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High Diversity in DNA of Soil Bacteria

VIGDIS TORSVIK,* JOSTEIN GOKSØYR, AND FRIDA LISE DAAE

Department of Microbiology and Plant Physiology, University of Bergen, Jahnebakken 5, N-5007 Bergen, Norway

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Soil bacterium DNA was isolated by minor modifications of previously described methods. After purification on hydroxyapatite and precipitation with cetylpyridinium bromide, the DNA was sheared in a French press to give fragments with an average molecular mass of 420,000 daltons. After repeated hydroxyapatite purification and precipitation with cetylpyridinium bromide, high-pressure liquid chromatography analysis showed the presence of 2.1% RNA or less, whereas 5-methylcytosine made up 2.9% of the total deoxycytidine content. No other unusual bases could be detected. The hyperchromicity was 31 to 36%, and the melting curve in 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate) corresponded to 58.3 mol% G+C. High-pressure liquid chromatography analysis of two DNA samples gave 58.6 and 60.8 mol% G+C. The heterogeneity of the DNA was determined by reassociation of single-stranded DNA, measured spectrophotometrically. Owing to the high complexity of the DNA, the reassociation had to be carried out in 6× SSC with 30% dimethyl sulfoxide added. Cuvettes with a 1-mm light path were used, and the A_{275} was read. DNA concentrations as high as 950 μg ml^{-1} could be used, and the reassociation rate of *Escherichia coli* DNA was increased about 4.3-fold compared with standard conditions. $C_0t_{1/2}$ values were determined relative to that for *E. coli* DNA, whereas calf thymus DNA was reassociated for comparison. Our results show that the major part of DNA isolated from the bacterial fraction of soil is very heterogeneous, with a $C_0t_{1/2}$ about 4,600, corresponding to about 4,000 completely different genomes of standard soil bacteria. The reassociation curves did not follow ideal second-order reaction curves, indicating that there are several different DNA fractions corresponding to common and more rare biotypes. This means that the $C_0t_{1/2}$ values give only approximate and probably low values for the genome number. Some of the DNA preparations had a rapidly reassociating fraction of about 5% of the total DNA. The reassociation rate for this fraction was about one-third of the rate of the *E. coli* genome. The fraction might be a population of plasmids and/or bacteriophages. Our results indicate that the diversity of the total bacterial community in a deciduous-forest soil is so high that diversity indices based on DNA heterogeneity can be determined only with difficulty. Most of the diversity is located in that part of the community which cannot be isolated and cultured by standard techniques.

Diversities in bacterial communities are normally determined by phenotypic characterization of isolated strains. A problem is that phenotypic methods can be used only on bacteria which can be isolated and cultured. Most (99.5 to 99.9%) of the soil bacteria observed in the fluorescence microscope cannot be isolated and cultured on laboratory media. They will therefore be excluded when phenotypic diversity is estimated. Isolated bacteria may therefore account for only a minor proportion of the total bacterial diversity in soil, while our knowledge about the dominant part is very scant.

DNA can be isolated from bacterial fractions containing 50 to 80% of the soil bacteria (17, 29, 31) and may provide genetic information about the nonculturable bacteria in soil. The heterogeneity of this DNA is a measure of the total number of genetically different bacteria in soil. DNA heterogeneity can be determined by thermal denaturation and reassociation. Renaturation of homologous single-stranded DNA follows second-order reaction kinetics. The fraction of renatured DNA is usually expressed as a function of the product (C_0t) of DNA nucleotide concentration (C_0) in moles per liter and the reaction time (t) in seconds. Under defined conditions C_0t for a half-completed reaction ($C_0t_{1/2}$) is proportional to the genome size or the complexity of DNA. The complexity is defined as the number of nucleotides in the DNA of a haploid cell, without repetitive DNA (3). The genetic diversity of a bacterial community can be expressed in a similar manner, by using $C_0t_{1/2}$ as a diversity index. This would be analogous to indices based on phenotypic analysis or species diversities.

The purpose of our work is to determine whether DNA heterogeneity as determined by reassociation kinetics can be used as a measure of genetic diversity. Results from work with isolated soil bacterial populations are presented in the accompanying article (30a). In the present work, the method is applied to soil bacterium DNA to estimate the diversity of genome richness of the total bacterial community and to compare this with data obtained from the population of isolated bacteria from the same soil.

MATERIALS AND METHODS

Soil. Samples were taken from the top 10 cm of soil from a beech forest at Seim, north of Bergen, Norway. The pH in distilled water was 4.5, the organic-matter content was 49% of the dry weight, and the water content was 213% of the dry weight. The water-holding capacity was 344 ml 100 g of dry soil^{-1}. The total bacterial count was 1.5×10^{10} g (dry weight) of soil^{-1}, as determined by fluorescence microscopy after staining with acridine orange. The plate count was 4.3 $\times 10^7$ g (dry weight) of soil^{-1} on Thornton agar with 10% soil extract. The soil was sieved (mesh size, 2 mm), mixed well, and kept in polyethylene bags at 4°C.

Isolation of DNA from the bacterial fraction in soil. The bacterial fractions were prepared from six 30-g samples of fresh soil (12). To increase the yield of DNA, the isolation procedure of Torsvik (31) was somewhat modified. The bacterial fractions were washed once with 200 ml of 2%

* Corresponding author.

sodium hexametaphosphate (pH 8.5), to extract naked DNA adsorbed to colloids, and once with 100 ml of Crombach buffer (5). The pellets were suspended in 30 ml of isopropanol (26) and homogenized with a Ystral homogenizer, speed setting at 2. The suspensions were stored in a refrigerator. Alternatively, the pellets could be stored frozen, but isopropanol seemed to increase the yield of DNA from soil bacteria.

To lyse the soil bacteria, we centrifuged the pellets, resuspended them in Crombach buffer, and homogenized them with an Ystral homogenizer. The volume was adjusted to 25 ml with Crombach buffer; 5 mg of lysozyme (Sigma Chemical Co.) ml^{-1} was added; and the suspension was incubated at 37°C for 1 h (4). Then 2 mg of proteinase K (Sigma) ml^{-1} was added, and the incubation was continued for another 1 h. The suspension was then heated to 60°C, sodium dodecyl sulfate was added to a final concentration of 1% (1 ml of a 25% solution), and the mixture was incubated for 5 min. The lysate was cooled on ice, and KCl was added to a final concentration of 1 M. The lysate was kept on ice for 2 h or in a refrigerator overnight and then centrifuged for 20 min at 10,000 rpm (Sorvall RC-5 SS34 rotor) at 5°C. The pellet was washed once with 10 ml of 0.02 M Tris–1 M KCl (pH 8.2). The supernatants were pooled and purified on a hydroxyapatite (HAP) column (Bio-Gel HT; Bio-Rad Laboratories) (31).

DNA in the pooled fractions from the HAP column was concentrated by cetylpyridinium bromide (CPB; Sigma) precipitation (14) to avoid coprecipitation of phosphate. The following procedure was used: 10 volumes of DNA solution were mixed carefully with 1 volume of 0.5 M EDTA, the mixture was adjusted with 0.5 M Tris to pH 6.0, and then 1.2 volumes of 1% CPB were added. The mixture was frozen at −20°C for 24 h, thawed slowly (to room temperature), and centrifuged for 10 min at 5,500 rpm (Sorvall RC-5 SS34 rotor). The pellet was washed once in 5 ml of distilled water, twice in 5 ml of 80% ethanol in 0.1 M sodium acetate buffer (pH 4.5), and twice in 5 ml of acetone. The pellet was dried in a vacuum desiccator and resolved in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The DNA prepared from bacteria of organic soil in this manner was often contaminated with some humic material. Therefore, a second run through HAP was applied. After an additional CPB precipitation and ethanol precipitation, the DNA was highly purified.

Escherichia coli ATCC 11775 DNA was isolated by the method of Marmur (23). Calf thymus DNA (type I; Sigma) was dissolved in 0.1× SSC.

Shearing of DNA. The DNA solutions were diluted to about 100 $\mu g\ ml^{-1}$ and sheared in a French press at 20,000 lb in^{-2}. The fragment size was determined by analytical ultracentrifugation (31). The molecular mass of the sheared DNA fragments was, on average, 420 kilodaltons. Sheared DNA was purified through HAP to remove short DNA fragments and single-stranded DNA. The DNA solution was made 0.14 M in sodium phosphate buffer (pH 6.8), loaded on a HAP column, which was washed with the same buffer, and eluted with 0.24 M sodium phosphate buffer (pH 6.8). It was then concentrated by precipitation with CPB as described above.

Restriction enzyme digestion of DNA. DNA was digested with *Eco*RI and *Hin*dIII (Promega Biotec). A 1-μg portion of DNA in 20 μl was digested with 5 U of enzymes overnight at 37°C in the appropriate buffer supplied by the manufacturer. The digested DNA was analyzed by 0.7% agarose gel electrophoresis (22).

Thermal denaturation and reassociation of DNA. Thermal denaturation and reassociation were determined in a UV-visible recording spectrophotometer (Shimadzu UV-240) equipped with a thermoelectric cell holder, an SPR-5 temperature controller, and a KPC-5 temperature programmer. The time in seconds and absorbance was registered by a BBC microprocessor. The data were transferred to an Olivetti M24 PC computer, and the melting curves were plotted with an ULTIMO program.

Melting was carried out with DNA samples of about 25 $\mu g\ ml^{-1}$ in 1.0× SSC or in 6× SSC–30% dimethyl sulfoxide (DMSO). The temperature was increased continuously at 0.5°C min^{-1}. The A_{260} was measured for SSC solutions, and the A_{275} was measured when DMSO was added, since DMSO has a significant absorbance below 270 nm which increases with increasing temperature. The A_{275} of DNA was about 75% of the A_{260}. The moles percent guanine plus cytosine was calculated from the melting point in 1.0× SSC by the method of De Ley (6).

Reassociation of DNA was measured as the decrease in absorbance of denatured DNA kept at constant temperature at about 25°C below the T_m, using the same equipment as for thermal denaturation. The absorbance was registered by a BBC computer at logarithmically increasing time intervals. Soil bacterium DNA was reassociated in 6× SSC–30% DMSO at 50°C, with the A_{275} being measured. *Escherichia coli* B DNA was renatured under the same conditions and also under standard conditions (2× SSC at 73°C) (16). Samples of sheared and purified DNA were diluted to concentrations of 25 to 80 $\mu g\ ml^{-1}$ for *E. coli* B DNA and 400 to 800 $\mu g\ ml^{-1}$ for soil bacterium and calf thymus DNA. Cuvettes with a 10- or 1-mm light path were used depending on the DNA concentration. The samples were covered with silicon oil (DC200; Dow Corning), and the cuvettes were closed with Teflon stoppers to avoid evaporation. One hundred percent reassociation (A_∞) was determined by measuring the A_{275} (or A_{260}) of native DNA in the reassociation buffer at the reassociation temperature, T_r. DNA was then melted at 85°C (T_m + 10°C) in the spectrophotometer, and the hyperchromicity was determined. The solution was then cooled rapidly (in <2 min) in a water bath at 25°C and transferred to the thermostatted cuvette holder held at T_r, and the A_{275} was measured to determine 0% reassociation (A_0).

($A_0 - A_\infty$) is the maximum decrease in A due to complete reassociation of the DNA. The percent reassociated DNA $(A_0 - A_t) \times 100/(A_0 - A_\infty)$ was plotted against $\log_{10} C_0t$. C_0 was calculated from the A_{260} of native DNA in 0.1× SSC, by assuming that 50 μg of native DNA ml^{-1} has an A_{260} of 1 in a 10-mm light path (21) and that the average molecular weight of nucleotides in DNA is 309. For long-term experiments the spectrophotometer was calibrated by using a guanine standard solution (50 $\mu g\ ml^{-1}$ in 50 mM NaOH).

The DNA complexity (synonymous with molecular weight) in base pairs (bp) was calculated relative to the *E. coli* B genome of 4.1×10^6 base pairs.

RESULTS

Soil bacterial DNA, purified twice on HAP and precipitated with CPB, inhibited restriction enzymes to some extent. After an additional ethanol precipitation, the DNA was highly purified and was digested by restriction enzymes. Lambda DNA mixed with this soil bacterial DNA was also completely digested by the restriction enzymes (Fig. 1). The A_{260}/A_{280} and A_{260}/A_{230} ratios for highly purified soil bacterium DNA were 1.80 to 1.95 and 2.10 to 2.50, respectively.

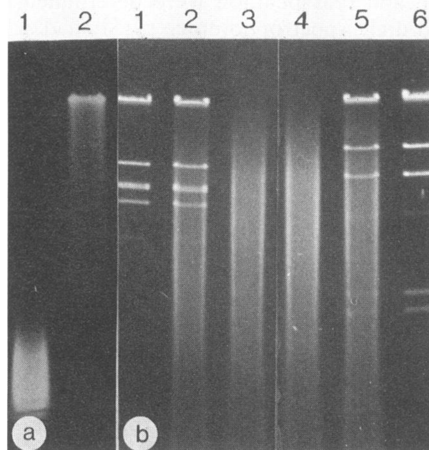

FIG. 1. Agarose gel electrophoresis. (a) Highly purified soil bacterium DNA. Lanes: 1, sheared DNA; 2, untreated DNA. (b) Restriction enzyme-digested soil bacterium and phage lambda DNA. Lanes: 1, *Eco*RI-digested lambda DNA; 2, *Eco*RI-digested lambda plus soil bacterium DNA; 3, *Eco*RI-digested soil bacterium DNA; 4, *Hin*dIII-digested soil bacterium DNA; 5, *Hin*dIII-digested lambda plus soil bacterium DNA; 6, *Hin*dIII-digested lambda DNA.

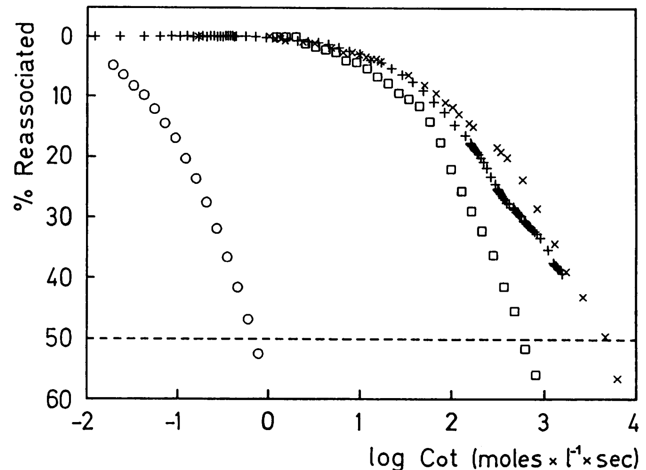

FIG. 3. Reassociation of soil bacterium DNA, purified three times on HAP (C_0t plot) in 6× SSC–30% DMSO (×, +). The reassociation of *E. coli* B (○) and calf thymus (□) DNA is included for comparison. The DNA was sheared to about 420 kilodaltons and reassociated at 50°C. The abscissa gives the log initial concentration of single-stranded DNA (in mole-nucleotides per liter) multiplied by time in seconds. The ordinate gives the percent reassociated DNA.

High-pressure liquid chromatography analysis, carried out by W. B. Whitman, University of Georgia (25), showed that the soil bacterium DNA that had been purified twice on HAP contained 2.1% RNA or less, which is typical for highly purified DNA. No unusual nucleosides were observed, but 5-methylcytosine accounted for about 2.9% of the total deoxycytidine content.

The melting curves for native soil bacterium DNA in 1× SSC and in 6× SSC–30% DMSO are shown in Fig. 2. The hyperchromicity of soil bacterial DNA in 1× SSC (measured at 260 nm) was 24 to 28% after the first purification on HAP and 31 to 36% after a second purification. This indicates that there probably were some impurities such as humic material or some single-stranded DNA in the samples after the first purification on HAP. The melting temperature, T_m, for soil bacterium DNA was calculated relative to the T_m for *E. coli* B DNA and was 93.2 ± 0.1°C in 1× SSC. This corresponds to 58.3 ± 0.2 mol% G+C. The moles percent G+C of two different DNA preparations was also determined by high-pressure liquid chromatography (25). Their base compositions were 58.6 ± 0.2 and 60.77 ± 0.11 mol% G+C. The melting profile of soil bacterium DNA was relatively broad, with a standard variation, 2σ (8, 10), of 10.0 to 12.5°C, whereas the *E. coli* B DNA melting profile had a standard variation of 3.0 to 4.8°C. The T_m for *E. coli* B DNA and soil bacterium DNA was reduced by 17.2 to 17.3°C in 6× SSC–30% DMSO compared with 1× SSC.

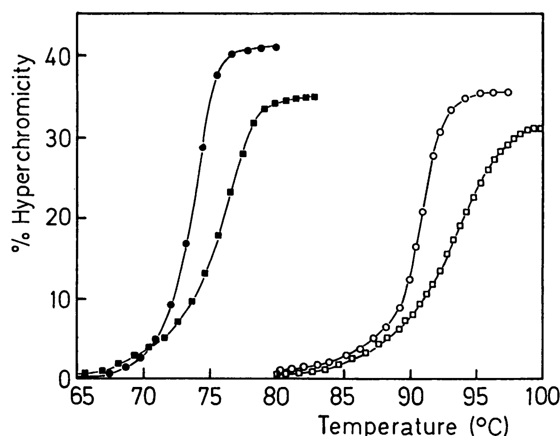

FIG. 2. Melting profiles for *E. coli* B DNA and soil bacterium DNA purified three times on HAP. Symbols: ○, *E. coli* B DNA in 1× SSC; ●, *E. coli* B DNA in 6× SSC–30% DMSO; □, soil bacterium DNA in 1× SSC; ■, soil bacterium DNA in 6× SSC–30% DMSO. When 1× SSC was used, the A_{260} was measured; when 6× SSC–30% DMSO was used, the A_{275} was measured.

Figure 3 shows some typical reassociation curves (C_0t curves) of soil bacterium DNA. Renaturation of *E. coli* B DNA and calf thymus DNA is included for comparison. There was a rapid drop in absorbance in the beginning, which is not shown in the figure since it is due to nonspecific base stacking or collapse (1). This was followed by a slower decrease in absorbance, shown in the figure, which is due to reassociation. Bendich and Anderson (1) found that the collapse was about 12% at T_m − 25°C and depended on the reassociation temperature, base composition, and, to some extent, the salt concentration. Under our conditions the collapse hypochromicity was 17 to 20%. This collapse could be due in part to the hypochromicity of DMSO. By cooling the melted DNA rapidly (in 2 to 3 min) to the reassociation temperature, the collapse was completed before any significant reassociation took place.

To ensure that complex DNA reassociated normally under the conditions used, calf thymus DNA was reassociated under the same conditions. The calf genome consists of about 60% nonrepetitive DNA with a complexity of about 3.2×10^9 bp (3). In our system the nonrepetitive calf thymus DNA renatured with a $C_0t_{1/2}$ of approximately 600, which corresponds to about 3.4×10^9 bp, relative to *E. coli* B DNA (Tables 1 and 2).

Impurities such as humic material probably inhibited reassociation to some extent. DNA that had been purified twice on HAP and still contained traces of impurities had a lower reassociation rate than DNA purified three times

TABLE 1. Reassociation kinetics for *E. coli* B DNA under different conditions[a]

Reassociation buffer	$C_0t_{1/2}$
4× SSC–30% DMSO	0.85 ± 0.01
4× SSC–30% DMSO[b]	0.87
6× SSC–30% DMSO	0.72 ± 0.01

[a] The DNA heterogeneity was 4.1 × 10^6 bp (2.71 × 10^9 daltons). The average fragment size was 660 bp (420 kilodaltons). The reassociation temperature was 53°C in 4× SSC–30% DMSO and 50°C in 6× SSC–30% DMSO.

[b] Containing a mixture of 30% *E. coli* B DNA and 70% soil bacterium DNA purified three times on HAP.

(Table 2). To ensure that soil bacterium DNA purified three times on HAP did not contain any impurities which could influence the reassociation rates, 30% *E. coli* B DNA was mixed with this DNA and renatured. The renaturation rate for *E. coli* B DNA was nearly the same with and without soil bacterium DNA (Table 1).

Soil bacterium DNA which was reassociated in 6× SSC to a C_0t of ca. 1,600 (40% reassociation) was melted again. The melting curve shows that reassociated DNA melted to give the same absorbances as the native DNA (Fig. 4). Covalent cross-binding, which could inhibit the process, was not produced during the incubation, since the reassociation was fully reversible. The slope of the melting curve for reassociated DNA was less steep than for native DNA. This shows that the reassociated DNA had a certain degree of mismatch, i.e., that it contained some heteroduplex DNA from different types of bacteria.

In some of the soil bacterium DNA samples there was a small fraction of rapidly reassociating DNA (about 5% of the total) (Fig. 5). The reassociation rate for this fraction was about one-third the rate of the *E. coli* genome.

The reassociation of DNA from the soil bacterium fraction purified three times on HAP was compared with the reassociation of a mixture of DNA from 206 bacterial strains isolated from the same soil (Table 2). In this experiment the reassociation buffer was 4× SSC–30% DMSO and T_r was 53°C (30a), and under these conditions the reassociation rates were about 1.2 times higher than in 6× SSC–30% DMSO.

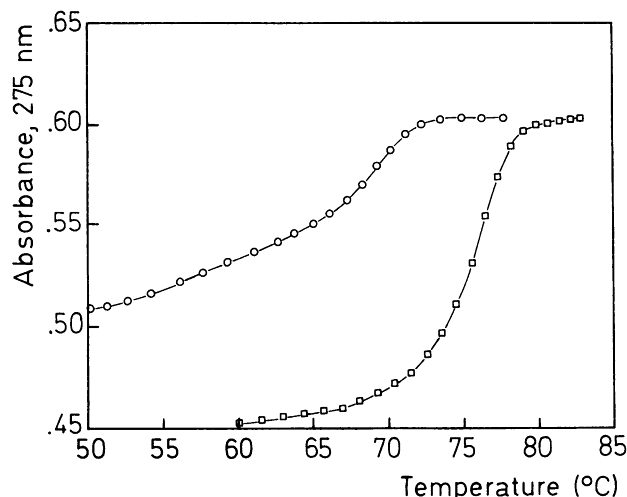

FIG. 4. Melting profiles for native (□) and reassociated (○) soil bacterium DNA in 6× SSC–30% DMSO. The reassociated DNA was run to a C_0t value of about 1,600 (about 40% reassociation).

DISCUSSION

Under stringent conditions in 2 to 6× SSC, the reassociation rates of soil bacterial DNA were so low that it was very difficult to determine the $C_0t_{1/2}$. Incubation times of several weeks were needed to reach 50% reassociation. Factors which could inhibit the reassociation rates were the occurrence of unusual bases, impurities in the DNA, or degradation of DNA during the incubation. No unusual bases were detected, except 5-methylcytosine in normal concentrations for the DNA of many bacteria. To remove humic material and other soil impurities, the DNA used in reassociation experiments was normally purified twice on HAP before shearing and once after shearing. Restriction enzymes such as *Eco*RI or *Hin*dIII were somewhat inhibited by DNA preparations purified twice on HAP and concentrated by CPB. After an additional ethanol precipitation the DNA preparations did not inhibit restriction enzymes. Holben et al. (17) and Steffan and Atlas (29) have demonstrated that DNA isolated by procedures similar to ours reacts normally with restriction enzymes.

Sheared DNA contained some single-stranded DNA, probably due to severely damaged DNA (9). We found that about 10% of the DNA did not bind to HAP after shearing. It was therefore necessary to remove single-stranded DNA after shearing, by purifying the DNA for a third time on HAP. When *E. coli* B DNA was renatured in a mixture with soil DNA purified in this way, the renaturation rate of *E. coli* B DNA was not affected.

Under stringent conditions in 2 to 6× SSC, the risk of DNA degradation increased due to relatively high T_r (<70°C) and long incubation times (18). DNA is unstable at elevated

TABLE 2. Reassociation kinetics and heterogeneity of DNA from the soil bacterium fraction and from a mixture of 206 bacterial strains isolated from the same soil[a]

DNA source	Reassociation buffer	$C_0t_{1/2}$	Heterogeneity (bp)	No. of standard genomes
Bacterial fraction, rapid reassociating fraction	6× SSC–30% DMSO	0.24	1.4 × 10^6	0.21
Bacterial fraction[b]	6× SSC–30% DMSO	8,700	5.0 × 10^{10}	7,200
Bacterial fraction[c]	6× SSC–30% DMSO	4,670	2.7 × 10^{10}	4,000
206 bacterial isolates	4× SSC–30% DMSO	28	1.4 × 10^8	20.6
Calf thymus	6× SSC–30% DMSO	602	3.4 × 10^9	500

[a] Nonrepetitive calf thymus DNA with known heterogeneity was used as a control. The average fragment size was 660 bp (420 kilodaltons). Heterogeneities were calculated relative to *E. coli* B DNA by using the $C_0t_{1/2}$ values under the same reassociation conditions. The reassociation temperature was 53°C in 4× SSC–30% DMSO and 50°C in 6× SSC–30% DMSO. The standard genome (6.8 × 10^6 bp, 4.5 × 10^9 daltons) is the average genome size of the isolated soil bacteria.

[b] Purified twice on HAP.

[c] Based on four experiments with DNA purified three times on HAP.

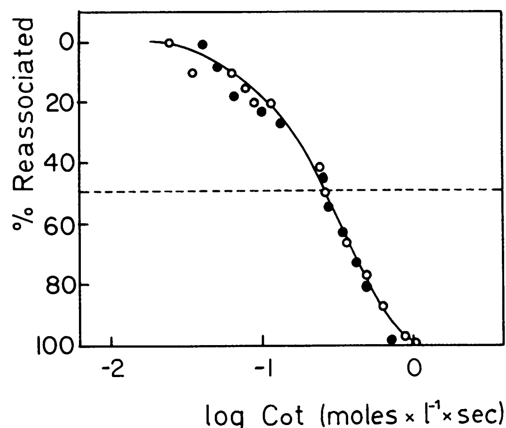

FIG. 5. Reassociation kinetics (C_0t plot) for the rapidly reassociating fraction of soil bacterium DNA. The start concentration, C_0, was adjusted to 5% of the total C_0 used in the experiments, as the rapidly reassociating fraction accounted for about 5% of the DNA (calculated from Fig. 3). The conditions were the same as for Fig. 3. The abscissa gives the logarithm of the initial concentration of single-stranded DNA (in mole-nucleotides per liter) multiplied by the time in seconds. The ordinate gives the percent reassociated DNA.

temperatures due to depurination and shearing (19, 20). Under such conditions the reassociation might terminate due to degradation of DNA. To lower the T_r, organic solvents which reduce the thermal stability of DNA are added to the reassociation mixture. Normally, formamide is used (9, 18, 24), but then the reassociation rate decreases due to increased viscosity of the solution. DMSO has also been used to lower the T_m (7). In this solvent the reassociation rate is increased partly because the dielectric constant is reduced (11). We have used 30% DMSO in the reaction mixture. We found that 6.7× SSC (1.3 M Na^+) was the highest SSC concentration which could be used in 30% DMSO. Trisodium citrate precipitated from this solution at 20°C but remained in solution at the T_r. To increase the reassociation rate, we used the highest possible DNA concentrations. With our equipment we could measure absorbances up to 2.0. By using cuvettes with a 1-mm light path and measuring the A_{275}, DNA concentrations as high as 950 μg ml^{-1} could be used. The reassociation rate of *E. coli* DNA was increased about 4.3-fold under these conditions compared with the standard conditions.

Reassociation rates of highly heterogeneous DNA such as eucaryotic DNA are usually measured by incubating DNA samples to certain C_0t values and determining the fraction of reassociated DNA by separating single and double strands on HAP columns. The HAP method measures the fraction of DNA strands which form duplexes, even if the base pairing is not complete throughout the strands. The optical method measures the fraction of bases that have paired. The HAP method normally gives higher initial reassociation rates than the optical method does, due to foldback and self-reassociation of the DNA strands (2, 3). With the HAP method (2), the reaction rates can be increased by using very high DNA concentrations. The problem is that high DNA concentrations are difficult to obtain from soil bacterium fractions.

Bendich and Anderson (1) found that C_0t curves for bacterial and bacteriophage DNA deviated from an ideal second-order curve at about 50% reassociation and approached about 90% of complete reassociation. This pseudoideal curve represents the reassociation of randomly sheared DNA containing a single kinetic component. The deviation is due to interactions between partly reassociated duplexes and single-stranded fragment regions.

All the reassociation rates were measured relative to *E. coli* B DNA, whose genome size is 4.1 × 10^6 bp or 2.71 × 10^9 daltons (15). The average genome size of the isolated bacteria from our soil sample was about 6.8 × 10^6 bp (4.5 × 10^9 daltons) (30a). This value was used as the standard genome size of soil bacteria.

Some of the DNA preparations had a rapidly reassociating fraction of about 5% of the total soil bacterial DNA. The genetic diversity of this fraction was about 1.4 × 10^6 bp (9 × 10^8 daltons). The fraction might be a population of plasmids and/or phages.

We conclude that the main fraction of soil bacterium DNA was very heterogeneous, with a $C_0t_{1/2}$ of about 4,600. The reassociation curves for the main DNA fraction did not follow ideal second-order reaction kinetics. The experimental curves indicate that there are several different DNA fractions, corresponding to common and rare genotypes. This means that the $C_0t_{1/2}$ values give only approximate and probably low values for the genome number (30a). The genetic diversity corresponds to about 4,000 different genomes of standard soil bacterial size. This is about 200 times the genetic diversity found in the isolated strains and indicates that the phenotypic variants that can be isolated by standard plating techniques make up only a minute fraction of the soil bacterium flora. The nonplatable K-selected population must consist of a large number of different, highly specialized bacteria. These results are consistent with a theory which regards bacteria growing on plates as the *r*-selected bacterial population having a fairly high growth rate and growing on high nutrient concentrations. The main bacterial fraction counted in the microscope is the K-selected population, composed of a large number of different bacteria, having a lower growth rate than the colony-forming bacteria.

The total number of bacteria per gram of dry soil was ca. 1.5 × 10^{10}. If these were distributed among about 4,000 clones with standard genome sizes, there would be a mean number of about 3.8 × 10^6 bacteria clone^{-1} g of dry soil^{-1}. This seems to imply that the bacterial population in soil is composed of a large number of genetically separate clones.

By isolating the total DNA from a bacterial community and determining the reassociation rate, the heterogeneity of the DNA in the community can be estimated. This can be used to determine a diversity index based on DNA types, analogous with the phenotypic diversity indexes. Procedures for isolating DNA from soil (17), river sediments (29), and seawater (13, 28) have recently been described. In addition, different procedures for isolating DNA from soils and sediments have been discussed (30). This opens up the possibility of using this method with systems other than soil. Our results indicate that the genetic diversity of the total bacterial community in a deciduous-forest soil is so high that diversity indices based on DNA heterogeneity can be determined only with difficulty, and not very precisely. In environments with more homogeneous populations, due to pollution or extreme environmental conditions, the genetic diversity might be easier to determine. On the other hand, the results indicate that soil contains a vast number of bacteria which are virtually unknown. The genes of these bacteria ought to be available now, through isolation of their DNA.

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