bacteria in the fish farm and natural sediment was 8.2×10^9 and 1.6×10^9 per g, respectively (unpublished). The organic content in these sediments was similar, 27 and 20%, therefore the quality rather than the quantity of the organic matter must have been important for the diversity. In the organic polluted fish farm sediment there was an input of a relatively small range of readily utilized substrates (proteins, carbohydrates, lipids) which sustained a higher bacterial biomass compared to the natural sediment. The organic substrates exerted a selection pressure favoring a few fast growing bacteria (r-selection) which become dominant. Another explanation for the reduced diversity could be that antibacterial agents used in fish farming had a bacteriostatic/bacteriocidal effect on some bacteria, with a corresponding selective advantage on those which became resistant. However, in mesocosm experiments where antibacterial agents were added to fish farm sediments, no significant differences in diversity were observed between sediments with these agents and the control sediments without antibacterial agents [25]. It is frequently observed that bacterial diversity is reduced in response to environmental stress and perturbation [6, 8, 20]. Atlas *et al* [7] using the reassociation method, found reduced genetic diversity in the bacterial community of soil exposed to the herbicide 2,4,5-T (trichlorophenoxyacetic acid) compared to untreated soil.

How can the diversity have evolved?

A key to the high genetic diversity can be that most natural bacterial populations have a clonal population structure and thus consist of many different clonal lines [1, 12, 23, 29].

This implies that bacteria, even those that remain functionally related may have diverged genetically. Along with nucleotide substitution, DNA rearrangements are important for the generation of high generic variability within bacterial populations [4, 22]. Large populations located in heterogeneous habitats comprise a high number of genetically divergent clones, each adapted to the habitat conditions [35], consequently the genetic diversity within such populations can be high.

Gene transfer between bacteria may provide both genetic variance and genetic cohesion in bacterial populations. By bringing together genes from different species, horizontal gene transfer may increase the genetic variability. Information on secondary metabolism and antibiotic resistance suggests that gene transfer between bacteria has been an important factor in the evolution of new genotypes and new traits [29]. Many secondary metabolic pathways seem to have arisen by modification of existing primary metabolic pathways and have been spread among bacteria. After transfer to new species, genes for the primary pathways have diverged due to different selective conditions, and have become highly variable [52]. Gene transfer and recombination between chromosomal genes of related bacteria can destroy the clonal population structure and link the lineages genetically. Normally such recombination is infrequent and involves only short DNA fragments, and hence does not affect the clonal population structure as detected by protein electrophoresis pattern [29]. Therefore in nature there obviously must be strong barriers against recombination between bacterial strains, probably due to restriction- and repair-mechanisms, or to lack of gene expression.

Ecological factors regulating the diversity in soil and sediments

The high bacterial diversities observed in pristine soil and sediments are probably related to the multiplicity of ecological niches in time and space present in these ecosystems. High microhabitat variations create adaptive variability in bacterial populations and may lead to divergence in the reproductive success (genetic fitness) among replicating bacterial clones [23]. This will promote genetic polymorphism and create genetic barriers between different bacterial clones. Thus at high habitat variations, the conditions for divergence of bacterial clones and selection of novel genotypes are favorable. Pristine soil and sediments are complex structural systems that normally have a high range of different organic substrates. Habitat variability is created by physico-chemical gradients, nutrient concentrations [58] and interactions between microorganisms. In soil and sediments where the bacterial numbers are high and the competition is strong, many bacteria inhibit their strongest competitors by producing antibiotics or bacteriocins. Such antagonism may create discontinuities or boundaries between related populations. On the other hand a large part of the bacterial flora form associations of closely interacting microorganisms of different types that respond differently to environmental conditions. Zavarzin *et al* [61] argue that the high diversity seen in many habitats could only have evolved by interactions between bacteria that can modify their environment, creating new niches and thereby selecting for new traits.

Because ecosystems are dynamic and fluctuating, variations in bacterial populations occur not only spatially but also temporally. The time factor may contribute to the high diversity because bacteria are only active at different time intervals due to temporal discontinuity of their functional niches. At a specific time most of the bacteria, although they are alive, may not be actively growing but are in a resting stage until their functional niche may reappear. Much of the genetic diversity demonstrated in soil and sediments, may therefore be represented by inactive organisms. The situation can be illustrated by comparing it to the discrepancies between the biodiversity of soil seed banks and plants. It has been shown that the species diversity of seeds found in the soil in a defined area is much higher than the diversity of plants actually growing there [18, 44]. Like the seed bank, the genetic diversity represents the total potential diversity, of which only a minor part is expressed and active at a given time and space.

In addition to environmental factors such as habitat variations, the bacterial diversity is also controlled by biological factors. The biological control is exerted through close interactions between organisms at different trophic levels in the ecosystem. In soil there is a direct linkage between plant and bacterial activity through bacterial growth on root exudates, dead root cells and litter. Therefore it is conceivable that the soil bacterial diversity is related to the plant diversity, as demonstrated by the difference in bacterial diversity in the grazing field with a complex plant community, and the crop field with a monoculture. The activity of predators (grazing) and viruses may also have an impact on the bacterial diversity. They will feed on, or multiply most efficiently on bacteria with the largest population size, thereby decreasing the population size of any dominating bacteria. Moderate predator and virus activity will reduce the density of the dominant bacteria (reduce their fitness) so that less competitive bacteria get a better chance to proliferate. The relative abundance of the different bacterial populations becomes more equal and the diversity increases. In the forest soil studied, high numbers of different bacteriovore slime molds have been observed [14].

The relative importance of the factors controlling the diversity may be different in different ecosystems. In ecosystems like soil and sediments, environmental factors such as the complex physico-chemical structure providing great habitat and niche variations may be the most important. This is in contrast to more homogeneous ecosystems such as bulk water. In such ecosystems biological control through the functional relationship between trophic levels, may be of relatively greater importance.

We conclude that the bacterial diversity in a community can be expressed as the heterogeneity of directly-isolated bacterial DNA. The pristine soil and sediments studied by us contain in the order of 10000 different 'species' with approximately the same population size. Such anthropogenic influences as agricultural management (tilling, manuring and cropping) and pollution can have a profound impact on bacterial communities, leading to reduced bacterial diversity, even though the bacterial biomass may be increased. The reduced diversity is probably due to reduced

structural and chemical complexity of the environments. Disturbed bacterial communities seem to be able to regain the diversity again if the perturbation is reduced.

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