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Simple, Rapid Method for Direct Isolation of Nucleic Acids from Aquatic Environments

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Direct isolation of nucleic acids from the environment may be useful in several respects, including the estimation of total biomass, detection of specific organisms and genes, estimations of species diversity, and cloning applications. We have developed a method that facilitates the concentration of microorganisms from aquatic samples and the extraction of their nucleic acids. Natural water samples of 350 to >1,000 ml are concentrated on a single cylindrical filter membrane (type SVGS01015; Millipore Corp., Bedford, Mass.), and cell lysis and proteolysis are carried out within the filter housing. Crude, high-molecular-weight nucleic acid solutions are then drawn off the filter. These solutions can be immediately analyzed, concentrated, or purified, depending on the intended application. The method is simple, rapid, and economical and provides high-molecular-weight chromosomal DNA, plasmid DNA, and speciated RNAs which comigrate with 5S, 16S, and 23S rRNAs. The methods presented here should prove useful in studying both the ecology and the phylogeny of microbes that resist classical culture methods.

One of the major limitations to research in microbial ecology has been the inability to isolate and grow in culture the vast majority of bacteria found in nature. Ferguson et al. (3) studied the effect of confinement on marine bacteria and found that >99.9% of the bacteria present in the initial samples were nonculturable. Hoppe (8) found that metabolically active microorganisms that did not form colonies on agar media represented the predominant marine flora and estimated that culturable bacteria represented only 0.01 to 12.5% of the viable bacterial population. Microbial ecologists, therefore, have been limited to describing a portion of the culturable population or working with ill-defined, mixed cultures under conditions that are meant to mimic natural systems. Both approaches are less than satisfactory because either a major portion of the population is ignored or the functional roles of specific microorganisms cannot be determined. Further complicating the picture is the existence of microbes that are able to grow on laboratory media but become recalcitrant to culture, though still metabolically active, after exposure to the environment (2, 19). Among the organisms that have been shown to become viable but nonculturable are *Escherichia coli*, *Salmonella typhimurium*, and certain *Vibrio* spp. (1, 2, 19). Because of the extensive research done with these organisms, they are likely candidates for use as genetically engineered microorganisms (2). Tracking such organisms, when and if they are released into the environment, will require methods which are able to detect microorganisms that culture methods do not.

Microbial ecologists have recently begun to apply molecular techniques to the study of microorganisms in natural systems, obviating the need for cell culture. Paul and Myers (17) demonstrated that DNA could be isolated and quantitated directly from aquatic samples. More recently, Furhman et al. (4) described a different method for the extraction of microbial DNA from natural water samples, and Ogram et al. (13) as well as Holben et al. (7) have devised methods for the direct isolation of microbial DNA from sediments. The utility of these methods lies in the fact that the purified DNAs can be used to identify, classify, and measure the abundance of microbes that cannot be studied in any other way. Examples include the detection of *Bradyrhizobium japonicum* at very low numbers in soil samples (7), the detection of *nif* genes in aquatic sediments (13), the study of the response of ruminal flora to antibiotics (21), the identification of single microbial cells (5), and the production of gene libraries from environmental DNA (15).

Microbial ecologists for many years have struggled with the limitations imposed by sampling methodology. Typically, samples collected from natural ecosystems are limited to milliliters or grams of water or sediment, respectively, mainly because of constraints imposed by culture techniques. In cases where much larger samples are obtained, e.g., when microcosms are used, the number of replicates that can be taken is constrained by the volume of the given microcosm. Thus, new sampling methods that can increase sample size while holding collection and processing time to a minimum, allowing for greater repeatability, are needed.

In this paper, we describe a method by which (i) the microbial biomass can be concentrated from relatively large volumes of water and (ii) high-molecular-weight DNA, as well as intact RNA species, can be easily and rapidly recovered. The method combines the advantages of rapid sample collection, stable sample transport, and cost effectiveness and can be used to produce a large number of replicates per sample site. In addition, the method is readily used on board ship or in the field and should prove amenable to automation in the near future.

MATERIALS AND METHODS

Sampling sites. Water samples were collected in May and June 1988 at two sites in the Chesapeake Bay. One site is located near Love Point (39°3.1′ N, 76°17.0′ W), at the mouth of the Chester River, and the second site is in Baltimore Harbor (39°16.6′ N, 76°35.6′ W). Samples were collected by using ethanol-sterilized 10-liter Niskin bottles deployed from the research vessel R/V *RIDGELY WARFIELD* at depths of 1 to 5 m.

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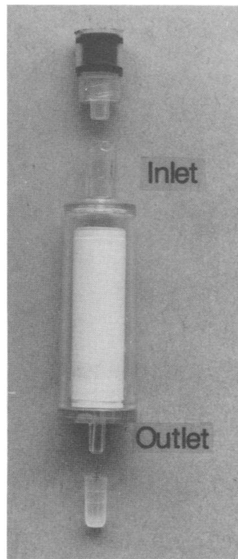

FIG. 1. Sterivex-GS filter unit, showing inlet and outlet port caps. The inlet port cap is the luer-lock end of a 3-ml disposable syringe, and the outlet port cap is a syringe tip cover cut in half.

Concentration by filtration. After having been brought on deck, water samples were transferred to sterile 2-liter Nalgene flasks (Nalgene Labware DN., Nalge/Sybron Corp., Rochester, N.Y.) and aseptically pumped through Sterivex-GS filters (Millipore Corp., Bedford, Mass.) via a peristaltic pump. The Sterivex-GS unit is a mixed cellulose ester filter of 0.22-μm pore size mounted inside a cylindrical polyvinyl chloride housing designed for pressure-driven filtration (Millipore products guide; Fig. 1). Water was pumped at a flow rate of approximately 100 ml/min and a pressure of 15 to 20 lb/in^2 until the desired sample volume was collected or the flow rate slowed perceptibly. Without the use of prefiltration, sample volumes in copiotrophic bay water ranged from 350 ml to >1 liter. Total sampling time, once the water sample was brought aboard, was less than 15 min in all cases. Filters were washed with 10 ml of sterile SET buffer (20% sucrose, 50 mM EDTA, 50 mM Tris hydrochloride [pH 7.6]; 18). Excess buffer was forced off the filter by using an air-filled 50-ml syringe, the inlet and outlet were capped, and the filters were stored at −20°C until processed.

Cell lysis and nucleic acid extraction. Nucleic acids were extracted essentially by the method of Marmur (12), with modifications for extractions to be accomplished within the Sterivex-GS filter housing. All water, reagents, and buffers used in the extractions were prepared free of RNase activity as described by Maniatis et al. (11). The filter units were thawed, and 1.8 ml of SET buffer was added into the inlet of the filter unit with a 25-gauge, 5/8-in. (ca. 1.6-cm) needle (this needle size will not puncture the filter membrane that covers the top of the internal cylinder). After addition of SET buffer, 62 μl of a freshly made lysozyme solution (5 mg/ml in 10 mM Tris hydrochloride [pH 8], 1 mM EDTA, 10 mM NaCl) was added by micropipettor. The inlet was recapped, the contents were mixed by inversion, and the filter unit was placed on ice for 15 min. Sodium dodecyl sulfate (16 μl of a 25% stock solution) was added, and the filter unit was incubated at room temperature with constant rotation to keep the entire filter in contact with the reagents.

The roller unit was designed and built by one of us (W. L. Straube) specifically for use with the Sterivex-GS filter units. The unit consists of four steel roller bars 257 mm in length and 19 mm in diameter. The bars are arranged in a parallel array at a distance of 29 mm on center. Each bar has a small cog at one end, and all are chain driven by a 115-V, 60-cycle electric motor. The unit will hold up to nine filter units at a time, and each is rotated at a rate of 32 rpm.

After 1 h, 50 μl of proteinase K solution (20 mg/ml in double-distilled H_2O) was added, and the filter units were placed on the roller at room temperature for an additional 3 to 4 h. Longer incubation times did not result in increased nucleic acid yield. To remove the crude lysate from the filter unit, a 5-ml syringe was attached to the inlet, and the lysate was drawn into the syringe. After addition of 1 ml of SET buffer to the filter unit, the unit was placed on the roller for 5 min to wash the filter. The wash buffer was removed as described above and pooled with the crude lysate. The lysates were then purified and concentrated immediately or stored at −20°C until processed.

Purification and concentration. Nucleic acids were purified and concentrated from the crude lysates by one of three methods: (i) ethanol precipitation, (ii) ammonium acetate treatment followed by ethanol precipitation, or (iii) buoyant density centrifugation followed by ethanol precipitation.

Ethanol precipitation was performed as described by Maniatis et al. (11). Precipitates were dried in a Savant SpeedVac (Savant Instruments, Inc., Farmingdale, N.Y.) and suspended in 200 μl of TE buffer (10 mM Tris hydrochloride [pH 7.6], 1 mM EDTA).

Ammonium acetate treatment was performed by adding 0.5 volume (approximately 1.5 ml) of 7.5 M ammonium acetate to the crude lysate, mixing the contents gently, and incubating the mixture at room temperature for 15 min. The mixture was centrifuged at 14,500 × g for 5 min at room temperature to pellet dissociated proteins. The supernatants were carefully transferred to clean tubes and ethanol precipitated as described above. Pellets were suspended in 300 μl of sterile water, treated a second time with ammonium acetate, and ethanol precipitated. The final pellets were suspended in 100 μl of TE buffer.

Buoyant density centrifugation in CsCl-ethidium bromide gradients was performed as described elsewhere (11). Although distinct bands of linear DNA were obtained from sample sizes as small as 350 ml, in many cases the crude lysates did not yield a visible band in the density gradients. In such instances, the entire aqueous phase of the gradient was removed, leaving behind the RNA pellet and protein floc. The collected aqueous phase was dialyzed against TE buffer (11), followed by ethanol precipitation. The ethanol-precipitated pellets were dried as before and suspended in 40 to 200 μl of TE buffer.

Nucleic acid extraction from pure cultures. Pure cultures of *E. coli* HB101, *Vibrio cholerae* 62746 (supplied by J. Kaper, Center for Vaccine Development, University of Maryland, Baltimore), *Plesiomonas shigelloides* ATCC 14029, *Vibrio alginolyticus* ATCC 17749, and *Aeromonas hydrophila* SSU were grown overnight in LB broth (11). A 5-ml amount of each culture was added to 50 ml of 1% saline. The mixture was drawn into a sterile 60-ml syringe and concentrated on a Sterivex-GS filter. Nucleic acids were extracted and purified as described above.

DNase and RNase treatments. A stock solution of DNase-free pancreatic RNase (RNase A; Boehringer-Mannheim Biochemicals, Indianapolis, Ind.) was prepared as described by Maniatis et al. (11). RNase was added to nucleic acid preparations at a final concentration of 250 ng/μl. The

reactions were incubated at room temperature for 15 min and stored at −20°C until analyzed.

RNase-free DNase (23 U/μl) was obtained from Boehringer-Mannheim Biochemicals. Digests were carried out in 5 mM $MgSO_4$ and 100 mM sodium acetate (pH 5.0) for 1 h at room temperature at an enzyme concentration of 2.3 U/μl. Digests were held at −20°C until analyzed.

Digestion with restriction enzymes. DNAs that had been purified by treatment with ammonium acetate or by centrifugation in CsCl-ethidium bromide gradients were digested with restriction endonucleases. All digestions were performed as recommended by the enzyme manufacturers. Negative controls, in which a sample of DNA was incubated in restriction endonuclease buffer for at least 1 h at 37°C, were run with each digestion.

Preparation of plasmid DNA by alkaline lysis. Plasmid DNA was obtained from pure cultures by filtering 3 ml of an overnight culture diluted to 50 ml in 1% saline, using Sterivex-GS filters. Filters were washed with 10 ml of SET buffer as described above, followed by addition of 1.5 ml of SET buffer, 280 μl of 1 N NaOH, 40 μl of sodium dodecyl sulfate solution (25% in double-distilled H_2O), and 6 μl of DNase-free RNase (10 mg/ml). The filters were incubated on the roller for 30 min at 4°C. Crude lysates were withdrawn, and the filters were washed as before. Lysates were placed in sterile 15-ml Corex tubes, and 1.5 ml of 3 M sodium acetate was added. The tubes were mixed gently and incubated on ice for 30 min, followed by centrifugation at 17,000 × *g* and 4°C for 10 min. The supernatants were transferred to new tubes, and an equal volume of isopropyl alcohol was added. The tubes were mixed gently and incubated at −70°C for 30 min. Plasmid DNA was pelleted by centrifugation at 20,000 × *g* and 4°C for 10 min. Pellets were washed successively with 1 ml each of 70, 95, and 100% ethanol and then were dried and suspended in 100 μl of sterile double-distilled H_2O.

Agarose gel electrophoresis. The nucleic acids derived from the procedures described above were analyzed by agarose gel electrophoresis, using known DNA and RNA as reference standards. Gel concentrations ranged from 1.5 to 2.0% SeaKem GTG-type agarose (FMC Corp., Philadelphia, Pa.) in 1× TAE buffer (11). Gels typically were run between 5.0 and 5.5 V/cm.

Determination of DNA yield. The concentrations of DNA in purified samples and DNase-treated controls were determined by fluorometric assay, using Hoechst 33258 as described by Paul and Myers (17) and an Aminco-Bowman spectrophotofluorometer. Total cell counts in water samples were determined by acridine orange direct counting (6). Correlation of DNA concentration with cell counts was determined by the general linear models procedure of the statistics and analysis system available on the University of Maryland IBM 4381 computer.

RESULTS

After analysis of test results obtained for a number of environmental samples, it was concluded that the concentration and extraction method presented here yields a positive, linear correlation between cell number and recovered DNA. Figure 2 represents triplicate samples of various volumes collected at both Love Point and Baltimore Harbor and extracted and purified by treatment with ammonium acetate as described in Materials and Methods. The regression line shown fits the empirical data with a correlation coefficient of 0.96. When the samples were treated with

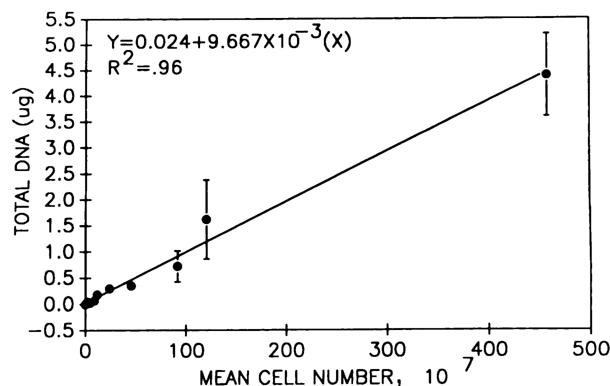

FIG. 2. DNA recovery from water samples collected in Baltimore Habor and Love Point in the Chesapeake Bay.

DNase before quantification, they yielded background fluorometric response, with no correlation observed with cell number or sample volume. Crude lysates treated by ethanol precipitation alone did not show a correlation between cell numbers and DNA concentration (data not shown). The lack of correlation may have arisen from the presence of impurities interfering with the fluorometric assay. From the regression line, recoveries of 1.0 ng of DNA per 10^6 cells were calculated for the environmental samples. Extraction of DNA from a pure culture of *V. cholerae* by this method yielded a recovery of 10.4 ng of DNA per 10^6 cells.

Figure 3 shows results of extractions from both environmental samples and pure cultures, along with DNA and RNA standards. Environmental samples yielded high-molecular-weight DNA as well as RNAs which comigrated with 23S, 16S, and 5S rRNAs. Pure cultures of *E. coli*, *V. cholerae*, *V. alginolyticus*, *P. shigelloides*, and *A. hydrophila* also yielded high-molecular-weight DNA and RNA species. Nucleic acids isolated from pure cultures of *E. coli* and *V. cholerae* are shown in Fig. 3. Treatment of the samples with DNase or RNase differentiated RNA and DNA and confirmed the presence of speciated RNAs (Fig. 3B and C).

DNAs purified from crude lysates by CsCl-ethidium bromide gradient centrifugation were treated with restriction endonucleases (Fig. 4). DNAs incubated in enzyme buffer at 37°C without added enzyme were not digested, which indicated the absence of contaminating nucleases. Digesting the DNA with a four-base cutter (*Sau*3AI) resulted in a predominance of fragments of less than 300 base pairs. Digestion with a six-base cutter (*Ssp*I) yielded predominantly larger fragments. These results are consistent with the known specificities of these enzymes. DNAs purified by treatment with ammonium acetate could also be cleaved with restriction endonucleases, although the extent of cleavage was reduced (data not shown). DNAs purified by either CsCl-ethidium bromide centrifugation or ammonium acetate treatment have also proven useful as targets for DNA probes or as templates for polymerase chain elongation (C. C. Somerville, I. T. Knight, W. L. Straube, and R. R. Colwell, Abstr. 1st Int. Conf. Release Genet. Eng. Micro-organisms 1988, no. 40, p. 20, and no. 70, p. 36).

Plasmid DNA was recovered from bacterial cultures after collection and lysis on Sterivex-GS filters (Fig. 5). Plasmid DNA could be detected by using the general lysozyme-sodium dodecyl sulfate lysis method described in Materials and Methods and the alkaline lysis designed specifically for plasmid isolation.

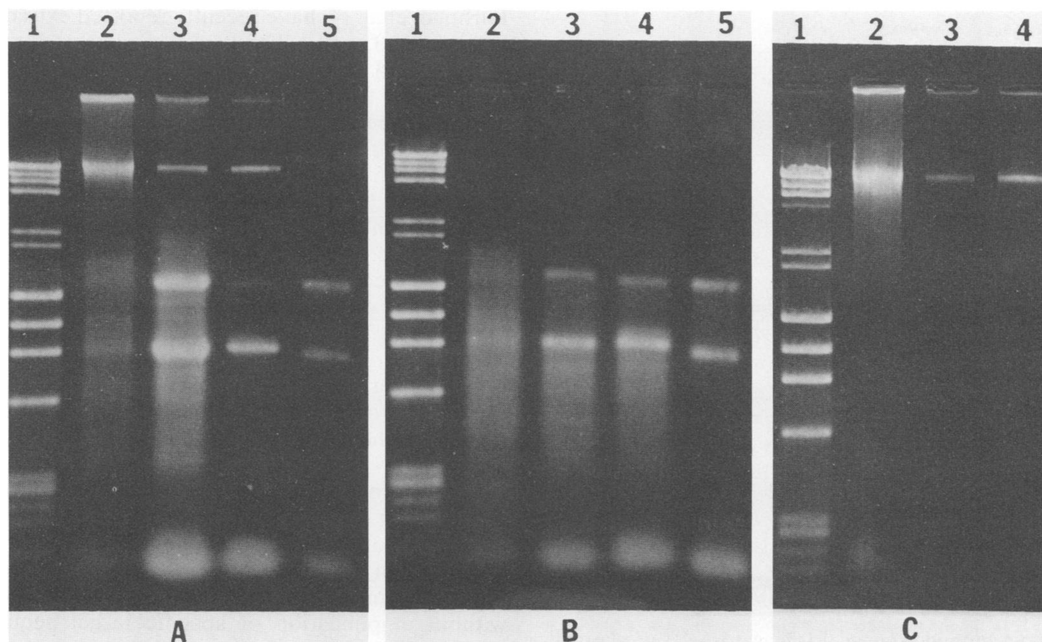

FIG. 3. Ethidium bromide-stained 2.0% agarose gels of nucleic acid extracts. (A) Extracts untreated; (B) extracts treated with DNase; (C) extracts treated with RNase. Lanes: 1, molecular weight ladder composed of lambda *Hin*dIII and φX174 *Hae*III restriction fragments; 2, 15 μl of ammonium acetate-purified extract obtained from a 500-ml water sample collected at Love Point in the Chesapeake Bay; 3 and 4, 10 μl of concentrated extracts of *E. coli* HB101 and *V. cholerae* 62746, respectively; 5 (A and B), 23S, 16S, and 5S rRNA from *E. coli* MRE600.

DISCUSSION

Microbial ecologists now recognize the enormous potential benefits accruing from application of molecular techniques to the study of the environment. New methods designed specifically for ecological applications have begun to emerge. The method presented here combines several important advantages: (i) a readily portable collection and extraction method which can easily be performed on board ship; (ii) sampling times sufficiently rapid to minimize population changes; (iii) sample volumes 30 to 100 times larger than those usually used for microbiological enumeration and identification; (iv) compact and stable transport of fixed samples to the laboratory without chemical additives; and (v) recovery of high-molecular-weight chromosomal DNA, plasmid DNA, and RNAs within 1 day.

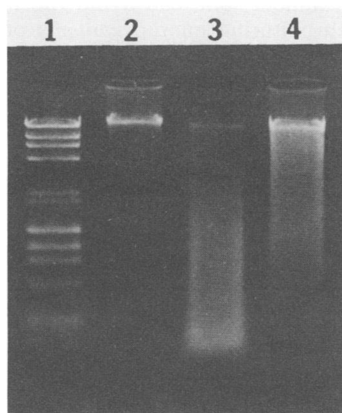

FIG. 4. Ethidium bromide-stained 1.5% agarose gel of purified extracts treated with restriction endonucleases. Lanes: 1, molecular weight ladder composed of lambda *Hin*dIII and φX174 *Hae*III restriction fragments; 2, chromosomal DNA extracted from 350 ml of Baltimore Harbor water and purified by buoyant density centrifugation; 3 and 4, the same DNA digested with *Sau*3AI and *Ssp*I, respectively.

Given the inability to obtain most environmental isolates in pure culture, a logical approach is to isolate and study the nucleic acids of microorganisms directly, without culturing the organisms. However, the isolation of nucleic acids first requires that the bacterial cells be harvested from the environment. In the laboratory, cells are typically harvested by centrifugation from liquid culture, but bacteria that are incubated under oligotrophic conditions often resist pelleting (4; unpublished observations). We have found that a more efficient means of recovering procaryotes from aquatic environments is through the use of ultrafiltration. Filtration is limited, however, by the capacity of the filtration apparatus. The Sterivex-GS filter has a much higher filtration capacity than do other membrane filters of similar pore size and surface area. Furhman et al. (4) used ultrafiltration to collect bacteria from aquatic samples and were able to work with larger samples than those collected in the study reported here, in part because of the use of prefiltration. We have chosen not to recommend prefiltration with this method because the results of preliminary experiments indicated that bacteria were retained on membrane prefilters with pore sizes as large as 8 μm. In subsequent tests with glass-fiber filters (type A/E; Gelman Sciences, Inc., Ann Arbor, Mich.), 10 to 15% of the total bacteria from the sample, as determined by acridine orange direct counts, were retained on the prefilters. Avoiding prefiltration reduces the total volume of sample that can be concentrated but ensures that all bacteria in the sample, with the exception of those that pass through 0.22-μm membranes (24), are collected. This factor may be important for detecting bacteria colonizing particulates and microinvertebrates which would be selectively retained on most prefilters. Filter systems that remove particulates with-

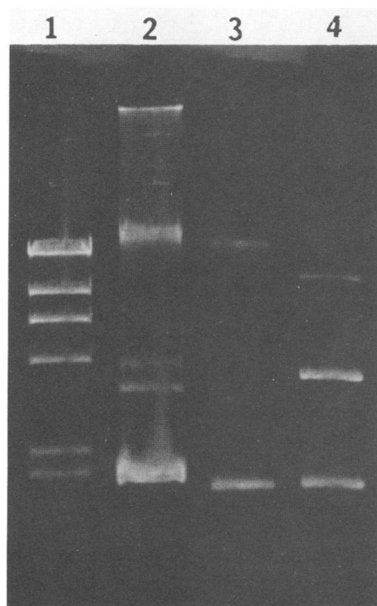

FIG. 5. Ethidium bromide-stained 1.0% agarose gel of plasmid DNA extracts. Lanes: 1, molecular weight ladder composed of lambda *Hin*dIII restriction fragments; 2 and 3, concentrated extracts of *E. coli* JM101, transformed with pUC8, obtained by filtering equal amounts of cells onto Sterivex-GS units and by using enzymatic lysis (lane 2) and alkaline lysis (lane 3) to obtain plasmid DNA; 4, purified pUC18 DNA.

out significantly decreasing the biomass collected, efficiently separate eucaryotes and procaryotes, and/or collect the ultrafilterable bacteria would improve our ability to study marine microbiota and should be the subjects of continued research.

The sample size used in this study is advantageous in that sampling time is kept to a minimum. Ferguson et al. (3) noted that aquatic samples held in containers underwent shifts in species composition in as little as 5 h. Samples requiring transport to the laboratory for processing, therefore, can show significant changes in population structure during transit.

Sampling by the method reported here requires very little specialized equipment and is readily completed on site. Also, large numbers of samples can be collected, and the frozen filters can be transported stably and conveniently. The number of samples or replicates that can be collected is limited mainly by the number of available peristaltic pumps or pump heads. We have found that the use of two pumps, each with a single pump head, enables us to collect a minimum of eight samples per hour. Approximately 40 or more samples per day can be collected easily.

The cell lysis and nucleic acid purification methods used are modifications of recognized techniques (11, 12, 18). Addition of reagents to the filter chamber makes the method easier, less expensive, and less prone to nuclease contamination than are traditional extraction procedures or extractions from flat filters. Currently, the procedure yields 1.0 ng of purified, high-molecular-weight DNA per 10^6 cells from environmental samples. Pure cultures grown in the laboratory yielded 10.4 ng/10^6 cells. Paul and Myers (17) reported yields of 8.4 and 13.6 ng of DNA per 10^6 cells in pure culture and 10.0 ng/10^6 cells from the environment. Paul et al. (16) later reported a lower estimate of 5.7 ng/10^6 cells, and Furhman et al. (4) have recently reported extracting 2.8 ng of DNA per 10^6 cells. These results suggest that the method described in this paper provides an extraction efficiency comparable to those of other methods that have been reported. Indeed, DNA quantitation can be an efficient means of estimating microbial numbers. Unlike the case with other methods for DNA extraction from aquatic samples, RNA species can be purified simply and rapidly from both pure cultures and environmental samples by the method described here.

High-molecular-weight DNA recovered from environmental samples, concentrated by ethanol precipitation and without further purification, provides a target for DNA or RNA probes. A conservative estimate of yield is ca. 30 to 100 ng of DNA per filter unit (based on 10^5 organisms per ml, sample size of 300 to 1,000 ml, and yield of 1 ng of DNA per 10^6 cells). The DNA yield from a single filter extraction when assayed by probe methods capable of detecting specific DNAs within 1 pg of target (7) should enable the detection of organisms which represent 0.003% or less of the microbes present. In other words, it is theoretically possible to detect organisms in the aquatic environment that are present at 1 to 3 cells per ml by using a single Sterivex-GS filter unit and without amplification of specific target sequences. Even greater sensitivity could be gained by pooling extracts or by using the polymerase chain reaction method to amplify target DNAs (20; R. J. Steffan and R. M. Atlas, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, Q6, p. 284).

With further purification, DNAs extracted by this method can be cut with restriction endonucleases. Figure 4 shows DNA recovered from Baltimore Harbor which has been cleaved with *Sau*3AI and *Ssp*I restriction enzymes. Restriction digests of pure, homogeneous DNA preparations produce distinct banding on agarose gels. Heterogeneous mixtures of DNAs recovered from the environment in this study did not yield distinct bands upon digestion but did appear to have been cleaved with proper specificity. As mentioned above, cleavage was not a function of contaminating nucleases.

Purified DNAs can be cut with restriction enzymes and ligated into conventional vector DNAs to produce environmental gene libraries, which can be useful in environmental studies (4, 15). By cloning and replicating DNAs directly from the environment, one can avoid the many problems associated with culturing microorganisms present in environmental samples. Instead, it is possible to detect and identify environmentally and phylogenetically important genes of organisms that were previously unobtainable.

An exciting application is the rapid and easy isolation of high-molecular-weight RNAs, from both pure cultures and environmental samples, that comigrate with rRNA standards. These RNAs can be used as targets for the detection and monitoring of specific microorganisms and to make cDNA libraries of rRNA genes. In fact, DNA probes specifically targeted to rRNAs or their genes have already found important application in microbial ecology (5, 14, 15). The abundance of ribosomes in bacterial cells means that rRNAs are highly amplified relative to their encoding genes. In an actively growing *E. coli* cell, rRNAs have been reported to account for as much as 20% of the total dry weight of the cell (15). Thus, targeting probes to specific rRNAs increases the possibility of detecting a specific organism. One strategy that can be followed in monitoring genetically engineered microorganisms released to the environment is to induce a site-specific mutation in a noncritical area of an rRNA gene to serve as a specific marker or signature sequence for the

organism, which can then be detected by specific oligomer probes. Rapid recovery of rRNAs from environmental samples without the need for cell culture would be integral to an effective monitoring protocol.

Several studies have used RNAs and DNA isolated directly from environmental samples to estimate species diversity, measure relative species abundance or activity, and classify member species of the microbial community (15, 21–23). The approach used to describe high-diversity habitats was to produce a gene library from total environmental DNA, screen the library for the presence of ribosomal DNAs, and sequence those clones to identify community members (15). We propose instead that purified RNAs be used to produce cDNA libraries composed exclusively of ribosomal DNAs. With this method, 16S rRNA can be isolated and purified and, by using one of the universal 16S probe-primers (15), used to produce cDNA as previously described (11). Such oligomers have already been used to sequence 16S rRNAs (9), which also requires the polymerization of a nascent chain from the rRNA template. The primer which anneals at bases 1392 to 1406, according to the *E. coli* 16S rRNA nucleotide numbering system, could be used to generate a nearly full-length cDNA. Such a scheme would vastly increase efficiency in creating rDNA libraries relative to shotgun cloning of total DNA. Sequences derived from these cDNAs should contain enough information to support phylogenetic comparisons. Alternatively, Lenstra et al. (10) have determined conditions necessary for production of long cDNAs applicable to both synthetic oligonucleotide primers and random priming with pentanucleotides. Random primers should be helpful in producing nested sets of cDNAs, including full-length copies, which would facilitate sequence determinations.

Detection of both plasmid and chromosomal DNA in purified lysates suggests that the method described here may be useful in studying the fate of extrachromosomal elements in culture or in the environment without the need for separate chromosomal and plasmid isolation procedures. Plasmid extraction by alkaline lysis is more rapid and yields plasmid DNA of higher purity than does extraction by enzymatic lysis, but plasmid yields are somewhat reduced and the chromosomal DNA is denatured (Fig. 5). To date, we have observed plasmid DNA only in pure culture lysates, but plasmid DNA isolated from environmental samples would not be expected to be present in high enough concentration for visualization by agarose gel electrophoresis. Highly sensitive hybridization probes, coupled with extraction as described here, should permit detection of plasmid DNAs that may be present at very low concentrations. Research is in progress to investigate plasmid carriage in nonculturable bacteria (2). Since viable but nonculturable cells are not easily harvested by centrifugation, the combination of efficient plasmid extraction and cell concentration by ultrafiltration should prove valuable in overcoming this obstacle.

The methods described herein were designed to provide a rapid, simple, and economical means of recovering nucleic acids from environmental isolates without the need for cell culture. Application of the method for recovery of high-molecular-weight DNA, plasmid DNA, and RNA species from the sample should permit a better understanding of both the phylogeny and the physiology of microbiota in aquatic environments.

ACKNOWLEDGMENTS

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