Isolation of Intact High-Molecular-Weight DNA by Using Guanidine Isothiocyanate

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A method for obtaining high-molecular-weight chromosomal DNA from Bacteroides intermedius and Bacteroides gingivalis is described. This technique is a modification of the guanidine isothiocyanate isolation procedure for RNA and should be useful for isolating intact DNA from organisms with high endogenous nuclease activity.

The standard preparative procedure for extraction and purification of intact high-molecular-weight chromosomal DNA from procaryotic organisms has required an enzyme detergent lysis and an organic solvent treatment (3). In previous studies, as well as in our own attempts to isolate intact DNA from the anaerobic periodontal pathogens Bacteroides intermedius and Bacteroides gingivalis, the standard procedure of Marmur or published modifications of the method have not yielded intact or large amounts of nucleic acid (2, 3).

In an effort to develop a method to isolate large amounts of intact high-molecular-weight DNA, we have adapted a technique routinely used for isolation of RNA from eucaryotic cells (1).

The initial identification and characterization of the procedure used cultures of B. intermedius, the organism from which we have experienced the most difficulty in isolating intact DNA. A comparative test isolation procedure for the B. intermedius DNA which used guanidine isothiocyanate and the Marmur procedure was done with duplicate samples. A 1-liter culture of B. intermedius was grown to log phase anaerobically at 37°C in mycoplasma broth base (BBL Microbiology Systems) supplemented with 0.5% glucose, 0.5 µg of hemin per ml, and 0.3 µg of menadione per ml. The optical density at 600 nm was read, and the culture was split into two aliquots. The cells were centrifuged at 8,000 rpm for 10 min in a Sorvall RC-2B centrifuge (Ivan Sorvall, Inc.) at 4°C. DNA was extracted from one aliquot by the standard lysis chloroform-phenol extraction procedure of Marmur. DNA was extracted from the second aliquot by the guanidine isothiocyanate procedure of Chirgwin et al. (1) as follows. The cell pellet was resuspended by vortexing in 5 volumes of a solution of 5 M guanidine isothiocyanate (Bethesda Research Laboratories)-50 mM Tris chloride (pH 7.6)-10 mM EDTA-2% S-lauryl sarcosinate (Sigma Chemical Co.)-140 mM 2-mercaptoethanol. A 1-g amount of CsCl was added to each 2.5 ml of homogenate. The homogenate was layered over a 2-ml cushion of 5.7 M CsCl in a 12-ml polyallomer centrifuge tube (Sorvall; model 03699), and the top was overlaid with glycerol. The samples were spun for at least 16 h in a Sorvall RC70 ultracentrifuge at 35,000 rpm at 20°C in a swinging bucket TH641 rotor. After ultracentrifugation, the contents of the tube were fractionated into 500-µl aliquots and 5-µl portions were spotted on a 1% agarose plate containing 0.5 µg of ethidium bromide per ml. The DNA-containing fractions were visualized under UV light (Photodyne), pooled, and dialyzed at 4°C against 10 mM Tris-1 mM EDTA overnight, and 1 µg of DNA from each procedure was electrophoretically analyzed (Fig. 1). The profile from the procedure of Marmur shows a degraded heterogenous size distribution of DNA fragments from the B. intermedius culture extract (Fig. 1, lane 3). DNA obtained by using the guanidine isothiocyanate procedure yielded intact high-molecular-weight chromosomal DNA at a predominant size of 23 kilobases (Fig. 1, lane 2). Both preparations appear completely free of RNA.

DNA obtained by using the guanidine isothiocyanate procedure was then digested with restriction enzymes and electrophoretically analyzed. The size distribution of fragments generated by the restriction enzyme digest of DNAs isolated from B. intermedius and B. gingivalis is shown in Fig. 2. The complete digestion of DNAs isolated from B. intermedius and B. gingivalis is indicated in lanes 3 and 5, respectively. DNA isolated by the guanidine isothiocyanate purification method and then digested with restriction en-

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FIG. 1. Gel electrophoresis of B. intermedius DNA. DNA preparations were electrophoresed on a 0.8% agarose gel in a 0.089 M Tris borate-0.089 M boric acid-0.002 M EDTA buffer system containing 0.5 µg of ethidium bromide per ml. Kilobases. Lanes: 1, linear DNA size markers obtained from a HindIII restriction enzyd digest; 2, B. intermedius DNA extracted and purified by using the guanidine isothiocyanate procedure described in the text; 3, B. intermedius DNA extracted by the phenol-chloroform-isoamyl isolation procedure of Marmur.
FIG. 2. Gel electrophoresis of guanidine isothiocyanate-purified B. intermedius and B. gingivalis DNA. DNA preparations were electrophoresed on a 0.8% agarose gel system run in a solution of 0.089 M Tris borate–0.089 M boric acid–0.002 M EDTA containing 0.5 μg of ethidium bromide per ml. kb, Kilobases. Lanes: 1, linear DNA size markers obtained from a HindIII restriction enzyme digest; 2, B. intermedius DNA; 3, B. intermedius DNA digested with the restriction enzyme PstI; 4, B. gingivalis DNA; 5, B. gingivalis DNA digested with the restriction enzyme PstI.

zymes showed the appropriate size distribution for DNA cloning procedures.

Functional analysis of the DNA was performed. The ability of the isolated nucleic acid to be radiolabeled by nick translation was determined, followed by hybridization analysis (data not shown).

The guanidine isothiocyanate procedure is one which is rapid and quantitative and does not rely on labor-intensive standard methodology. We also used the procedure for the isolation of DNA from Actinobacillus actinomycetemcomitans and Eikenella corrodens (data not shown) and were also successful in the isolation of high-molecular-weight and RNA-free DNA preparations. We recommend this procedure for isolating DNA from any organism in which there are high levels of endogenous nucleases.

In summary, we have developed a rapid and convenient method of isolating DNA from anaerobic bacteria which have high levels of endogenous nucleases.

LITERATURE CITED