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The use of gene probes in the rapid analysis of natural microbial communities

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SUMMARY

Hybridization probes produced from DNA sequences have proven to be a powerful tool in the rapid and sensitive analysis of natural microbial communities. By using function-specific probes, such as those identifying genes coding for photosynthesis, the potential a microbial community has for performing a given function may be rapidly determined. Gene probes have also been used in the identification and isolation of a specific catabolic genotype in less than one-fourth the time required for the conventional culture enrichment technique. Species-specific probes constructed from portions of genes coding for ribosomal RNA have been used for the rapid identification and enumeration of bacterial species in environmental samples. The use of reassociation kinetics as a measure of community diversity and complexity is also discussed. The successful application of this technique to community analysis may reduce the time required from 1 year, for conventional analysis, to 2 weeks.

INTRODUCTION

The analysis of the structure and function of natural microbial communities has traditionally relied upon cultural, physiological and biochemical techniques that are frequently time-consuming and sometimes imprecise [18]. By incorporating the techniques of molecular biology into microbial ecology, it is possible to gain a great deal of information concerning the nature of microbial communities in a relatively short period of time and with a high degree of precision. In many cases, the time required for environmental analysis may be reduced by over half through the use of gene probes (Table 1).

Through nucleic acid hybridization technology and the use of specific gene probes, the identification and quantitation of specific genetic sequences within a microbial community is possible [28]. Examples of environmentally relevant gene probes are listed in Table 2. These nucleic acid probes generally fall into two categories: species-specific probes [8,13,27] and function-specific probes [22,29]. Species-specific probes are constructed

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Table 1

Estimated comparisons of time involved for the conventional and molecular analysis of microbial community structure[a]

Application	Conventional		Molecular	
	procedure	time	procedure	time
Enumeration and isolation of poorly selected catabolic communities	1. enrichment	2–4 weeks	1. cultivation	1–2 weeks
	2. isolation and cultivation	1–2 weeks	2. detection and confirmation	0.5–1 week
	3. phenotype	1–10 weeks	3. isolation	1 week
	total:	4–16 weeks		2–4 weeks
Community diversity/complexity	1. cultivation	2–4 weeks	1. cell harvesting	1 day
	2. isolation	2–4 weeks	2. DNA extraction and purification	1 week
	3. characterization	4–24 weeks	3. molecular analysis	3–4 weeks
	4. data analysis and reduction	4–24 weeks	4. probe-specific analysis	2–4 weeks
	total:	12–56 weeks		3–9 weeks

[a] Real comparison on identical samples have not been made; values given are estimates based on previously published research. Differences exist in the quantity and quality of information for either approach.

from genetic sequences, usually from genes encoding ribosomal RNA, that are highly specific to one taxonomic group. Depending upon the nature of the probe, it may be specific to a number of closely related species within the same genus, or to a specific strain within a species. Function-specific probes, such as those that identify genes for nitrogen fixation or pollutant degradation, are not specific to one taxonomic group, but rather measure the potential of the community as a whole to per-

Table 2

Examples of nucleic acid probes used in environmental analysis

Target	Probe	Application	Ref.
4-CB-degrading strains	pSS50	identification, enumeration, isolation	26–28
Naphthalene-mineralizing strains	NAH-7	enumeration	28
Gram-negative mercury-resistant strains	*mer* operon	identification, enumeration	3,4
Bacillus subtilis	cloned fragment of 23 S rRNA	identification	17
Rhizobium	*R. trifolii* chromosome	identification	13
Nitrogen-fixing strains	*nif K,D,H* genes from *Klebsiella pneumoniae*	enumeration	Sayler, unpublished
CO_2-fixing strains	Ribulose bisphosphate carboxylase gene	enumeration	Sayler, unpublished

form a given function. It should be noted that function-specific probes are usually qualitatively different from the more traditional measures of a given function, in that gene probes will provide a measure of the potential a community has for that function, but not the actual activity of the function. It would be necessary to use the more standard techniques if, for example, the total amount of nitrogen being fixed by a system must be known. This approach may also, however, be limited to potential activity if the analysis is conducted on samples taken from the environment.

Two recently developed gene probe techniques for use in molecular microbial ecology studies are DNA:DNA colony hybridization [4,29] and DNA:DNA hybridization of direct DNA extracts [14,22]. Each of these techniques has its weaknesses and strengths which may be exploited depending upon the type of information desired. For most applications in community structure and function analysis, the total extraction and probing of community DNA may be preferable to colony hybridization. Colony hybridization has the requirement that cells be cultured before analysis may begin. Since many of the species in nature are not readily culturable (perhaps fewer than 10% of environmental isolates may be cultivated by standard laboratory media and growth conditions) [20], most of the organisms in a typical sample may be excluded from analysis. Direct extraction and purification of DNA from an environmental sample may be more tedious than colony hybridization, but may yield a more truly representative sample. The benefits and drawbacks of both of these techniques and their contribution to efficient and specific analysis of microbial communities will be discussed in the following sections.

EXPERIMENTAL APPROACHES

The colony hybridization technique for the detection of specific genetic sequences has been in use for over 12 years, but has only recently been applied to environmental analysis. The general procedure consists of growing cells on an appropriate solid medium, lysing the colonies and transferring the DNA to a DNA-binding membrane, followed by

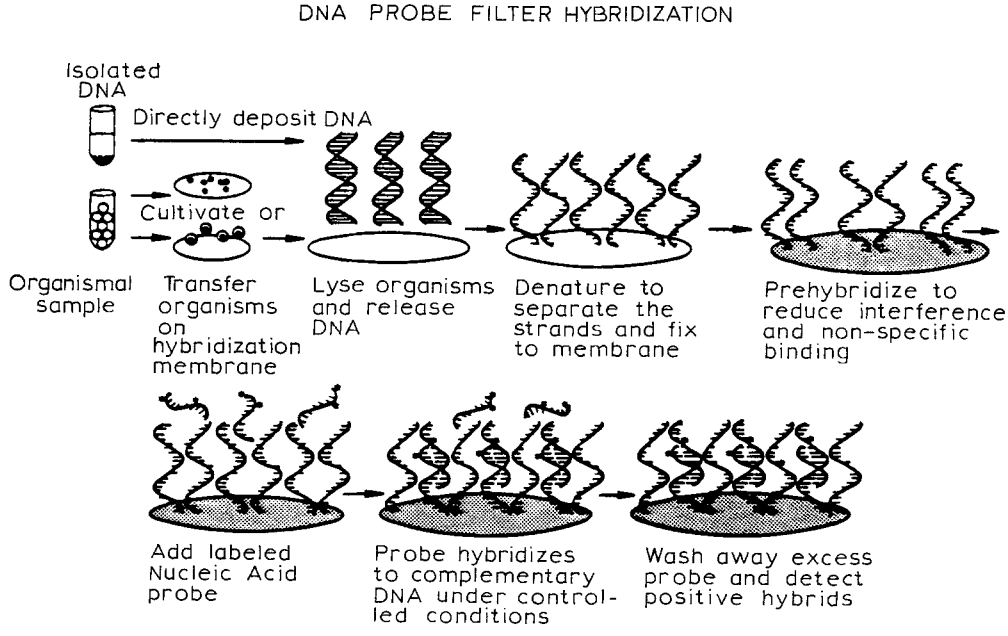

Fig. 1. Description of the colony hybridization technique. Reproduced with permission from Ref. 15.

hybridizing a ^{32}P-labeled probe to specific sequences of the bound DNA. The number of colonies containing the target sequences are then enumerated by autoradiography (Fig. 1) [12].

The technique was originally developed to facilitate the isolation of specific recombinant DNA clones in *Escherichia coli* [12]. As mentioned previously, one of the major limitations of this procedure is the requirement that cells be cultured in the laboratory. There is no single growth medium available that will allow the cultivation of the majority of the hundred or more strains that may be present in a typical environmental sample [20]. Strict anaerobes and species with unusual growth requirements, such as the iron and sulfur bacteria, are automatically excluded from analysis unless specific care is taken to grow these organisms. These factors are frequently limitations in traditional methods as well.

Another requirement implicit in colony hybridization is that all cells must be dislodged from the particulates (such as clay and silt) that are ubiquitous in environmental samples. This is essential in soil and sediment samples, and is frequently necessary in aquatic samples as well. A number of procedures have been developed for the separation of cells from particulates, most of which are based on blending in the presence of surfactants and the subsequent fractionation of cells from the particulates [7,21,33], but none of these procedures is completely successful [2,21]. Since most environmental isolates produce an extracellular polysaccharide that facilitates the irreversible adhesion of the cell to surfaces, bacteria may become permanently bound to the organic matrix of particulates [9]. This would make it difficult, if not impossible, to separate all viable cells from the environmental matrix. A sampling bias may then result from only the easily dislodged species being available for colony hybridization.

An alternative strategy to the colony hybridization technique is the direct extraction and isolation of DNA from the environment [14,22,33]. By isolating microbial DNA directly from environmental samples, some of the limitations of the colony hybridization technique may be bypassed. Direct isolation and purification of DNA obviates the need for cultivation of organisms, thereby eliminating the major source of sampling error inherent in the colony hybridization technique. Direct extraction, like colony hybridization, has the requirement that all cells be efficiently lysed so that the recovered DNA will not be biased in favor of those species that are easily lysed. Direct extraction is also faster than colony hybridization. Colonies isolated from environmental samples are usually allowed 2 weeks to grow before gene probing commences, while only 3 days or less are required to directly isolate DNA. A comparison between these two techniques is presented in Table 3.

This technique allows a more accurate analysis of many parameters than standard techniques allow because no 'bottle effect' is observed. Analysis are performed directly on environmental samples with no incubation time in the laboratory required. For example, when samples are removed from the en-

Table 3

Comparison of colony hybridization and direct DNA extraction and probing

Colony hybridization	Direct DNA extraction and probing
Requires cultivation, does not yield representative sample	Does not require cultivation, yields representative sample
May isolate specific strains	Cannot isolate strains
Requires 2–2.5 weeks, including sampling	Requires 1–1.5 weeks, including sampling
Sampling is simple, requiring only small sample size	Sampling may require processing of large ($\geq$1000 liters) volumes

vironment to be used as microcosms for determining the potential for biodegradation of a given pollutant, the microcosm cannot be considered truly representative of conditions in the field. DNA extracted from a sample would give a more accurate assessment of the biodegradation potential in the field at the moment of sample collection. Direct extraction of DNA also has the advantage over other techniques in that DNA is very easy to store. One extraction may yield enough DNA for analysis with several probes. This probing may be done at any time after extraction, with no loss of validity if the DNA has been stored properly.

The major obstacle to direct extraction of DNA is frequently a logistic one: large samples must occasionally be dealt with, particularly when aquatic samples are to be analysed. Since cultivation of organisms is by-passed in this technique, enough sample must be collected so that sufficient DNA may be isolated to perform the experiment. This is usually not a problem when dealing with sediments and soils, where the biomass is usually high enough that samples of less than 100 g contain more than enough DNA for analysis. Over 50 μg of DNA have been recovered from 1 g of fresh water sediment, yielding enough DNA for at least five probings (Sayler et al., unpublished data). Aquatic samples typically have lower cell counts than sediments and soils, and volumes in excess of 1000 liters may be required in order to isolate enough DNA for analysis. Two methods of concentrating water samples for this type of analysis are currently in use: ultrafiltration and continuous centrifugation.

Concentration by ultrafiltration requires that the sample be passed repeatedly, under pressure, through a chamber containing a packet of filtration membranes. The pore size of the filters may range from 0.2 μm to a molecular weight cutoff of 100 000. The sample is continually passed through this chamber and filtrate is removed from the sample until the sample has been concentrated to the desired volume. Acridine orange direct counts of concentrates from marine and estuarine samples have revealed a high percentage of fragmented cells, indicating that cells are lysed during concentration. While this method appears to be satisfactory for laboratory applications, it may not be an efficient method of concentrating environmental samples (Sayler et al., unpublished data).

Continuous centrifugation seems to be the most gentle and efficient method of concentration currently available, although it is rather time-consuming. Using a small, portable continuous centrifuge, approximately 600 liters of mesotrophic lake water were processed in 6 h. In the preliminary experiments, 20 μg DNA were purified (Sayler et al., unpublished data).

Two general methods for the direct isolation and purification of DNA from environmental samples are currently in use. The primary difference between the two procedures is that one removes the microbial cells from particulates prior to lysis [14,33], while the other lyses the cells directly in the presence of the particulates and then extracts the liberated DNA from the sample [22]. The former technique requires that the cells be removed from particulates by successive blendings and fractionations, followed by a gentle lysis procedure using enzymes and detergents. Large fragments (greater than 48 kb) of DNA are then isolated [14]. The latter procedure does not attempt to separate cells from particulates, but mechanically lyses the cells in the sediment, and then extracts the DNA through a series of alkaline extractions (Fig. 2). It is believed that DNA is extracted from particulates with a higher efficiency than from whole cells. Up to 99.9% of labeled DNA added to a clay soil was successfully extracted [23]. The mechanical lysis of the latter procedure is harsh, and the recovered DNA is sheared to less than 10 kb. This small fragment size does not interfere with hybridization probing, and the DNA appears to be of sufficiently high quality for most applications.

The direct lysis procedure has the advantage of being capable of separating intracellular DNA from extracellular DNA [22]. By preextracting sediments prior to lysis, sufficient extracellular DNA may be obtained for probing. Extracellular DNA may have a very short half-life in some environments [25], but may be significantly protected by adsorption to certain clays in other environments [1,11,19]. If extracellular DNA does persist in some sediments and

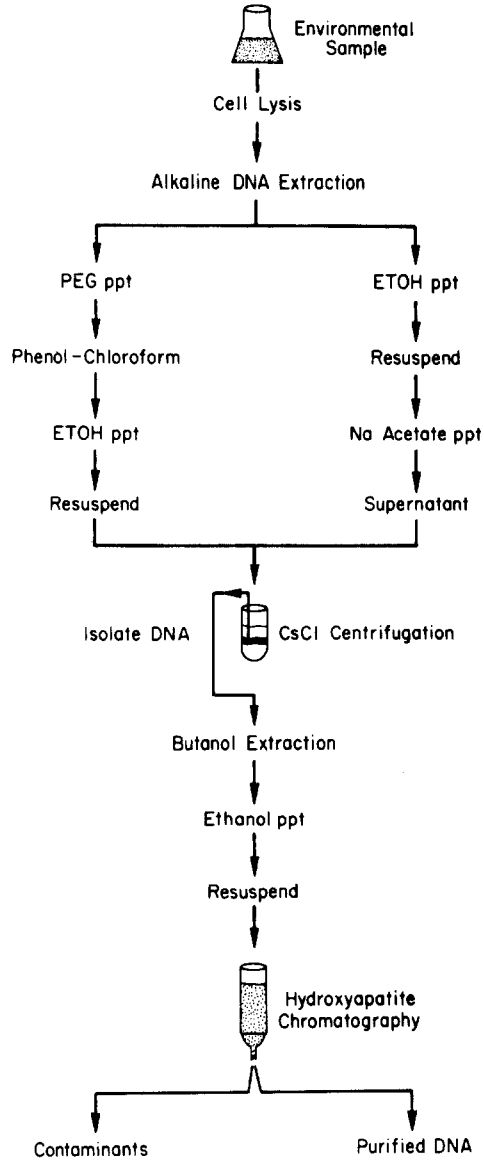

Fig. 2. A procedure for the direct lysis, extraction and purification of DNA from natural samples containing large amounts of particulates. Reproduced with permission from Ref. 22.

soils, it may represent a kind of fingerprint left behind by a prior state of a dynamic community. Preliminary data collected from a fresh water sediment indicated that the genetic composition of the extracellular fraction contained a significantly greater amount of sequences related to a catabolic plasmid (pSS50) [31] than was found in the intracellular fraction, either by colony hybridization or by total

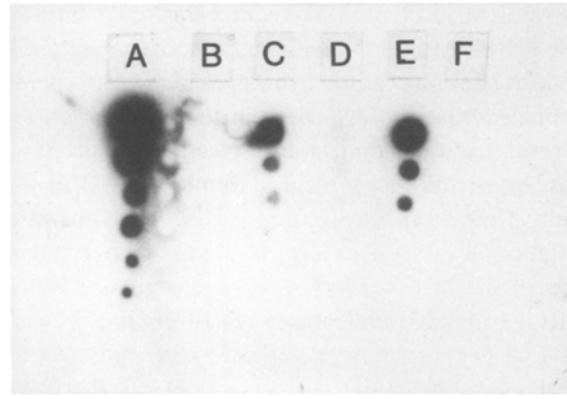

Fig. 3. Intracellular and extracellular DNA extracted from a fresh water sediment probed with pSS50, a plasmid containing genes coding for the catabolism of 4-CB. (A) pSS50: 0.1 μg, 0.01 μg, 0.001 μg, 0.0001 μg, 0.00001 μg. (B) Intracellular DNA: 10 μg, 5 μg, 1 μg, 0.5 μg, 0.05 μg. (C) Intracellular DNA (a) spiked with pSS50 DNA, (b): 5 μg a + 0.05 μg b, 2.5 μg a + 0.005 μg b, 0.5 μg a + 0.0005 μg b, 0.025 μg a + 0.00005 μg b. (D) Extracellular DNA; 5 μg, 1 μg, 0.18 μg. (E) *Alcaligenes* sp. A5 chromosomal DNA contaminated with pSS50 DNA: 10 μg, 5 μg, 1 μg, 0.5 μg, 0.1 μg, 0.05 μg. (F) *Bacillus cereus* DNA (as negative control): 10 μg, 5 μg, 1 μg, 0.5 μg, 0.1 μg. Note the presence of positive signals in the extracellular fraction and the lack of signal in the intracellular fraction.

DNA extraction (Fig. 3). pSS50-harboring strains had been found in this sediment 2 years before these recent data were collected, but repeated attempts to locate it with colony hybridization have failed. It is possible that strains harboring this plasmid are demonstrating significant population dynamics, undergoing a period of die-off, and that their DNA passed from the intracellular to the extracellular fraction. These data are preliminary and the conclusions are highly speculative. Further data must be collected before any of it can be substantiated.

Total DNA extraction is not superior to colony hybridization in all circumstances. If the isolation of a strain possessing a certain genotype is desired, it would not be possible to use direct DNA isolation techniques. It is also conceivable that in certain circumstances, colony hybridization may be more sensitive than direct isolation. If, for example, the numbers of a specific genotype are below the limits of detection of a direct isolation and probing procedure, but the strain is efficiently removed from its

environmental matrix and is culturable, colony hybridization may be more likely to detect its presence. The limits of detection possible using labeled probes are continually decreasing; it has been reported that 0.1 fg viral DNA can be detected in environmental samples (C. Gerba, personal communication).

PROBE SELECTION AND SPECIFICITY

Regardless of whether colonies or total DNA extracts are probed, the usefulness of the technique is largely determined by the probe. Probe specificity is a crucial factor in any environmental probing technique, and the proper controls must be performed to ensure that the investigator truly understands the data received. The most useful probes are usually those that contain only specific genes that are well characterized, so that when homology is detected between a probe and an environmental sample, the probability that one has actually detected a copy of the target sequence is quite high. Ambiguity frequently enters data interpretation when the exact nature of the probe is unknown. An example of this might be when a large uncharacterized plasmid encoding a particular function is used as a probe. The genes of interest may take up only a few kilobases, leaving the rest of the plasmid as largely uncharted territory. When using such a probe, one cannot be sure whether one has identified the target plasmid, or detected homology with another plasmid or the chromosome of another strain. When specific genes are cloned into a cloning vector, care must be taken to ensure that there is no homology between the vector and the environmental sample. This may be accounted for by initially probing the environmental sample with the vector alone. If no homology is detected, the vector and insert may be used directly as probe. If, however, there is homology between the vector and the sample, two options are available. The insert containing the sequence may be removed from the vector and used as a probe, or the samples may be prehybridized with the vector alone prior to hybridization with the labeled vector with insert. The latter option has the effect of allowing the vector to hybridize to all homologous sequences prior to the introduction of the probe, so that the only available sequences that are homologous to the probe are those homologous to the insert.

There are also instances where a probe may be too specific. In the case of function-specific probes, genes used as probes may not be strongly conserved across genera, and the probe may fail to detect the function in species other than the one from which the probe was originally cloned. In such a case, it may be necessary to use more than one gene coding for a specific function, cloned from a range of organisms possessing this function.

ENVIRONMENTAL ANALYSIS USING GENE PROBES

Among the earliest reported applications of colony hybridization in the analysis of environmental samples was in the detection and enumeration of pathogenic *E. coli* in water samples collected from homes in rural Thailand [6]. By screening colonies with a cloned gene coding for a specific enterotoxin, the need for a live mouse assay was bypassed, and up to a 10000-fold increase in sensitivity over conventional methods was achieved.

Gene probes have been used extensively in environmental microbiology and biodegradation research. Both colony hybridization and the hybridization of directly isolated DNA have been used in the determination of cells capable of degrading naphthalene in activated sludge [28], which may then be used for the calculation of second-order degradation rate coefficients. The potential for the degradation in sediments of environmental pollutants as diverse as simple aromatic hydrocarbons, such as toluene [29], and polychlorinated biphenyls [28] has also been calculated with the use of specific gene probes. Conventional techniques for these types of measurements would require the cultivation of the environmentally isolated bacteria with the pollutant incorporated into the growth medium. Such studies are time-consuming, and the results may be ambiguous due to the possibility of

cross-feeding or the appearance of false positives due to the growth of colonies on medium contaminants or micronutrients [29].

One of the most powerful environmental applications of the colony hybridization technique has been in the isolation of specific catabolic genotypes from environmental samples. Classical techniques require a time-consuming series of successive enrichment cultures to isolate cultures of organisms capable of degrading specific pollutants. By using colony hybridization, Pettigrew and Sayler [26] isolated strains (both singly and in a catabolic consortium) capable of degrading 4-chlorobiphenyl (4-CB) in approximately one-fourth the time required by the enrichment technique. The probe used for this study was pSS50, a 53.2 kb plasmid coding for the catabolism of 4-CB. Even though this is a rather large plasmid and undoubtedly codes for more genes than the 4-CB pathway, in this study it appeared to be fairly specific to the 4-CB catabolic phenotype. A comparison between gene probe and conventional methods for the isolation of specific catabolic phenotypes is presented in Table 4.

Gene probes may also be used in conjunction with more conventional techniques for the isolation and characterization of novel environmentally important genotypes. A highly specific probe for mercury resistance in Gram-negative cells has been developed [3] and used to study the adaptation of microbial communities to mercury contamination [4]. In addition to yielding valuable information on the response of natural communities to pollution, this study isolated Gram-negative colonies that were resistant to mercury, but did not share homology with the probe. This may represent a previously unknown mechanism for mercury resistance among Gram-negative bacteria. One of the areas of intense research in environmental microbiology concerns the efficacy and fate of genetically engineered microorganisms (GEMs) in the environment [32]. Gene probes are proving to be an indispensable method for evaluating both the fate of GEMs and the effects that they may have on the indigenous community [15,30]. Jain et al. [16] recently showed the applicability of the colony hybridization technique in measuring the maintenance and stability of introduced catabolic plasmids in ground water microcosms, while Holben et al. [14] probed direct DNA extracts to track the fate of genetically modified *Bradyrhizobium japonicum* in soil microcosms.

Evaluation of the effects of GEMs on the structure and function of the microbial communities they invade is providing the impetus for development of function-specific probes directed toward ecological parameters such as nitrogen fixation and photosynthesis. Until recently, the use of environmental nucleic acid probes has largely been restricted to applied concerns, such as biodegradation and heavy metal resistance. In an ongoing experiment to determine the effects of an introduced bacterial species on several ecological parameters in lake

Table 4

Comparison of times required by colony hybridization and enrichment technique for isolation of 4-CB-degrading bacteria[a]

Colony hybridization		Culture enrichment	
procedure	time (weeks)	procedure	time (weeks)
Cultivation	1	Enrichment	4
Detection and confirmation	0.5	Isolation and cultivation	1
Isolation	1	Phenotype confirmation	1
total:	2.5	total:	6

[a] Real comparisons on identical samples have not been made; values given are estimates based on previously published results.

microcosms (Sayler et al., in preparation), isolated DNA will be probed with DNA sequences specific for genes coding for CO_2 fixation, nitrogen fixation, and photosynthesis. The probe used for CO_2 fixation will be a combination of genes isolated from two different organisms because this gene is not completely conserved over the range of possible target organisms. It is felt that a probe made from these two organisms will give a wider base for probe specificity, thereby making it more general to CO_2 fixation.

The preceding discussion has almost exclusively concerned function-specific probes; a number of species-specific probes have also been developed for environmental analysis. Due to the highly conserved nature of ribosomal RNAs, most species-specific probes are constructed from fragments coding for either 16S rRNA or for 23S rRNA. Examples of this type of probe have been constructed for *Bacillus subtilis* [17] and the fluorescent group of *Pseudomonas* [8]. Another probe, specific to an *Arthrobacter* sp. isolated from a contaminated groundwater aquifer [27], has not been characterized as to its genetic origin, although it seems likely that this, too, is related to sequences coding for rRNA.

As the data base for rRNA sequences expands, more species- and genus-specific probes will become available. At present, kingdom-specific probes for identification of archaebacteria, eukaryotes and eubacteria may be constructed from signature sequences derived from comparisons of 16S rRNA (D. Stahl, personal communication). These synthetic oligonucleotides may be used to define the structure of natural communities relative to the representation of each of the three kingdoms. Synthetic oligonucleotides specific to such ecologically important groups as sulfate-reducing bacteria and methanogens may soon become available through research into rRNA sequences. Probing community nucleic acid extracts with probes directed toward rRNAs might be particularly useful due to the increased sensitivity that can be attained. Ribosomes are present at levels up to 10000 copies per cell, greatly increasing the number of targets for the probe [24].

A new genetic approach for the rapid characterization of microbial community complexity employing DNA:DNA reassociation kinetics is currently being developed [30]. The two complementary strands of DNA may be separated from each other by heat, and complementary strands will reassociate with one another when the temperature of the system is reduced. Reasssociation of the two strands follows second-order kinetics, with the rate of reassociation being dependent upon the number of similar sequences; the greater the number of similar sequences, the faster the reassociation [5]. This technique has been employed in the past for the determination of genome size in bacteria [10], and for estimating the number of repetitive sequences present in eukaryotic genomes [34]. If one considers a natural bacterial community as possessing a community genome, then reassociation kinetics may be used to determine the genetic complexity of this community. DNA sequences that are common in the community genome will reassociate faster than those which are not as well represented, yielding a reassociation curve that may be used as an index in describing that community. The genetic complexity of a natural community is determined by the numbers of each species present in the community. The more species present in the community, the greater the complexity, and the greater the second-order reassociation coefficient. This complexity is also dependent upon the relative numbers of species within the community. If a few species dominate the community in terms of population size, the complexity will be lower than if each species is equally represented.

Conventional techniques of assessing community diversity and complexity are difficult, time-consuming, and ultimately suspect because of the need for cultivation. Reassociation kinetics provides a very rapid method of analysis that does not require cultivation. A typical analysis of community diversity may take up to a year [20], while community reassociation kinetics may be completed within 2 weeks (Table 5). An example of preliminary reassociation kinetics data is shown in Figure 4. Curve C represents the reassociation kinetics of total DNA extracted from activated sludge. While the

Table 5

Comparison of times required by reassociation kinetics and conventional techniques for community complexity analysis[a]

Reassociation kinetics		Conventional analysis	
procedure	time	procedure	time
Cell harvesting	1 day	Cultivation	2–4 weeks
DNA extraction and purification	1 week	Isolation	2–4 weeks
Preparation of DNA for reassociation kinetics	2 days	Characterization	4–24 weeks
Reassociation kinetics and data analysis	2–3 weeks	Data analysis and reduction	4–24 weeks
total:	3–4 weeks		12–56 weeks

[a] Real comparisons on identical samples have not been made; values given are estimates based on previously published results. Differences exist in the quantity and quality of information obtained for either approach.

data are preliminary and incomplete, the results show the utility of the technique. The reassociation rate constant derived from these data may be used for comparison with other communities, or to monitor the change of this community over time. A practical application of this technique might also be to measure quantitatively the perturbation in community structure as a result of the introduction of a novel species, such as a GEM.

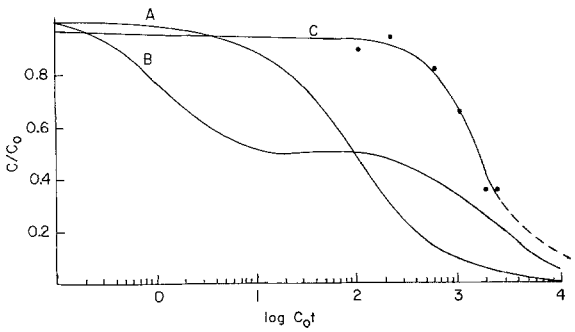

Fig. 4. Preliminary data showing the relative genetic complexity of activated sludge by reassociation kinetics (C/Co = fraction single-stranded DNA; C_oT = initial concentration of single-stranded DNA (mol/l) × time (min)). Curve A: theoretical reassociation curve of 10 species with equal representation. Curve B: theoretical reassociation curve of 200 species, with five species represented at a concentration 40 times greater than that of the remaining 195 species. Curve C: preliminary reassociation curve of activated sludge DNA.

FUTURE PROSPECTS FOR GENE PROBES IN ENVIRONMENTAL ANALYSIS

The possibilities of community structure and function analysis through the use of gene probes are almost unbounded. The major limitation of the technique, in its current state, is the lack of useful probes. As the molecular biology of naturally occurring bacteria becomes better understood, more probes will become available. New applications for molecular genetics in environmental microbiology are continually being found, and as the study of molecular genetics progresses, environmental microbiology will undoubtedly benefit.

It is not suggested that gene probes will replace all conventional biochemical and physiological tests, but that they may be used in conjunction with these tests to provide a more complete picture of the state of a microbial ecosystem. There are many instances, however, where the use of gene probes will greatly facilitate environmental analysis. Gene probes will yield a more complete description of the genetic content of a microbial community, quickly and efficiently, than can be possible with conventional techniques.

REFERENCES

1 Aardema, B.W., M.G. Lorenz and W.E. Krumbein. 1983. Protection of sediment-adsorbed transforming DNA against enzymatic inactivation. Appl. Environ. Microbiol. 46: 417–420.

2 Bakken, L.R. 1985. Separation and purification of bacteria from soil. Appl. Environ. Microbiol. 49: 1482–1487.

3 Barkay, T., D.L. Fouts and B. H. Olson. 1985. Preparation of a DNA gene probe for detection of mercury resistance in Gram-negative bacterial communities. Appl. Environ. Microbiol. 49: 686–692.

4 Barkay, T. and B.H. Olson. 1986. Phenotypic and genotypic adaptation of aerobic heterotrophic sediment bacterial communities to mercury stress. Appl. Environ. Microbiol. 52: 403–406.

5 Britten, R.J. and D.E. Kohne. 1968. Repeated sequences in DNA. Science 161: 529–540.

6 Echeverria, P., J. Seriwatana, O. Chityothin, W. Chaicumpa and C. Tirapat. 1982. Detection of enterotoxigenic *Escherichia coli* in water by filter hybridization with three enterotoxin gene probes. J. Clin. Microbiol. 16: 1086–1090.

7 Faegri, A., V.L. Torsvik and J. Goksoyr. 1977. Bacterial and fungal activities in soil: separation of bacteria and fungi by a rapid fractionated centrifugation technique. Soil Biol. Biochem. 9: 105–112.

8 Festl, H., W. Ludwig and K.H. Schleifer. 1986. DNA hybridization probe for the *Pseudomonas fluorescens* group. Appl. Env. Microbiol. 52: 1190–1194.

9 Geesey, G.G. 1982. Microbial exopolymers: ecological and economic considerations. Am. Soc. Microbiol. News 48: 9–14.

10 Gillis, M., J. De Ley and M. De Cleene. 1970. The determination of molecular weight of bacterial DNA from renaturation rates. Eur. J. Biochem. 12: 143–153.

11 Greaves, M.P. and M.J. Wilson. 1970. The degradation of nucleic acids and montmorillonite-nucleic acid complexes by soil microorganisms. Soil Biol. Biochem. 2: 257–268.

12 Grunstein, M. and D.S. Hogness. 1975. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci USA 72: 3961–3965.

13 Hodgson, A.L.M. and W.P. Roberts. 1983. DNA colony hybridization to identify *Rhizobium* strains. J. Gen. Microbiol. 129: 207–212.

14 Holben, W.E., J.R. Jansson and J.M. Tiedje. 1987. Methods for assessing the fate of genetically engineered microorganisms in soil. Abstr. 87th Annu. Meet. Am. Soc. Microbiol., Q-131, p. 303.

15 Jain, R.K., R.S. Burlage and G.S. Sayler. 1987. Methods for detecting recombinant DNA in the environment. CRC Crit. Rev. Biotechnol. (in press).

16 Jain, R.K., G.S. Sayler, J.T. Wilson, L. Houston and D. Pacia. 1987. Maintenance and stability of introduced genotypes in groundwater aquifer material. Appl. Environ. Microbiol. 53: 996–1002.

17 Kraus, J., W. Ludwig and K.H. Schliefer. 1986. A cloned 23S rRNA gene fragment of *Bacillis subtilis* and its use as a hybridization probe of conserved character. FEMS Microbiol. Lett. 33: 89–93.

18 Litchfield, C.D. and P.L. Seyfried (eds.) 1979. Methodology for Biomass Determinations and Microbial Activities in Sediments. ASTM, Philadelphia.

19 Lorenz, M.G., B.W. Aardema and W.E. Krumbein. 1981. Interaction of marine sediment with DNA and DNA availability to nucleases. Marine Biol. 64: 225–230.

20 Mallory, L.M. and G.S. Sayler. 1983. Heterotrophic bacterial guild structure: relationships to biodegradative populations. Microb. Ecol. 9: 41–55.

21 McDaniel, J.A. and D.G. Capone. 1985. A comparison of procedures for the separation of aquatic bacteria for subsequent direct enumeration. J. Microbiol. Methods 3: 291–302.

22 Ogram, A.V., G.S. Sayler and T. Barkay. 1987. The extraction and purification of microbial DNA from sediments. J. Microbiol. Methods 7: 57–66.

23 Ogram, A.V., G.S. Sayler, D. Gustin and R. Lewis. 1987. DNA sorption to soils and sediments. Environ. Sci. Technol. (in press).

24 Olsen, G.J., D.J. Lane, S.J. Giovanoni, N.R. Pace and D.A. Stahl. 1986. Molecular ecology and evolution: a ribosomal RNA approach. Annu. Rev. Microbiol. 40: 337–355.

25 Paul, J.H., W.H. Jeffrey and M. De Flaun. 1987. Dynamics of extracellular DNA in the marine environment. Appl. Environ. Microbiol. 53: 170–179.

26 Pettigrew, C.A. and G.S. Sayler. 1986. The use of DNA: DNA colony hybridization in the rapid isolation of 4-chlorobiphenyl degradative bacterial phenotypes. J. Microbiol. Methods 5: 205–213.

27 Sayler, G.S., C. Harris, C. Pettigrew, D. Pacia, A. Breen and K. Sirotkin. 1987. Evaluating the maintenance and effects of genetically engineered microorganisms. Dev. Ind. Microbiol. 27: 135–149.

28 Sayler, G.S., R.K. Jain, L. Houston, A. Ogram, C. Pettigrew, J. Blackburn and W. Riggsby. 1987. Applications for DNA probes in biodegradation research. In: Proceedings of the 4th International Conference on Microbiology and Ecology (in press).

29 Sayler, G.S., M.S. Shields, E. Tedford, A. Breen, S. Hooper, K. Sirotkin and J. Davis. 1985. Application of DNA-DNA colony hybridization to the detection of catabolic genotypes in environmental samples. Appl. Environ. Microbiol. 49: 1295–1303.

30 Sayler, G.S. and G. Stacey. 1986. Methods for evaluation of microorganism properties. In: Biotechnology Risk Assessment: Issues and Methods for Environmental Introduction (Fiskell, J. and V.T. Covello, eds.), pp. 35–55, Pergamon Press, New York.

31 Shields, M.S., S.W. Hooper and G.S. Sayler. 1985. Plasmid

mediated mineralization of 4-chlorobiphenyl. J. Bacteriol. 163: 882–889.

32 Stotzky, G. and H. Babich. 1984. Fate of genetically engineered microbes in natural environments. Recomb. DNA Tech. Bull. 7: 163–188.

33 Torsvik, V.L. 1980. Isolation of bacterial DNA from soil. Soil Biol. Biochem. 12: 15–21.

34 Wills, J.W., B.A. Lasker, K. Sirotkin and W. Riggsby. 1984. Repetitive DNA of *Candida albicans*: nucleae and mitochondrial components. J. Bacteriol. 157: 918–924.