Rapid Extraction of DNA and rRNA from Sediments by a Novel Hydroxyapatite Spin-Column Method

K. J. PURDY,1* T. M. EMBLEY,2 S. TAKI,3 and D. B. NEDWELL1

Department of Biological and Chemical Sciences, University of Essex, Colchester, Essex CO4 3SQ,1 and Department of Zoology, Natural History Museum, London SW7 5BD,2 United Kingdom, and Department of Biology, Tokyo Metropolitan University, Hachioji-Shi, Tokyo 192-03, Japan3

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We describe a novel hydroxyapatite spin-column method of nucleic acid extraction from natural sediments by which DNA and rRNA can be extracted separately. This very rapid method produces pure nucleic acid that can be utilized in some of the most common molecular biological procedures used in the analysis of natural microbial communities.

Molecular analysis of natural populations of microorganisms requires the extraction of high-quality nucleic acids directly from the environment. It is generally accepted that direct lysis and extraction of nucleic acid from an environmental sample is necessary in order to obtain nucleic acid that is representative of the microbial community but that this may be complicated by poor extraction efficiency and coextraction of contaminating organic material (16, 18). Numerous direct lysis methods exist and range from enzymatic lysis to mechanical lysis with bead beating (3, 9, 11, 12, 19). More et al. (9) demonstrated that mechanical lysis was the most efficient and least selective method of lysing cells in sediments.

Hydroxyapatite (HTP) has been used for many years as a high-performance liquid chromatography column matrix to bind and separate nucleic acids and proteins (1, 6, 7). Different types of DNA molecules as well as different types of RNA can be separated with HTP (2, 7). Here we describe a method which utilizes HTP in a spin column to extract, either together or separately, high-purity DNA and rRNA from natural sediment samples.

Sediment samples from Colne Point Salt Marsh, Essex, United Kingdom (fine, silty clay sediment with a high organic content [10]), were shaken at 150 rpm with equal volumes of 120 mM sodium phosphate, pH 8.0, for 15 min in order to remove extracellular nucleic acids, pelleted by centrifugation at 6,000 × g for 10 min (19), and stored in 2-g samples at −20°C.

We compared our method with the widely used (3, 5, 20) Tsai and Olson method (19), which involves enzymatic and freeze-thaw lysis steps and phenol and phenol-chloroform purification of extracted nucleic acids. The Tsai and Olson method was slightly modified in that ethanol was used instead of isopropanol to precipitate the nucleic acid. Humic contaminants were removed with a 2.5-ml Sephadex G-75 spin column as described by Moran et al. (8), since the column used in the original method (Elutip-D; Schleicher & Schuell, Dassel, Germany) was specific for DNA.

Our new extraction method, which also includes elements of previously published procedures (11, 16, 19), was developed as follows. Samples of sediment (1 g) were aliquoted (0.5 g) into two 2-ml screw-cap Eppendorf tubes, each with 0.5 g of 0.1-mm-diameter glass beads baked at 260°C. The following solutions were then added to each tube: 0.70 ml of 120 mM sodium phosphate (pH 8.0) plus 1% (wt/vol) acid-washed polyvinyl-polypyrrolidone (4), 0.5 ml of Tris-equilibrated phenol (pH 8.0) (14), and 50 μl of 20% (wt/vol) sodium dodecyl sulfate. The samples were bead beaten (Mikrodiaphragmator U; Braun Biotech International, Melsungen, Germany) three times at 2,000 rpm for 30 s, with 30 s on ice in between bead beatings. They were then centrifuged at 12,000 × g for 2 min, and the supernatants were pooled and stored on ice. In order to extract residual nucleic acid from the sediment pellet, the pellet was resuspended in 0.70 ml of 120 mM sodium phosphate, pH 8.0, bead beaten at 2,000 rpm for 30 s, and then centrifuged again. The supernatants from both the first and second extractions were pooled.

An HTP (Bio-Gel HTP; Bio-Rad Laboratories Ltd., Hemel Hempstead, United Kingdom) spin column was made from a 1-ml plastic hypodermic syringe plugged with a sterile pyrex glass wool plug (14) supporting 0.6 to 0.7 ml of HTP. (DNA-grade Bio-Gel HTP cannot be used in spin columns as the small particle size and slower flow rate make loading times extremely long.) The extracted samples were loaded onto a prepared HTP spin column by spinning 0.7-ml aliquots of extract at 100 × g in a swing-out rotor at room temperature for 2 to 4 min until all the sample was loaded. The column was then washed by spinning three times with 0.5-ml aliquots of 120 mM sodium phosphate, pH 7.2, to remove any protein binding to the HTP.

The concentration of the elution buffer was dependent on whether total nucleic acid or separate DNA and RNA were required. Total nucleic acid was eluted into a sterile 1.5-ml Eppendorf tube with 0.4 ml of 300 mM K2HPO4, pH 7.2. (Potassium phosphate is much more effective than sodium phosphate at eluting nucleic acid from HTP columns [2].) The eluent was desalted with a 2.5-ml Sephadex G-75 spin column (8), and the nucleic acid was precipitated with 2.5 volumes of ethanol (14). If, after ethanol precipitation, the pellet was white (indicating no humic contamination), then the pellet was resuspended in 50 μl of TE (10 mM Tris, 1 mM EDTA [pH 8.0]) and stored at −70°C, but if the pellet was brown (indicating some humic contamination), then the pellet was resuspended in 200 μl of sterile double-distilled water and precipitated with polyethylene glycol 8000 at 4°C for 1 h (14). After centrifugation at 12,000 × g for 15 min, the supernatant was
removed by aspiration and the pellet was washed with 70% ethanol, resuspended in 50 μl of TE, and stored at −70°C. The typical processing time, including precipitation, for a single sample was 4 h compared with a minimum of 7 h by the Tsai and Olson method (19).

Separate elution of DNA and rRNA requires that the rRNA be eluted from the column prior to DNA elution. Therefore, rRNA was eluted into three sterile 1.5-ml Eppendorf tubes. Batch elution from the column prior to DNA elution. Therefore, and Olson method (19).

A agarose gel of these samples showed similar DNA and rRNA content. A visual examination of an ethidium bromide-stained gel electrophoresis (Fig. 1), and the A260/A280 and A260/A230 ratios of DNA extracted by this method indicated that it was heavily contaminated with humic substances (16) (Table 1). Humic contaminants affected spectrophotometric quantification of nucleic acids to the extent that, in our work, the yield from the Tsai and Olson method could not be accurately determined. However a visual semiquantitative analysis of Fig. 1 appeared to indicate that the yield was substantially less than that of the HTP spin-column method.

In our hands the modified Tsai and Olson method (19) extracted substantially less nucleic acid from replicate sediment samples, as seen by ethidium bromide-stained agarose gel electrophoresis (Fig. 1), and the A260/A280 and A260/A230 ratios of DNA extracted by this method were lower than those from the Tsai and Olson method (Table 1). The separation of DNA and rRNA by this method is shown in Fig. 2. Purification of RNA requires only an extra 45- to 60-min processing time.

Samples of nucleic acid extracted from sediment by the HTP spin-column method have been used successfully in PCRs and restriction digestions and also in slot blot hybridizations to radiolabelled 16S rRNA-targeted oligonucleotide probes (data not shown).

To separate DNA from the sediment samples from Colne Point, we used a method similar to the one described here for reproducibly extracted nucleic acid not only in larger cells but also in the form produced by the method of Tsai and Olson (19). The speed with which samples can be processed greatly facilitates the extraction of rRNA by minimizing exposure to RNases released during cell lysis. The extracted DNA and rRNA can be used with some of the most common molecular biological methods. The nucleic acid yield obtained from sediment, 43.2 μg/g (wet weight) of sediment, was greater than the best yields reported by other researchers (9, 12, 19). Nucleic acids have been extracted successfully by this HTP method from a variety of riverine, estuarine, marine, and lake sediments (17) as well as from coarse sediments from Antarctica (13).

A major advantage of HTP as a purification matrix is the ease with which rRNA can be extracted separately from DNA without the need to use additional steps, such as enzymatic purification. The ability to extract separately the rRNA and DNA from the same sample provides the possibility of assessing the relative metabolic activities of a community by probing rRNA (15) as well as the possibility of investigating the community structure by analyzing DNA from the same sample.

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