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Comparison of Methods of DNA Extraction from Stream Sediments†

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In Upper Three Runs Creek (Aiken, S.C.) and many other environments, less than 1% of bacteria visible microscopically can be cultured. Exploitation of molecular biology techniques has led to development of new methods, such as extraction of nucleic acids from soils or sediments, to study the dominant, nonculturable bacteria. The purpose of this study was to compare three published methods of DNA extraction that fall into two general categories: those in which cells are lysed in sediments (the Ogram and Tsai and methods [A. Ogram, G. S. Sayler, and T. Barkay, J. Microbiol. Methods 7:57–66, 1987; Y. L. Tsai and B. H. Olson, Appl. Environ. Microbiol. 57:1070–1074, 1991]) and those in which cells are removed from sediments prior to lysis (the Jacobsen method [C. S. Jacobsen and O. S. Rasmussen; Appl. Environ. Microbiol. 58:2458–2462, 1992]). DNA yield varied with extraction method; the Ogram method had a significantly higher yield than the other methods. However, DNA extracted via the Ogram method was badly sheared and contained a smaller proportion of eubacterial DNA. The Tsai method was less time consuming than the other methods, but DNA samples were of lower purity. If DNA purity is of paramount concern (as would be the case if PCR was to be performed) and quantity is not important, the Jacobsen method is recommended because of the low concentration of contaminants. If DNA is to be used directly in DNA-DNA hybridizations, the Ogram method is recommended since it gives maximal yields. However, if a Southern blot is to be performed, the Tsai method is recommended because of the high degree of DNA fragmentation observed with the other methods.

Widespread use of nucleic acid-specific stains in bacterial ecology (i.e., direct bacterial counts) (3) revealed that in many ecosystems, culturable bacteria represented only a fraction of the total bacterial assemblage. To study these nonculturable bacteria, which may contain species never previously discovered, scientists have frequently relied on extraction of nucleic acids from environmental samples. As this approach has become more popular, a number of different methods have been developed to extract DNA from soils and sediments (5–7, 9, 12–14).

Methods of DNA extraction from sediments and soils can be divided into two categories: those in which cells are lysed following removal from sediments, and those in which cells are lysed within the sediments. In this study, three DNA extraction techniques (6, 9, 14) were compared on the basis of yield, purity, quality, and taxonomic composition. When each method was published, the strengths and weaknesses of the method were discussed, but a comparison of all of these methods has not been reported (4, 7, 12, 13), making method selection difficult.

Approximately 600 g (wet weight) of sediment was collected from Upper Three Runs Creek on the U.S. Department of Energy's Savannah River Site near Aiken, S.C. Sediment, consisting of sand, clay, and detritus, was homogenized in the laboratory and subdivided into 50-g (wet weight) subsamples. DNA was extracted from four subsamples by each of the three methods.

In the method of Ogram et al. (subsequently referred to as the Ogram method), a bead beater was used to disrupt cells following incubation in 1.25% sodium dodecyl sulfate (SDS; in sodium phosphate buffer [pH 8]) at 70°C for 1 h (9). After centrifugation to remove glass beads and sediment particles, polyethylene glycol (Sigma) was added to precipitate DNA. Polyethylene glycol was removed by phenol-chloroform extraction. Following extraction, CsCl-ethidium bromide density gradient ultracentrifugation was used to concentrate and purify the DNA.

In the method of Tsai and Olson (subsequently referred to as the Tsai method), sediments were treated with lysozyme, and cells were lysed by rapid freezing and thawing (−70 to 65°C three times) (14). Following phenol-chloroform extraction, DNA was precipitated with isopropanol, and impurities were removed by gel filtration with Sephadex 100 (Pharmacia) as described by Moran et al. (8).

The method of Jacobsen and Rasmussen (subsequently referred to as the Jacobsen method) differed from the other two methods in that cells were removed from sediments prior to lysis (6). A cation-exchange resin (Chelex 100; Bio-Rad) was used to break the attraction of the cells for sediment particles. Resin and sediment were removed by centrifugation, and cells were treated with lysozyme and pronase. CsCl-ethidium bromide density gradient ultracentrifugation was used to further purify the extracted DNA.

Following DNA extraction, DNA concentrations were determined by the addition of Hoescht 33258 dye, which intercalates specifically with DNA (10). Intercalation was detected with a fluorescence spectrophotometer (Perkin-Elmer model 650-40). Contamination of extracted DNA by humic compounds and other organic materials was assessed by determining the A_{260} and A_{280} (4) with a spectrophotometer (Beckman DU-40).

The relative amounts of eubacterial DNA obtained by the three extraction procedures were determined with a eubacterium-specific oligonucleotide. Environmental DNA (1.0, 0.25, and 0.0625 μg per sample) was blotted onto Hybond-N nylon (Amersham) with a Mini-Fold II slot blot apparatus (Schleicher & Schuell). The eubacterium-specific oligonucleotide probe (5′-GCTGCCTCCCGTAGGAGT-3′), which hybridizes

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† Dedicated to the memory of Jim Dana, 1965–1993.

TABLE 1. Comparison of processing times, yields, and purities among DNA samples extracted from stream sediments

Extraction method	Time[a] (days)	DNA yield (μg of DNA/g of sediment)[b]	Purity[c]
Jacobsen	4.0	0.35 (0.06)	1.21
Ogram	4.5	1.74 (0.29)	1.03
Tsai	2.5	0.62 (0.06)	0.99

[a] Time required to extract and purify DNA from four samples.
[b] DNA yield from fluorometry. Values are means of four independently extracted samples with standard errors within parentheses.
[c] Ratio of A_{260} to A_{280}.

to positions 338 to 355 of the 16S rRNA gene (1), was end labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase (11). Filters were prehybridized in a mixture of 6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.7]), 1% SDS, 10× Denhardt's solution, and 100 μg of yeast tRNA at 55°C for 2 h, hybridized in 6× SSPE–1% SDS at 55°C overnight, washed three times for 8 min each at room temperature in 6× SSPE–1% SDS, and washed once at 55°C in 1× SSPE–1% SDS for 2 min. Hybridization strengths were determined with an AMBIS radioanalytical imaging system, and hybridization strength increased linearly with amount of DNA blotted.

DNA samples were also hybridized to an archaebacterium-specific probe and a universal probe as described above (2). Controls on all blots consisted of 1.0, 0.25, and 0.0625 μg of DNA from *Methanococcus aeolicus*, *Myxococcus xanthus*, *Escherichia coli*, and calf thymus or salmon sperm.

The three extraction techniques differed in the time required to process four samples (Table 1). DNA extraction required 1 to 1.5 days, and purification required 1 day for gel filtration and 3 days for CsCl-ethidium bromide density gradient ultracentrifugation. Costs incurred by the three techniques were similar, although the expense of the Chelex resin (about $10 per sample) made the Jacobsen method slightly more costly.

DNA yield varied significantly among methods (Table 1). The Ogram method had the greatest total DNA yield, and the Jacobsen method had the lowest.

Although DNA yield was greatest for the Ogram method, the DNA was highly fragmented because of shearing by the bead beater (Fig. 1). The Jacobsen method also provided fragmented DNA, perhaps because of digestion of DNA during the initial steps of the procedure prior to EDTA addition. The Tsai method provided DNA with the least fragmentation. Extent of fragmentation did not differ among samples processed by the same method (data not shown).

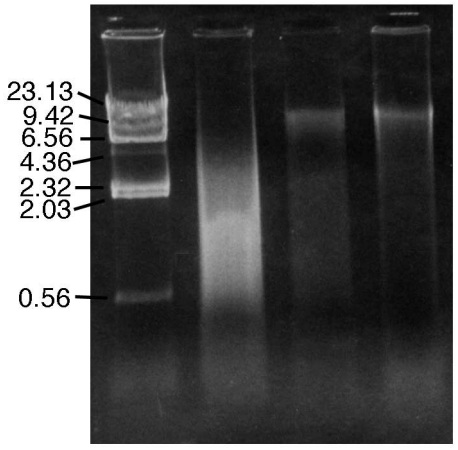

FIG. 1. Comparison of fragmentation of environmental DNA by extraction using three methods. Lanes: 1, lambda digested with *Hin*dIII; 2, Ogram sample; 3, Jacobsen sample; 4, Tsai sample. The electronic image was created with Adobe Photoshop for the Apple Macintosh after scanning the gel image.

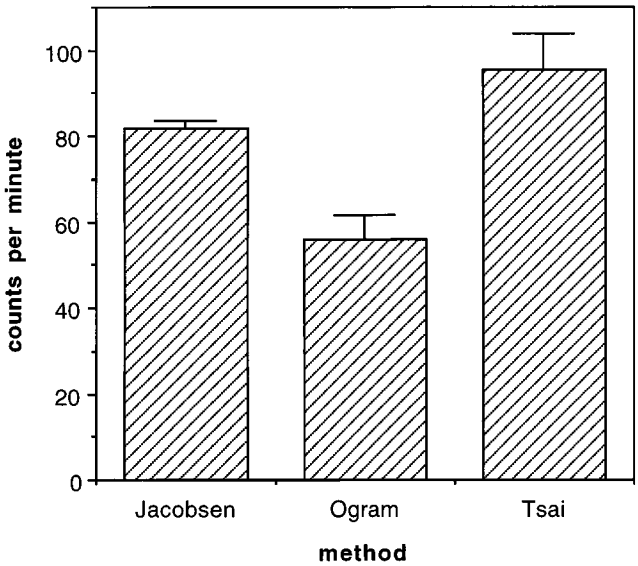

FIG. 2. Comparison of hybridization strengths of environmental samples with eubacterium-specific probe as quantified by AMBIS radioanalytical imaging system. Values are means (with standard errors) of four samples.

The purity of the extracted DNA varied as determined by the ratio of A_{260} to A_{280} (Table 1). In all cases, DNA samples were of low purity, apparently because of contamination with humic materials, but to different degrees; the Jacobsen samples had the lowest amount of contamination, and the Tsai samples had the greatest amount.

The relative amounts of eubacterial DNA obtained by the methods also varied (Fig. 2). The Tsai samples contained the greatest fraction of eubacterial DNA, and the Ogram samples contained the smallest. Non-eubacterial DNA in the Ogram samples was apparently eukaryotic because these samples had minimal hybridization with an archaebacterium-specific probe (data not shown) (2). Environmental DNA samples were also hybridized with a universal probe (2) in an effort to convert hybridization strengths to more useful units (i.e., micrograms of eubacterial DNA per gram of sediment). However, the universal probe had a higher hybridization strength per microgram of DNA for the *Methanococcus aeolicus* control compared with that of the eubacterial and eukaryotic controls, and thus the conversion could not be performed accurately.

Each of the three methods of extraction has advantages and disadvantages, and method choice will vary with the goals of the study. If DNA must be of high purity and quantity is not important, the Jacobsen method is recommended because of the low concentration of contaminants. However, recovery of cells by this method may be differential, depending on the strength and nature of the attachment of the cell to the sediment particles. If DNA is to be used directly in DNA-DNA hybridizations, the Ogram method is recommended since it gives maximal yields. If purity and contamination with eukaryotic DNA is of concern, the Tsai method is recommended. Also, the Tsai method is recommended if a Southern blot is to

be performed because of the high degree of DNA fragmentation observed with the Ogram method. Comparatively lower levels of fragmentation with the Jacobsen method may make the DNA obtained also suitable for Southern blots.

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